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*Original article (Orijinal araştırma)*

# Tachinid (Diptera: Tachinidae) parasitoids reared from lepidopterous and hymenopterous hosts in southern forests of Turkey

## Türkiye'nin güneyindeki ormanlarda lepidopter ve hymenopter konukçulardan elde edilen tachinid (Diptera: Tachinidae) parazitoitler

**Fatih AYTAR[1]** **Kenan KARA[2]** **Turgut ATAY[2*]**

## Abstract

This study was conducted to determine the tachinid (Diptera: Tachinidae) parasitoids of lepidopterous and hymenopterous hosts from forests of southern Turkey (Adana, Hatay, Karaman, Mersin, Niğde and Osmaniye) in 2002-2019. For this purpose, host larvae were collected from forests and herbaceous plant communities and brought to the laboratory with their food-plants. As a result of the study, six tachinid species were reared from nine hosts. Four new hosts for Turkey were recorded. These are *Vanessa cardui* (L., 1758) and *Polygonia c-album* (L., 1758) (Lepidoptera: Nymphalidae) for *Sturmia bella* (Meigen, 1824) (Diptera: Tachinidae), *Utetheisa pulchella* (L., 1758) (Lepidoptera: Erebidae) for *Exorista segregata* (Rondani, 1859) (Diptera: Tachinidae) and *Thaumetopoea wilkinsoni* Tams, 1924 (Lepidoptera: Notodontidae) for *Compsilura concinnata* (Meigen, 1824) (Diptera: Tachinidae). In addition, two parasitoid-host couples were recorded for the second time in the world. These are *U. pulchella* for *E. segregata* and *T. wilkinsoni* for *C. concinnata*.

**Keywords:** Forest, host records, parasitoids, Tachinidae, Turkey

## Öz

Bu çalışma Türkiye'nin güneyindeki (Adana, Hatay, Karaman, Mersin, Niğde ve Osmaniye) ormanlarda bulunan lepidopter ve hymenopterlerin, tachinid (Diptera: Tachinidae) parazitoitlerini belirlemek için 2002-2019 yılları arasında yürütülmüştür. Bu amaç için konukçu larvaları orman ağaçları ve yabancı otlar üzerinden toplanarak beslendikleri bitki ile birlikte laboratuvara getirilmiştir. Çalışma sonucunda, dokuz farklı konukçudan altı tachinid tür elde edilmiş, 4 konukçu ise Türkiye için yeni konukçu kaydı olarak belirlenmiştir. Bunlar; *Sturmia bella* (Meigen, 1824) (Diptera: Tachinidae) için *Vanessa cardui* (L., 1758) ve *Polygonia c-album* (L., 1758) (Lepidoptera: Nymphalidae), *Exorista segregata* (Rondani, 1859) (Diptera: Tachinidae) için *Utetheisa pulchella* (L., 1758) (Lepidoptera: Erebidae) ve *Compsilura concinnata* (Meigen, 1824) (Diptera: Tachinidae) için *Thaumetopoea wilkinsoni* Tams, 1924 (Lepidoptera: Notodontidae)'dır. Ayrıca *E. segregata* için *U. pulchella* ve *C. concinnata* için *T. wilkinsoni* konukçu-parazitoit çiftleri dünya için ikinci kez kaydedilmiştir.

**Anahtar sözcükler:** Orman, konukçu kayıtları, parazitoit, Tachinidae, Türkiye

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## Introduction

Forests are terrestrial ecosystems that consists of various trees, shrubs, herbaceous plants, microorganisms, insects and other animals. There are also economically important pests in forests in Turkey. The activities and damage of such pests do not occur suddenly. Forest pests are generally not given sufficient consideration and are even of no interest to the general public with the destruction of the pests only understood after they become an epidemic. It is known that 50 species and more cause damage in Turkish forests. The plant protection operations are conducted against these pests on 205 kHa in 2019. About a million dollars is spent for these activities each year (Anonymous, 2020). In the past, chemical control measures were used intensely. In recent years, mechanical, biological (bird nest construction, ant augmentation, and parasitoid and predator mass production) and biotechnical (pheromone) methods that do not harm nature are applied instead of chemicals. Also, the use of pesticides is very difficult and expensive in huge areas. In addition, pesticides lead to soil, air and water pollution and resistance, and damaging side effects to natural enemies should not be neglected. It is critical to protect natural enemies, so that they can provide effective biological control. Therefore, it is necessary to support the activities of these insects. Declines in beneficial organisms can lead to increased outbreaks of pests. Therefore, it is important to investigate the biology, ecology and host relationships of natural enemies.

Tachinids are an important group of parasitoids and the majority of the hosts are insect pests. Many hosts are lepidopteran pests. Others hosts are species belonging to the orders Coleoptera, Hemiptera, Hymenoptera, Orthoptera, Diptera and Lithobiomorpha (Grenier, 1988; Stireman et al., 2006; Tschorsnig, 2017). Detailed information on Palearctic and Turkish hosts of tachinids can be found in Tschorsnig (2017) and Kara & Tschorsnig (2003), respectively. Kara et al. (2014) have prepared a comprehensive catalog of tachinid-host associations in Turkish forests. It comprises 27 tachinid species reared from 14 hosts belonging to three orders.

Applied biological control studies with tachinid flies against certain forest pests were successfully performed in Canada and the USA in the 1900s (Grenier, 1988). Augmentative releases were conducted with *Blepharipa pratensis* (Meigen, 1824) in 1933 and *Compsilura concinnata* (Meigen, 1824) (Diptera: Tachinidae) in 1976 against the gypsy moth, *Lymantria dispar* (L., 1758) (Lepidoptera: Erebidae). These two parasitoids have been successfully established and are important parasitoids of the gypsy moth in many states of the USA (Blumenthal et al., 1979). Against the winter moth, *Operophtera brumata* (L., 1758) (Lepidoptera: Geometridae), two tachinids, *Lypha dubia* (Fallén, 1810) and *Cyzenis albicans* (Fallén, 1810) (Diptera: Tachinidae), were used in Canada in 1955-1980. Only the latter species was successfully established (Pschorn-Walcher et al., 1969). In Turkey, *Phryxe caudata* (Rondani, 1859) (Diptera: Tachinidae) is produced by means of an islet, a wire cage and a water chambered wire cage technique and released against *Thaumetopoea pityocampa* (Denis & Schiffermüller, 1775) (Lepidoptera: Notodontidae) which causes significant damage to pine trees (Kanat & Türk, 2002; Özdal, 2002).

This paper focuses on the tachinid parasitoids of some forest pests in southern Turkey.

## Materials and Methods

This research was conducted to determine the tachinid parasitoids of lepidopterous and hymenopterous hosts from forests of southern Turkey (Adana, Hatay, Karaman, Mersin, Niğde and Osmaniye) in 2002-2019. Host larvae were collected from forests and herbaceous plant communities.

The collected host larvae were brought to the laboratory together with the plants they consume for rearing, transferred to distinct cages, and kept at 25 ± 2°C and 60-70% RH. Daily checks were made, and food was refreshed as needed. Also, parasitoid emergence was observed daily. After adult parasitoids were obtained, flies were prepared for identification. Reared parasitoids were described according to Mesnil (1944-1965), Tschorsnig & Herting (1994) and Tschorsnig & Richter (1998). Herting & Dely-Draskovits

(1993) was also followed for species nomenclature. Lepidopterous samples were identified using the keys of Doğanlar & Avcı (2001), Doğanlar et al. (2005), and Mazzei et al. (2020). Hymenopterous host was identified using the paper of Smith (1974). Plants were identified by Dr. Mehtap Öztekin (Systematic Botany, Department of Collections, National Botanical Gardens Directorate, Ministry of Agriculture and Forestry, Republic of Turkey, Ankara).

The date of emergence, the number of male and female individuals, the host species, the place where the hosts were collected and the plant where the host insect fed are given for each parasitoid separately. In addition, general information is given on distribution, hosts, and biology of reared parasitoids.

## Results and Discussion

Six tachinid species were reared as parasitoids of eight lepidopteran and one hymenopteran host.

**Subfamily: Exoristinae**

**Tribe: Exoristini**

***Exorista segregata*** (Rondani, 1859)

Reared specimens. 07.10.2017, ♂, reared from *Utetheisa pulchella* (L., 1758) (Lepidoptera: Erebidae), collected in Yenişehir, Mersin from *Heliotropium* sp. (Boraginaceae); 06.07.2009, ♀, reared from *L. dispar* collected in Tarsus, Mersin from *Quercus coccifera* L., 1753 (Fagaceae); and 03.08.2017, 7♂♂, 4♀♀, reared from *Thaumetopoea ispartaensis* Doğanlar & Avcı, 2001 (Lepidoptera: Notodontidae) collected in Toroslar District, Mersin from *Cedrus libani* A. Rich. (Pinaceae).

Distribution in Turkey. İstanbul (Schimitschek, 1944), Trakya (Gürses, 1975), Erzurum (Doğanlar, 1975; Doğanlar, 1982; Kılıç & Alaoğlu, 1996; Özbek & Çoruh, 2012), Ankara, Kırşehir, Niğde (Kansu et al., 1986), Tokat (Kara, 1998; Kara & Alaoğlu, 2001; Atay & Kara, 2014), Isparta (Avcı & Kara, 2002), Belen (Mückstein et al., 2004), Lakes District (Avcı, 2009), Nevşehir (Bartsch & Tschorsnig, 2010), Mersin (Akdağcık, 2010) and Muğla (Lutovinovas et al., 2018).

Hosts in Turkey. *Thaumetopoea pityocampa* (Schimitschek, 1944), *Euproctis chrysorrhoea* (L., 1758) (Lepidoptera: Erebidae) (Gürses, 1975), *Leucoma salicis* (L., 1758), *Malacosoma castrensis* (L., 1758), *Malacosoma franconica* (Denis & Schiffermüller, 1775) (Lepidoptera: Lasiocampidae), *Simyra* sp. (Lepidoptera: Noctuidae) (Herting, 1960; Doğanlar, 1975), *Euproctis* sp., *Phalera bucephala* (L., 1758) (Lepidoptera: Notodontidae), *Simyra dentinosa* Freyer, 1838 (Lepidoptera: Noctuidae) (Doğanlar, 1982; Atay & Kara, 2014), *Hyles centralasiae* (Staudinger, 1887) (Lepidoptera: Sphingidae) (Bartsch & Tschorsnig, 2010), *L. dispar* (Kara & Tschorsnig, 2003; Avcı, 2009), *L. salicis* (Kansu et al., 1986; Kılıç & Alaoğlu, 1996; Kara & Alaoğlu, 2001), *Malacosoma neustria* (L., 1758) (Lepidoptera: Lasiocampidae) (Kara & Alaoğlu, 2001; Özbek & Çoruh, 2012), *Parocneria terebinthi* (Freyer, 1838) (Lepidoptera: Erebidae) (Kara & Alaoğlu, 2001), *Aporia crataegi* (L., 1758) (Lepidoptera: Pieridae) (Kansu et al., 1986; Kara & Tschorsnig, 2003), *T. ispartaensis* (Avcı & Kara, 2002), *Pieris* sp., *Aglais io* (L., 1758) (Lepidoptera: Nymphalidae), *Zygaena carniolica* (Scopoli, 1763) (Lepidoptera: Zygaenidae) (Kara & Tschorsnig, 2003), *Cucullia lanceolata* (Villers, 1789) (Lepidoptera: Noctuidae) (Mückstein et al., 2004), *Pieris brassicae* (L., 1758) (Lepidoptera: Pieridae) (Akdağcık, 2010) and *Hyles siehei* Püngeler, 1903 (Lepidoptera: Sphingidae) (Bartsch & Tschorsnig, 2010).

*Utetheisa pulchella* is a new host for *E. segregata* in Turkey. There is only a single record of *E. segregata* reared from *U. pulchella* in the world (Kugler, 1980).

Remarks. In southern Europe has been seen from March to December, in several generations, visits flowers, hosts are species belonging to Erebidae, Zygaenidae, Noctuidae, Lasiocampidae, Notodontidae, Nymphalidae, Pieridae and Saturniidae (Tschorsnig & Herting, 1994).

***Diplostichus janitrix*** (Hartig, 1837)

Reared specimens. 07.10.2019, ♀, reared from *Diprion pini* (L., 1758) (Hymenoptera: Diprionidae), collected in Aladağlar, Adana from *Pinus nigra* Arnold subsp. *pallasiana* (Lamb.) Holmboe var. *pallasiana* (Pinaceae).

Distribution in Turkey. Ankara (Tunca et al., 2009).

Hosts in Turkey. *Diprion pini* (Tunca et al., 2009) and *Neodiprion sertifer* Geoffroy (Aksu, 2010).

Remarks. This parasitoid is seen till mid-September from end of June in pine forests, it does not visit flowers and probably has only one generation in Europe. It has been only rarely collected in the field, but is usually reared from its hosts. *Diplostichus janitrix* has a narrow host range. Diprionidae (Hymenoptera) is the usual host family and it was mostly reared from *Diprion* spp. (Tschorsnig & Herting, 1994; Tschorsnig, 2017).

**Tribe: Blondeliini**

***Compsilura concinnata*** (Meigen, 1824)

Reared specimens. 28.04.2017, 2♂♂, 10♀♀, reared from *Thaumetopoea wilkinsoni* Tams, 1824 (Lepidoptera: Notodontidae), collected in Central District, Mersin from *Pinus brutia* Ten. (Pinaceae).

Distribution in Turkey. Ankara (Tuatay et al., 1972), Uşak, Denizli (Öncüer et al., 1977), Erzurum (Doğanlar, 1982; Kılıç & Alaoğlu, 1996), Ankara, Kırşehir, Niğde (Kansu et al., 1986), Artvin, Erzurum, Gümüşhane, Trabzon (Eroğlu, 1995), Samsun (Tuncer & Ecevit, 1996; Sullivan et al., 2012), Tokat (Kara, 1998; Atay & Kara, 2014), Bursa (Kovancı et al., 1999), Eskişehir (Kara & Özdemir, 2000; Aksu, 2005), Isparta (Avcı & Kara, 2002), Hatay (Kaya & Kornoşor, 2008), Lakes District (Avcı, 2009), Hatay (Kaya et al., 2016), Sakarya (Balkan et al., 2015), Muğla (Lutovinovas et al., 2018) and Edirne (Tek & Okyar, 2018).

Hosts in Turkey. *Euproctis* sp. (Lepidoptera: Erebidae) (Tuatay et al., 1972), *E. chrysorrhoea* (Öncüer et al., 1977; Soydanbay, 1978; Eroğlu, 1995; Kara, 1998), *L. salicis* (Doğanlar, 1982; Kansu et al., 1986; Kılıç & Alaoğlu, 1996), *M. neustria*, *L. dispar* (Kansu et al., 1986; Avcı, 2009), *Hyphantria cunea* (Drury, 1773) (Lepidoptera: Erebidae) (Tuncer & Ecevit, 1996; Sullivan et al., 2012), *Parnassius apollo* (L., 1758) (Lepidoptera: Papilionide) (Kovancı et al., 1999), *P. brassicae* (Kara, 1998; Kaya & Kornoşor, 2008; Akdağcık, 2010), *Yponomeuta padella* (L., 1758) (Lepidoptera: Yponomeutide) (Kara & Özdemir, 2000), *T. pityocampa* (Oğurlu, 2000), *Autographa gamma* (L., 1758) (Lepidoptera: Noctuidae) (Kara & Tschorsnig, 2003), *P. terebinthi* (Kara & Alaoğlu, 2001), *T. ispartaensis* (Avcı & Kara, 2002), *Pontia daplidice* (L., 1758) (Lepidoptera: Pieridae), *Helicoverpa armigera* (Hübner, 1808) (Lepidoptera: Noctuidae) (Kaya & Kornoşor, 2008), *Helcystogramma triannulella* (Herrich-Schäffer, 1854) (Lepidoptera: Gelechiidae) (Kaya et al., 2016) and *Thaumetopoea solitaria* (Freyer, 1838) (Lepidoptera: Notodontidae) (Atay & Kara, 2014).

*Thaumetopoea wilkinsoni* is a new host for *C. concinnata* in Turkey. There is only one record of *C. concinnata* being reared from *T. wilkinsoni* (Tschorsnig, 2017).

Remarks. In Europe, this common parasitoid has two generations and is seen from May-September on flowers or foliage. It is reared from numerous Lepidoptera, whereas Microlepidoptera and Tenthredinidae are only rarely parasitized (Tschorsnig & Herting, 1994; Tschorsnig, 2017).

**Tribe: Eryciini**

***Phryxe caudata*** **(Rondani, 1859)**

Reared specimens. 20.10.2005, ♂, 3♀♀, reared from *T. wilkinsoni* collected in Mezitli, Mersin from *P. brutia*; 10.11.2005, ♀, reared from *T. wilkinsoni* collected in Beylice Village, Tarsus, Mersin from *P. brutia*; 11.11.2005, ♀, reared from *T. wilkinsoni* collected in Karaman on *Pinus nigra* Arnold (Pinaceae); 15.11.2005, ♂, reared from *T. wilkinsoni* collected in Çamalan Village, Tarsus, Mersin from *P. brutia*; 15.11.2005, ♂,

reared from *T. wilkinsoni* collected in Tekir, Adana from *P. nigra*; 21.04.2006, ♂, reared from *T. wilkinsoni* collected in Mut, Mersin from *P. brutia*; 21.04.2006, ♀, reared from *T. wilkinsoni* collected in Silifke, Mersin from *P. brutia*; 28.10.2007, ♀, reared from *T. wilkinsoni* collected in Pozantı, Adana from *P. nigra*; 25.04.2009, 2♀♀, reared from *T. wilkinsoni* collected in Sarıçam, Adana from *P. brutia*; 25.04.2009, ♂, ♀, reared from *T. wilkinsoni* collected in Osmaniye from *P. brutia* and *Pinus halepensis* Miller (Pinaceae); 25.04.2009, ♀, reared from *T. wilkinsoni* collected in Ceyhan, Adana from *P. halepensis*; and 26.04.2009, ♀, reared from *T. wilkinsoni* collected in Serinyol, Hatay from *P. brutia*.

Distribution in Turkey. Antalya (Tosun, 1976), İzmir (Soydanbay, 1978), Isparta (Avcı & Kara, 2002), Lakes Districts (Avcı & Oğurlu, 2002), Muğla (Özçankaya & Can, 2004) and Tokat (Atay & Kara, 2014).

Hosts in Turkey. *Thaumetopoea ispartaensis*, *T. pityocampa* (Denis & Schiffermüller, 1775) (Soydanbay, 1978; Avcı & Oğurlu, 2002, Kanat & Türk, 2002; Özdal, 2002; Kara & Tschorsnig, 2003; Özçankaya & Can, 2004; Atay & Kara, 2014), and *T. wilkinsoni* (Battisti et al., 2015).

Remarks. Thaumetopoeidae (currently placed in Notodontidae) is the usual host family for *P. caudata* and which is commonly reared from *Thaumetopoea* spp., especially *T. pityocampa* (Tschorsnig, 2017). In Turkey, *P. caudata* is produced and released in nature against *T. pityocampa* (Kanat & Türk, 2002; Özdal, 2002).

***Drino inconspicua*** (Meigen, 1830)

Reared specimens. 01.04.2005, ♀, reared from *D. pini*, collected in Mut, Mersin from *P. brutia*; 21.04.2006, ♀, reared from *D. pini*, collected in Mut, Mersin from *P. brutia*; 07.06.2007, 2♂♂, reared from *D. pini*, collected in Karaman from *P. nigra*d; and 20.10.2012, ♀, reared from *D. pini*, collected in Alihoca Village, Niğde from *P. nigra*.

Distribution in Turkey. Erzurum (Doğanlar, 1975; Doğanlar, 1982), Kırklareli (Haeselbarth, 1983), Konya (Tschorsnig, 2005), Bolu (Korkmaz, 2007), Lakes District (Avcı, 2009) and Burdur, Muğla (Lutovinovas et al., 2018).

Hosts in Turkey. *Malacosoma neustria* (Doğanlar, 1975), *L. dispar* (Herting, 1983; Avcı, 2009) *D. pini* (Tschorsnig, 2005), *Neodirion sertifer* (Geoffroy, 1785) (Hymenoptera: Diprionidae), (Akıncı & Avcı, 2016) and *P. bucephala* (Schimitschek, 1944; Doğanlar, 1982).

Remarks. In Europe, this parasitoid is commonly found in pine forests. It is collected from early June to Mid-September and mostly has two generations per year. In the field it is rather rare, but is more commonly reared from its hosts. *Diprion* spp. Schrank, 1802 (Hymenoptera: Diprionidae) are common hosts, but it is also reared from a few Lepidoptera, especially *L. dispar* and *Dendrolimus pini* L. (Lepidoptera: Lasiocampidae) (Tschorsnig & Herting, 1994; Tschorsnig, 2017).

**Tribe: Goniini**

***Sturmia bella*** (Meigen, 1824)

Reared specimens. 12.09.2002, ♀; 17.09.2002, ♀; 21.09.2002, 2♂♂; 30.09.2002, 2♂♂, reared from *Aglais urticae* (L., 1758) (Lepidoptera: Nymphalidae), collected in Aladağlar, Adana from *Urtica* sp. (Urticaceae); 16.07.2003, 2♀♀; 24.07.2003, 2♂♂, reared from *A. urticae*, collected in Alihoca Village, Niğde from *Urtica* sp.; 17.09.2002, ♂, reared from *Vanessa cardui* (L., 1758) (Lepidoptera: Nymphalidae) collected in Aladağlar, Adana from *Malva* sp. (Malvaceae); 29.03.2003, ♀, reared from *V. cardui* collected in Karabucak, Tarsus, Mersin from *Malva* sp.; 22.02.2003, ♂; 21.03.2003, ♀; 29.03.2003, ♂, ♀; 29.03.2003, ♀; 07.04.2003, ♂; 13.03.2004, ♂; 14.03.2004, ♂; 08.04.2007, ♂, 2♀♀; 16.04.2007, ♂, reared from *Vanessa atalanta* (L., 1758) (Lepidoptera: Nymphalidae) collected in Karabucak, Tarsus, Mersin from *Urtica* sp.; 2.07.2007, ♂, reared from *V. atalanta* collected in Alihoca Village, Niğde from *Urtica* sp.; 22.08.2002, 3♀♀;

23.08.2002, ♂, ♀, reared from *Polygonia c-album* (L., 1758) (Lepidoptera: Nymphalidae) collected in Aladağlar, Adana from *Urtica* sp.; 3.08.2005, 3♀♀; 3.08.2005, 2♂♂, ♀, reared from *P. c-album* collected in Alihoca Village, Niğde from *Urtica* sp.; and 12.09.2007, ♀, reared from *P. c-album* collected in Tekir, Adana from *Urtica* sp.

Distribution in Turkey. Erzurum (Doğanlar, 1975), Marmara Region (Atak & Atak, 1984), Tokat (Kara, 1998), Sakarya (Balkan et al., 2015) and Kayseri (Atay et al., 2018).

Hosts in Turkey. *Aglais urticae* (Doğanlar, 1975; Kara, 1998; Atay et al., 2018), *P. brassicae* (Atak & Atak, 1984) and *V. atalanta* (Atay et al., 2018).

*Vanessa cardui* and *P. c-album* are new hosts for *S. bella* in Turkey.

Remarks. This parasitoid is commonly found in warmer areas and is generally seen from mid-July to mid-September in meadows, bushes and forest edges in Europe. In warmer central Europe in open areas it is not rare and much more often reared from its hosts. This tachinid is usually reared from Nymphalidae, rarely from other Macrolepidoptera (Tschorsnig & Herting, 1994; Tschorsnig, 2017).

In this study, tachinid parasitoids of some species belonging to the order Lepidoptera and Hymenoptera were studied in the southern forests of Turkey. Six tachinid species were reared from nine hosts. These were *C. concinnata, D. inconspicua, D. janitrix, E. segregata, P. caudata and S. bella* from the subfamily Exoristinae. These species were previously reared from different hosts in Turkey. In addition, four new hosts for Turkey were recorded. These were *U. pulchella* for *E. segregata*, *P. c-album* and *V. cardui* for *S. bella*, and *T. wilkinsoni* for *C. concinnata*. In addition, two parasitoid-host couples were recorded for only the second time worldwide. These were *T. wilkinsoni* for *C. concinnata* and *U. pulchella* for *E. segregata*.

Turkey has a wide range of ecosystems under different climatic conditions. Forest areas are mostly natural ecosystems free from human activities. In these areas, natural balance usually occurs, but from time to time this balance deteriorates in favor of pests and irreversible ecosystem losses occur with this damage. For this reason, it is necessary to know the species diversity, habitat associations and the host complexes of harmful and beneficial organisms. Concurrently, it is important to support their populations. Although Tachinidae species, an important parasitoid group, are important for suppressing populations of many forest pests, studies on this family have been relatively limited in the Turkish forests. The areas studied have diverse habitats which have many insect species. Therefore, it is expected that more host-parasitoid interactions will be found in these forests with further research.

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*Original article (Orijinal araştırma)*

# Effects of 24-epibrassinolide on root-knot nematode, *Meloidogyne incognita* (Kofoid & White, 1919) Chitwood, 1949 (Tylenchida: Meloidogynidae) in tomatoes[1]

## 24-Epibrassinolidin domateslerde kök-ur nematodu, *Meloidogyne incognita* (Kofoid & White, 1919) Chitwood, 1949 (Tylenchida: Meloidogynidae) üzerine etkileri

**Çiğdem GÖZEL[2*]**

## Abstract

In this study conducted in 2020, three concentrations (1, 5 and 10 µM) of 24-epibrassinolide were applied to seedlings of *Lycopersicon esculentum* Mill. (Solanales: Solanaceae) cv. H2274, which is susceptible to root-knot nematodes, by immersion, spray and irrigation, and its effects against root-knot nematode *Meloidogyne incognita* (Kofoid & White, 1919) Chitwood, 1949 (Tylenchida: Meloidogynidae) were investigated. One-thousand second stage juveniles of *M. incognita,* collected from cucumber roots in a greenhouse located in Çardak, Çanakkale were inoculated on the roots of the plants per pot. After eight weeks, stem fresh weight, stem dry weight, root diameter and longest root length values, in addition to the stem length and stem diameter measured at the beginning and end of the experiment, were recorded. 24-Epibrassinolide, applied by an immersion method, gave similar or better results than the control even in the presence of nematodes. Distilled water plus nematode application showed the highest gall index whereas 5 µM 24-epibrassinolide plus nematode application gave the lowest gall index. The lowest number of egg mass was also obtained from the same concentration of 24-epibrassinolide applied by immersion. As a result, 24-epibrassinolide showed a beneficial effect in terms of reducing the damage caused by the nematodes in tomato plants, depending on the concentration and application method.

**Keywords:** Biotic stress, brassinosteroids, resistance, root-knot nematode, tomato

## Öz

2020 yılında yürütülen bu çalışmada nematoda duyarlı olan *Lycopersicon esculentum* Mill. (Solanales: Solanaceae) H2274 çeşidinin fidelerine daldırma, spreyleme ve sulama şeklinde 24-epibrassinolidin üç konsantrasyonu (1, 5 ve 10 µM) uygulanmış ve kök-ur nematodu, *Meloidogyne incognita* (Kofoid & White, 1919) Chitwood, 1949 (Tylenchida: Meloidogynidae)'ya karşı etkileri araştırılmıştır. Her bir saksıya *Meloidogyne incognita*'nın Çanakkale, Çardak'ta bir seradan alınan hıyar köklerinden elde edilen 1000 adet ikinci dönem larvası bitkilerin köklerine verilmiştir. Sekiz hafta sonunda, denemenin başlangıcında ve sonunda ölçülen gövde boyu ve gövde çapına ilave olarak gövde yaş ve kuru ağırlıkları ile kök çapı ve en uzun kök uzunluğu değerleri kaydedilmiştir. Daldırma şeklinde verilen 24-epibrassinolidin nematod varlığında dahi kontrol bitkilerine yakın veya daha iyi sonuçlara neden olduğu belirlenmiştir. Saf su+nematod uygulaması en yüksek gal indeksini, 5 µM 24-epibrassinolid+nematod uygulaması ise en düşük gal indeksini göstermiştir. Aynı konsantrasyondaki 24-epibrassinolid daldırma şeklinde uygulandığında da en az yumurta paketi sayısı tespit edilmiştir. Sonuç olarak, 24-epibrassinolid nematodun zararlarını azaltma yönünden konsantrasyona ve uygulama yöntemine bağlı olarak destekleyici bir etki göstermiş olup nematodların domates bitkileri üzerindeki etkilerini hafifletmiştir.

**Anahtar sözcükler:** Biyotik stres, brassinosteroidler, dayanıklılık, kök-ur nematodu, domates

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## Introduction

For supplying the nutritional needs of the increasing populations both in the world and in Turkey, it is essential to obtain more production per unit area, and agricultural activities and research are by conducted to achieve this goal. One of the most important of horticultural crops is tomato, which is a strategic and highly profitable crop for humanity. While there are many diseases and pests causing significant losses in tomato production, root-knot nematodes (*Meloidogyne* spp.) are especially known for causing serious damage. *Meloidogyne incognita* (Kofoid & White, 1919) Chitwood, 1949, *Meloidogyne javanica* (Treub, 1885) Chitwood, 1949, *Meloidogyne arenaria* (Neal, 1889) Chitwood, 1949 and *Meloidogyne hapla* Chitwood, 1949 (Tylenchida: Meloidogynidae) are the most common species (Moens et al., 2009). *Meloidogyne incognita* can infest almost all plant families, including vegetables (Trudgill & Blok, 2001). These nematodes cause weakening in plants by inhibiting water and nutrients uptake from the soil due to the large or small galls they form in the roots and can eventually lead to death of the plant in severe infestations (Williamson & Hussey, 1996; Karssen & Moens, 2006; Moens et al., 2009).

Nematicides are extensively used against root-knot nematodes all over the world, but the use of pesticides has been steadily declining because of the increasing environmental and human health problems. They are restricted due to toxic effects on both plants and organisms found in soil flora (Nyczepir & Thomas, 2009). Therefore, intensive research is being conducted on alternative methods to control root-knot nematodes. Among these methods, the use of plant-based compounds stands out. Brassinosteroids (BRs) elongate cells and increase cell division in stem, prevent root growth, induce vessel differentiation (Mandava, 1988; Nemhauser et al., 2004).

BRs can have key roles in stress-response systems demonstrated by research on the use of BRs against different types of stresses, such as heat, cold, salinity, heavy metal, and pathogens (including bacteria, fungi, and virus) (Hotta et al., 1998; Yi et al., 1999; De Vleesschauwer et al., 2013; De Bruyne et al., 2014). Research on BRs indicated that they may help overcome stress by inducing reactive oxygen species scavenging enzymes (Jasrotia & Ohri, 2014) or improving plant development (Grace et al., 2009). Lower incidence of infection by late blight, caused by *Phytophthora infestans* (Mont.) de Bary (Peronosporales: Pythiaceae) on potatoes sprayed with BRs was determined (Khripach et al., 1996). In barley, potato tubers and cucumber plants, brassinosteroid-induced disease resistance was also reported (Khripach et al., 2000). Induction of disease resistance to pathogens by BRs has also been shown in tobacco and rice (Nakashita et al., 2003). Effects of BRs on preventing damage caused by nematodes have also been under investigation. For example, *in vitro* tomato plants had better resistance when their seeds were pretreated with 28-homobrassinolide (Kaur et al., 2013) and 24-epibrassinolide (Jasrotia & Ohri, 2017) against *M. incognita*. The results of these studies have warranted further research on BRs for their inducing effects on plant health. However, since much of the research has been conducted *in vitro*, pot and/or field experiments are also needed. Therefore, in this study, tomato plants were raised in pots to observe the effects of 24-epibrassinolide at different concentrations (1, 5 and 10 µM) and different application methods (immersion, irrigation, and spray) against *M. incognita*.

## Materials and Methods

### Plant materials and the compounds

Seedlings (3-4 leaf) of tomato [*Lycopersicon esculentum* Mill. (Solanales: Solanaceae)] cv. Heinz 2274 (H2274), reported as susceptible against *M. incognita* (Barker et al., 1985), were obtained from a commercial company. The chemical 24-epibrassinolide (Sigma-Aldrich E1641, Merck, St Louis, MI, USA) was purchased and stored at -20ºC until the experiment started.

**Preparation of plants and 24-epibrassinolide solutions**

The study was conducted in 2020 in plastic pots in a control environment room at 27±2ºC. The growing medium, consisting of 70% sand and 30% soil, was sterilized and added to 1.4-l pots, then H2274 seedlings were transplanted to the pots. After 24-epibrassinolide was dissolved in pure ethanol, working solutions were prepared using distilled water at concentrations of 1, 5 and 10 µM. Solutions were stored in dark at +4ºC. Prior to the 24-epibrassinolide applications to the seedlings, 0.02% Tween-20 was added only to the spray solutions as a surface wetting agent.

**Mass production of *Meloidogyne incognita***

Çanakkale isolate of *M. incognita* was used in the study. Nematode-infected cucumber plants were taken from an infested greenhouse in Çardak, Çanakkale. Infected roots of were thoroughly washed and cleaned from soil and other substances. Then the egg masses were extracted from the roots and kept in Petri dishes in distilled water to obtain second stage juveniles (J2s). The distilled water in the Petri dishes was renewed at 24-h intervals and nematode suspension was stored. These J2s, found in suspension, were stored in 10 ml tubes at +4ºC until used for the inoculations. The viability of J2s was checked under the stereomicroscope (Leica DM 1000, Leica Microsystems, Germany) before inoculation.

**Inoculation of nematodes and application of 24-epibrassinolide**

After the seedlings were transplanted into the pots, J2s of *M. incognita* were inoculated as 1000 J2s/pot in all applications. The seedlings were grown at 27±2ºC in 18:6 h L:D photoperiod for 8 weeks. In the immersion method, the soil surrounding the roots was carefully washed with water, and the roots were immersed in a container with 24-epibrassinolide solution for 10 min before transplanting followed by nematode inoculation. Control groups of the immersion method were kept in distilled water for the same duration. In spray and irrigation methods, nematode inoculation was performed immediately after the 24-epibrassinolide application. 24-Epibrassinolide was applied three times (1, 7 and 14 d after planting) starting with 10 ml per plant, and increasing to 20 and 40 ml per plant, respectively. Control plants of both methods received the same amount of distilled water.

**Collecting data on nematode development and morphological characteristics**

At the end of the experiment (56 d), to determine the nematode damage, galls on the roots were evaluated according to the 0-10 scale by Piedra-Buena et al. (2011) adapted from Bridge & Page (1980). The number of egg masses was visually scored under the stereo microscope. Reproduction factor (R0) of the nematode was calculated using the formula of R0 = final population/initial population.

At the end of the first 24 h following planting, the stem length and the stem diameter was measured. When the plants stopped growing by the end of week 8 (56 d), they were uprooted, and the following data were obtained from the aboveground part: (a) increase in stem length (x fold) = final stem length (cm)/initial stem length (cm), (b) increase in stem diameter (x fold) = final stem diameter (cm)/initial stem diameter (cm), (c) stem fresh and dry weights (g) (after 48 h at 70ºC), (d) longest root length (cm), and (e) root diameter (cm).

**Statistical analysis**

The experiment was conducted with three replicates with one plant per pot in a completely randomized experiment. Comparison of means on both morphological and nematode development was done using analysis of means procedure (Mendeş & Yiğit, 2018; Mendeş, 2019). The analysis was implemented on R statistical package program (version 4.0.2; 2020-06-22) (Pallmann & Hothorn, 2016). The results are presented graphically. Treatments not receiving nematode addition were excluded during the analysis of the nematode development. Data for the root gall index and egg mass number were normalized by square root transformation.

## Results

Based on the data analysis, the interaction of 24-epibrassinolide and application method on root gall index, egg mass number and R0 was found significant (Figures 1-3). Significantly higher gall index was found compared to the other applications when T4 (distilled water+J2s) was applied by irrigation to the tomato plants (Figure 1). Although there was no significant difference between other applications, it was observed that T2 (5 µM 24-epibrassinolide+J2s) decreased the root gall index in all application methods. The highest concentration of 24-epibrassinolide T3 (10 µM 24-epibrassinolide+J2s) gave the same effect only with the irrigation and spray methods.

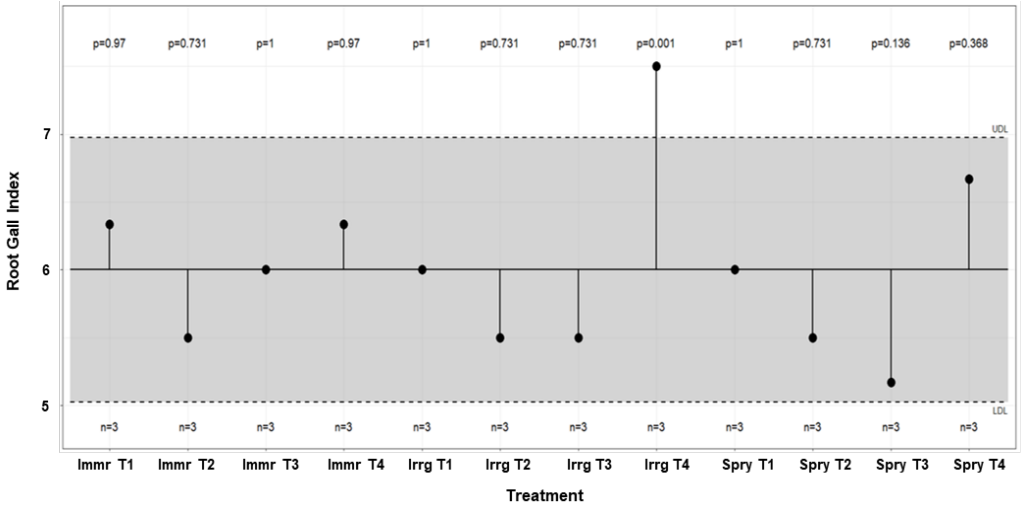


Figure 1. Interaction of application method and 24-epibrassinolide (EBL) rate on root gall index in tomato cv. H2274 (T1, 1 µM EBL+J2s; T2, 5 µM EBL+J2s; T3, 10 µM EBL+J2s; T4, distilled water+J2s; Immr, immersion; Irrg, irrigation; Spry, spray; LDL, lower decision limit; and UDL, upper decision limit).

Like gall index, the number of egg mass on roots reached the highest values with distilled water+J2s application (T4) by the irrigation method (Figure 2). The lowest number of egg masses was with 5 µM 24-epibrassinolide+J2s (T2) applied by irrigation. The most successful results in reducing the number of egg mass were obtained when 24-epibrassinolide was applied to the plants by the immersion method at all concentrations.

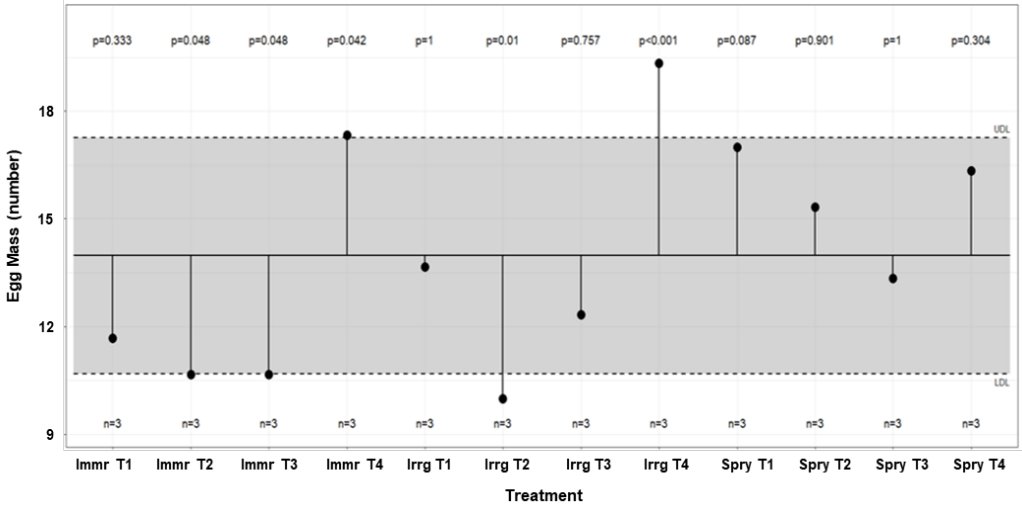


Figure 2. Interaction of application method and 24-epibrassinolide (EBL) rate on egg mass number in tomato cv. H2274 (T1, 1 µM EBL+J2s; T2, 5 µM EBL+J2s; T3, 10 µM EBL+J2s; T4, distilled water+J2s; Immr, immersion; Irrg, irrigation; Spry, spray; LDL, lower decision limit; and UDL, upper decision limit).

Nematode R0 was the highest with the application of distilled water+J2s (T4) by the irrigation method (Figure 3). Nematode R0 was reduced with 1 µM 24-epibrassinolide+J2s (T1) treatment applied by the immersion method. When 24-epibrassinolide was applied to the plants by the immersion and spray methods, even at low 24-epibrassinolide concentrations, R0 remained at a low level compared to other treatments.

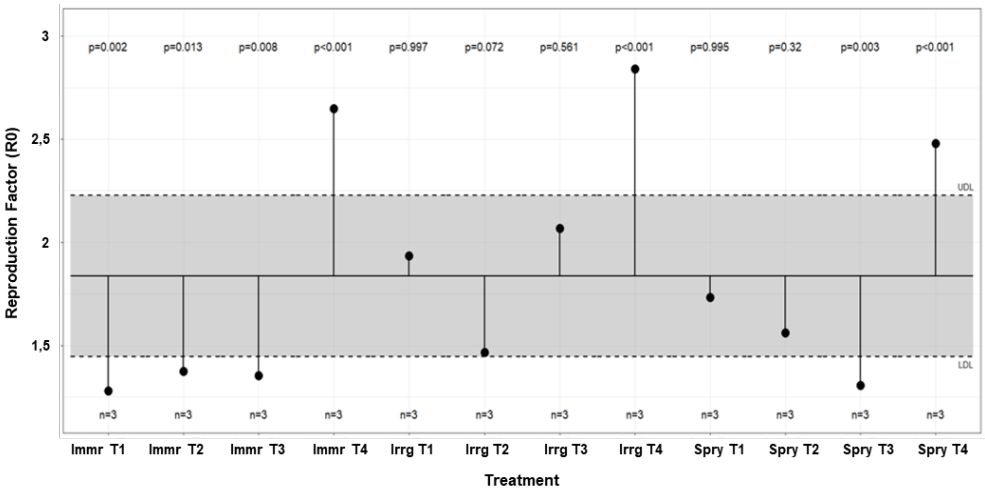


Figure 3. Interaction of application method and 24-epibrassinolide (EBL) rate on nematode reproduction factor in tomato cv. H2274 (T1, 1 µM EBL+J2s; T2, 5 µM EBL+J2s; T3, 10 µM EBL+J2s; T4, distilled water+J2s; Immr, immersion; Irrg, irrigation; Spry, spray; LDL, lower decision limit; and UDL, upper decision limit).

The data analysis revealed that the interaction between the application methods and 24-epibrassinolide rates were significant for changes in stem length and stem diameter, stem fresh and dry weights and the longest root length (Figures 4 to 8). Increase in stem length was highest with the immersion of seedlings in distilled water (T1), 10 µM 24-epibrassinolide (T8), 5 µM 24-epibrassinolide+J2s (T4) and 10 µM 24-epibrassinolide+J2s (T5) (Figure 4). Neither irrigated plants nor sprayed plants were significantly affected.

Increase in stem diameter did not significantly differ between the treatments, apart from with 10 µM 24-epibrassinolide+J2s applied as a spray (T5) (Figure 5). However, Figures 4 and 5 show that when the plants invested more on their longitudinal growth, they had a decreased radial growth.

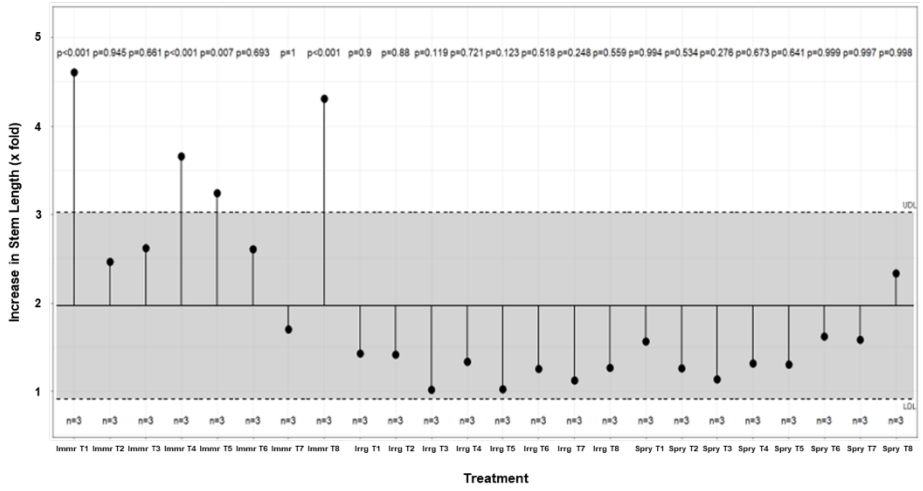


Figure 4. Interaction of application method and 24-epibrassinolide (EBL) rate on increase in stem length (x fold) in tomato cv. H2274 (T1, distilled water; T2, distilled water+J2s; T3, 1 µM EBL+J2s; T4, 5 µM EBL+J2s; T5, 10 µM EBL+J2s; T6, 1 µM EBL; T7, 5 µM EBL; T8, 10 µM EBL; Immr, immersion; Irrg, irrigation; Spry, spray; LDL, lower decision limit; and UDL, upper decision limit).

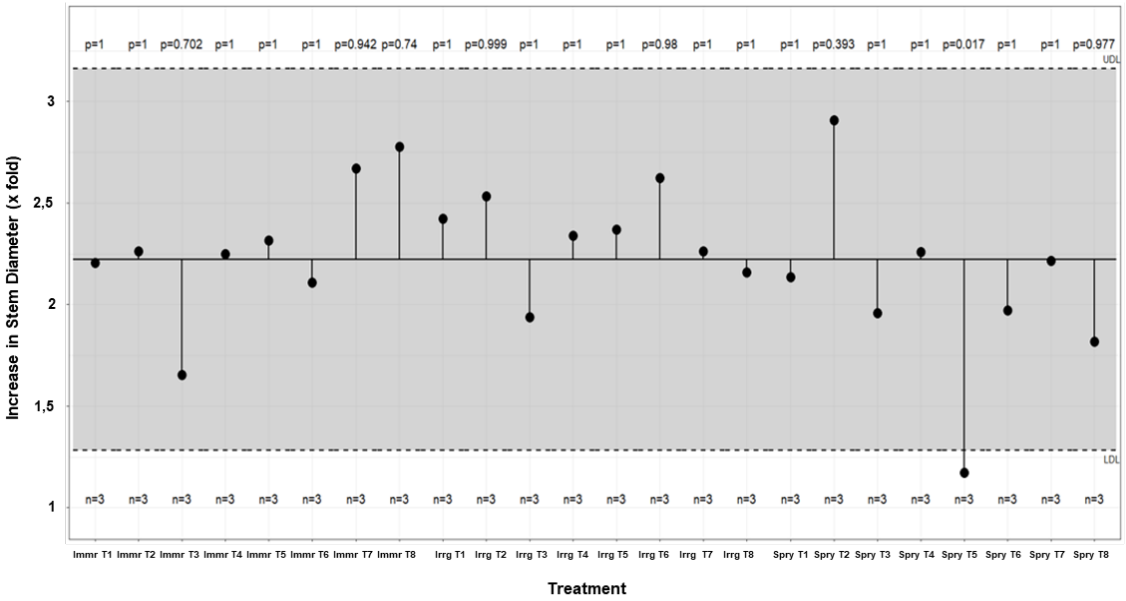


Figure 5. Interaction of application method and 24-epibrassinolide (EBL) rate on increase in stem diameter (x fold) in tomato cv. H2274 (T1, distilled water; T2, distilled water+J2s; T3, 1 µM EBL+J2s; T4, 5 µM EBL+J2s; T5, 10 µM EBL+J2s; T6, 1 µM EBL; T7, 5 µM EBL; T8, 10 µM EBL; Immr, immersion; Irrg, irrigation; Spry, spray; LDL, lower decision limit; and UDL, upper decision limit).

Stem fresh weight of the plants differed with application method for 24-epibrassinolide and/or nematode (Figure 6). For example, immersion in 24-epibrassinolide resulted the highest weight, but the same effect was not observed when this was applied by irrigation or spray, and nematode inoculation decreased fresh weight. Stem dry weight showed similar responses to stem fresh weight (Figure 7).

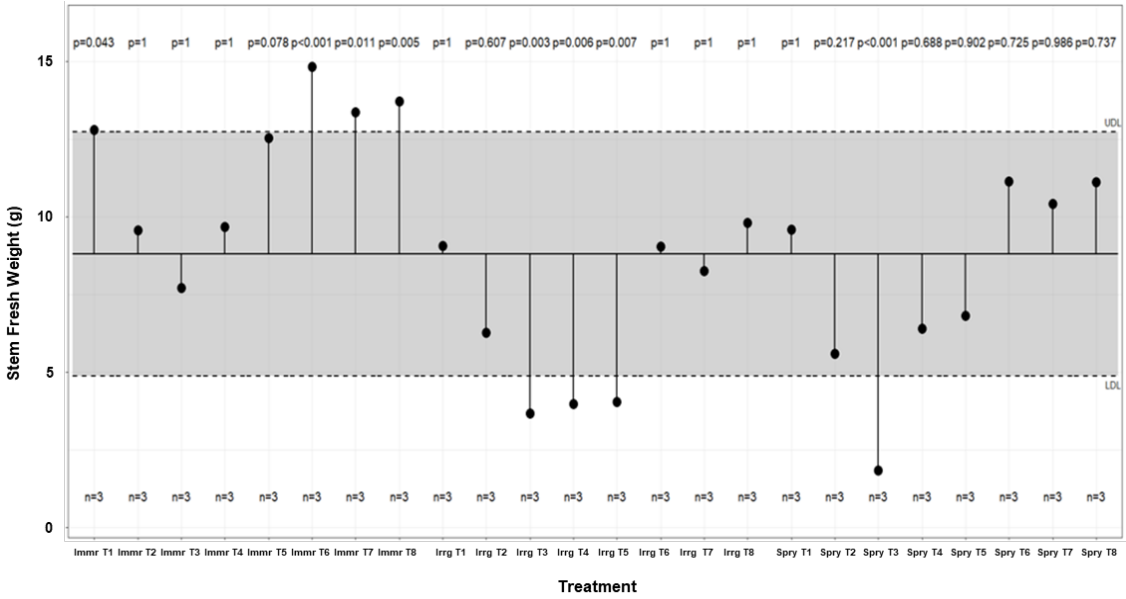


Figure 6. Interaction of application method and 24-epibrassinolide (EBL) rate on stem fresh weight (g) in tomato cv. H2274 (T1, distilled water; T2, distilled water+J2s; T3, 1 µM EBL+J2s; T4, 5 µM EBL+J2s; T5, 10 µM EBL+J2s; T6, 1 µM EBL; T7, 5 µM EBL; T8, 10 µM EBL; Immr, immersion; Irrg, irrigation; Spry, spray; LDL, lower decision limit; and UDL, upper decision limit).

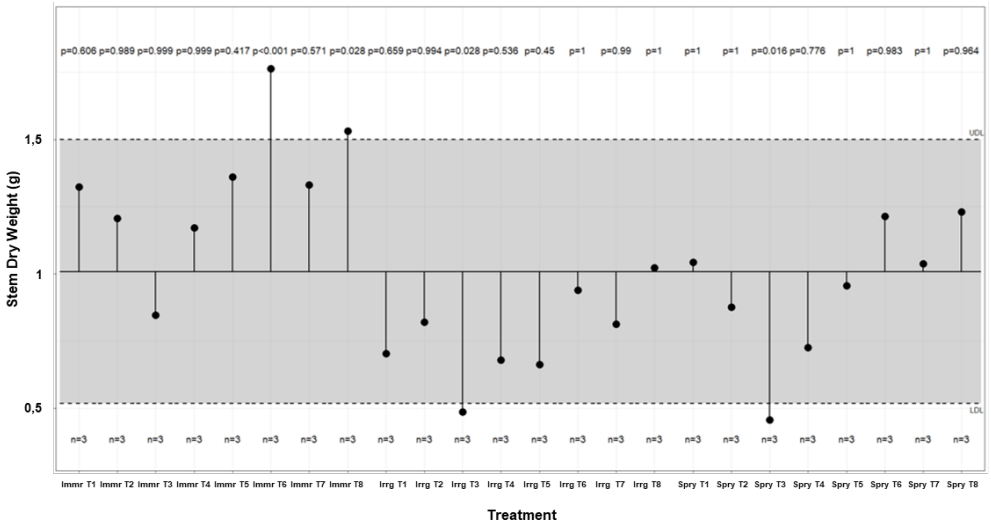


Figure 7. Interaction of application method and 24-epibrassinolide (EBL) rate on stem dry weight (g) in tomato cv. H2274 (T1, distilled water; T2, distilled water+J2s; T3, 1 µM EBL+J2s; T4, 5 µM EBL+J2s; T5, 10 µM EBL+J2s; T6, 1 µM EBL; T7, 5 µM EBL; T8, 10 µM EBL; Immr, immersion; Irrg, irrigation; Spry, spray; LDL, lower decision limit; and UDL, upper decision limit).

Roots were the longest and significantly different with 1 µM 24-epibrassinolide applied by immersion (T6) and the shortest with 1 µM 24-epibrassinolide+J2s applied as a spray (T3) (Figure 8). Overall, the figure indicates that nematode infection shortened the roots no matter how 24-epibrassinolide was applied. The data analysis of root diameter revealed that only application methods have a significant effect with immersion giving in longer than irrigation and spray (plot not shown).

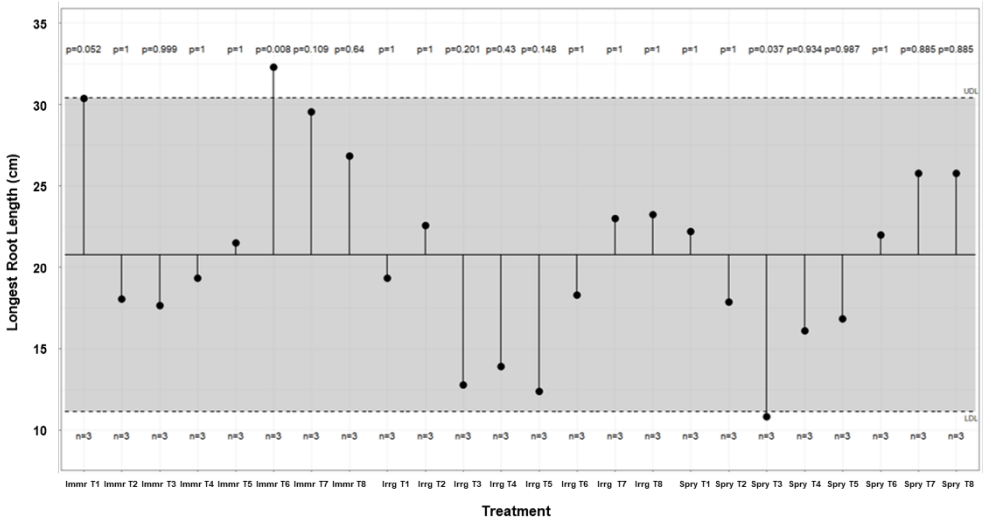


Figure 8. Interaction of application method and 24-epibrassinolide (EBL) rate on the longest root length (cm) in tomato cv. H2274 (T1, distilled water; T2, distilled water+J2s; T3, 1 µM EBL+J2s; T4, 5 µM EBL+J2s; T5, 10 µM EBL+J2s; T6, 1 µM EBL; T7, 5 µM EBL; T8, 10 µM EBL; Immr, immersion; Irrg, irrigation; Spry, spray; LDL, lower decision limit; and UDL, upper decision limit).

## Discussion

Although BRs are known to be affect stress response in plants, little is known about their effects against pathogens, particularly plant parasitic nematodes. Nakashita et al. (2003) reported that brassinolide induces disease resistance against tobacco mosaic virus, *Pseudomonas syringae* van Hall, 1902 (Pseudomonadales: Pseudomonadaceae) and *Oidium* sp. (Erysiphales: Erysiphaceae) in tobacco plants and *Pyricularia grisea* Cooke ex Sacc., 1886 (Magnaporthales: Pyriculariaceae) and *Xanthomonas oryzae*

(Ishiyama, 1922) Swings et al., 1990 (Xanthomonodales: Xanthomonodaceae) in rice. In a similar study by Ding et al. (2009) it was observed that foliar and root 24-epibrassinolide application significantly increased the resistance to *Fusarium* spp. (Hypocreales: Nectriaceae). In our study, root gall index, number of egg mass and nematode R0 were decreased with the application of 24-epibrassinolide depending on the concentration and application method. These results were consistent with the findings of Song et al. (2017), who reported that foliar application of 24-epibrassinolide reduced the gall numbers at different doses in tomato and it had an important effect on root resistance against nematodes. Reduction in number of galls were reported by Kaur et al. (2013, 2014a) with the use of 28-homobrassinolide. Effects of 24-epibrassinolide in reducing the number of galls in tomato were also reported by Jasrotia & Ohri (2017) in plants cultured *in vitro*.

It was observed in the current study that the increase in stem length was higher with the application of 24-epibrassinolide and radial growth of the roots were compromised at the expense of longitudinal growth. This effect of 24-epibrassinolide was also observed in carrot by Que et al. (2017). *Meloidogyne incognita* infected tomato plants had slight increase in plant height when exposed to 28-homobrassinolide (Kaur et al., 2013, 2014 a, b). Jasrotia & Ohri (2017) reported similar effects of 24-epibrassinolide on increasing stress tolerance of *in vitro* tomato plants through inducing antioxidant enzymes. These studies reveal that BRs can support aboveground plant growth and help alleviate nematode-induced stress in plants. Stem fresh weights of the plants differed with the method used to apply 24-epibrassinolide and nematodes. The immersion method resulted the highest weight, but the same effect was not observed with irrigation or spray application and nematode inoculation decreased stem fresh weights. Decreases in plant biomass with nematode infestation of tomato plants were also reported by Opoku-Asiama & Yeboah (2003) and Kaur et al. (2014b). Stem dry weight responded similarly to stem fresh weight. Ali et al. (2006) reported that the plant weight of tomato seedlings immersed in 28-homobrassinolide solution decreased as the concentration increased but was higher than the control. However, a similar effect was not observed in the plants, independent of the application method in the current study. Root length depended on the concentration of the 24-epibrassinolide applied and nematode infection shortened the roots irrespective of the application method. Müssig et al. (2003) found that BRs applied exogenously to *Arabidopsis thaliana* (L.) Heynh. (Brassicales: Brassicaceae) plants were effective in promoting root length. Uzunoğlu & Gökbayrak (2018) also reported that both 28-homobrassinolide and 24-epibrassinolide was successful in improving root characteristics in grapevine.

In conclusion, the results of this study confirm the potential of 24-epibrassinolide in enhancing plant defense against root-knot nematodes, even though any specific concentration or application method was not found optimal. However, it is of note that application of 10 µM 24-epibrassinolide by immersion was better at modulating of anti-stress responses in plant growth and tolerance to nematode infestation. This confirmation of the benefits of BRs in potted plants justifies the extension of the work to field experiments. In addition, the application of BRs in conjunction with other *Meloidogyne* spp. might provide a better understanding its capacity to induce nematode resistance in plants.

## Acknowledgments

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*Original article (Orijinal araştırma)*

# Investigation of the development of root lesion nematodes, *Pratylenchus* spp. (Tylenchida: Pratylenchidae) in three chickpea cultivars

Kök lezyon nematodlarının, *Pratylenchus* spp. (Tylenchida: Pratylenchidae) üç nohut çeşidinde gelişmesinin incelenmesi

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**İbrahim Halil ELEKCİOĞLU[1]**

## Abstract

In this study, penetration, population changes and reproduction rates of root lesion nematodes, *Pratylenchus neglectus* (Rensch, 1924), *Pratylenchus penetrans* (Cobb, 1917) and *Pratylenchus thornei* Sher & Allen, 1953 (Tylenchida: Pratylenchidae), at 3, 7, 14, 21, 28, 35, 42, 49 and 56 d after inoculation in chickpea Bari 2, Bari 3 (*Cicer reticulatum* Ladiz) and Cermi [*Cicer echinospermum* P.H.Davis (Fabales: Fabaceae)] were assessed in a controlled environment room in 2018-2019. No juveniles were observed in the roots in the first 3 d after inoculation. Although, population density of *P. thornei* reached the highest in Cermi (21 d), Bari 3 (42 d) and the lowest observed on Bari 2. *Pratylenchus neglectus* reached the highest population density in Bari 3 and Cermi on day 28. The population density of *P. neglectus* was the lowest in Bari 2. Also, population density of *P. penetrans* reached the highest in Bari 3 cultivar within 49 d, similar to *P. thornei*, whereas Bari 2 and Cermi had low population densities during the entire experimental period.

**Keywords:** Chickpea, penetration, population density, *Pratylenchus*, reproduction

## Öz

Bu çalışmada, Bari 2, Bari 3 (*Cicer reticulatum* Ladiz) ve Cermi [*Cicer echinospermum* P.H.Davis (Fabales: Fabaceae)] nohut çeşitlerinde Kök lezyon nematodlarının, *Pratylenchus neglectus* (Rensch, 1924), *Pratylenchus penetrans* (Cobb, 1917) ve *Pratylenchus thornei* Sher & Allen, 1953 (Tylenchida: Pratylenchidae), 3, 7, 14, 21, 28, 35, 42, 49 ve 56 günlerde popülasyon değişimleri, penetrasyon ve üreme oranları kontrollü oda koşullarında 2018-2019 yılları arasında incelenmiştir. İlk 3 günde nohut çeşitlerinin köklerinde larvalar görülmemiştir. *Pratylenchus thornei* en yüksek popülasyon yoğunluğuna Cermi çeşidinde 21 günde, Bari 3 çeşidinde 42 günde ulaşmasına rağmen Bari 2 çeşidinde üreme en düşük düzeyde kalmıştır. *P. neglectus* Bari 3 ve Cermi çeşidinde 28 günde en yüksek popülasyon yoğunluğuna ulaşmıştır. Bari 2'de ise en düşük popülasyon yoğunluğu *P. neglectus*'da görülmüştür. Buna ek olarak, *P. penetrans* popülasyon yoğunluğu, *P. thornei*'ye benzer şekilde, 49 günde Bari 3 çeşidinde en yüksek seviyeye ulaşırken, Bari 2 ve Cermi çeşitlerinde deneme süresince düşük popülasyon yoğunluğu göstermiştir.

**Anahtar sözcükler:** Nohut, penetrasyon, popülasyon yoğunluğu, *Pratylenchus*, üreme

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## Introduction

Chickpea [*Cicer arietinum* L. (Fabales: Fabaceae)] is one of the most important food legumes in the world. It is an ancient crop that has been grown in India, the Middle East and parts of Africa for many years. It may have been grown in Turkey in the twelfth century BC. (Singh & Ocampo, 1997). The chickpea growing area in Turkey is about 514 kha with a production of 630 kt and an average yield of 1.2 t/ha (TUIK, 2018). Chickpea production in Turkey has increased over recent decades. Turkey is ranked fifth in the world for chickpea production (FAO, 2019). It has been reported that plant parasitic nematodes can cause 21-40% crop losses in chickpea crops (Ali & Sharma, 2003; Reen et al., 2014). The strategies for nematode management hinge on detection and population density estimation to keep the nematode population below an economic threshold. However, this is difficult to achieve and needs to be modified under different cultivation conditions (Abd-Elgawad & Askary, 2015). Chemical control is not economic for use in crop production on large areas. Therefore, the most effective control method is to consistently use of resistant cultivars (Roberts, 2002).

Taylor et al. (2000) reported that there are differences between crops and cultivars in host susceptibility to root lesion nematode, *Pratylenchus neglectus* (Rensch, 1924) (Tylenchida: Pratylenchidae). For example, chickpea, wheat and canola were good hosts whereas field pea, faba bean and triticale were poor hosts. Thirty-three plant parasitic nematode species have been identified in association with chickpeas in Turkey (Kepenekçi & Ökten, 2000). Root lesion nematodes are one of the most important plant parasitic nematodes that causing damage to chickpea crops in Turkey (Di Vito et al., 1994b; Kepenekçi, 1999) and elsewhere in the world (Sharma et al., 1992).

There have been a range of studies on root penetration and population dynamics of root lesion nematodes. Rebois & Huettel (1986) indicated that there was a significant difference between the number of nematodes in the roots and agar in soybean and corn; the population density of eggs and individuals in the roots was greatest than nematode population in agar. *Pratylenchus thornei* Sher & Allen, 1953 (Tylenchida: Pratylenchidae) was found on 24 plant species that reproduced well on 97 chickpea lines in Syria and Turkey (Greco et al., 1988; Gomez-Barcina et al., 1996; Behmand et al., 2019). There are different factors that can affected on root lesion [*P. thornei* and *Pratylenchus penetrans* (Cobb, 1917) (Tylenchida: Pratylenchidae)] egg hatching. Pudasaini et al. (2008) indicated that the temperature of 20ºC had a greater effect on egg hatching among these factors. Population dynamics of *P. thornei* were observed in the chickpea genotypes grown in the greenhouse for 16 weeks (Thompson et al., 2015). The root lesion nematodes including *P. neglectus, P. penetrans* and *P. thornei*, have been commonly determined in fields in Turkey (Şahin et al., 2008; Söğüt & Devran, 2011; Akyazi et al., 2018). Also, Behmand et al. (2019) showed that these nematodes have been found widely distributed in 82% of the chickpea fields in many regions in Turkey.

Information on population density, population dynamics and growth rate in root is very important in the control of root lesion nematodes and to help keep the nematode population density below an economic threshold. The aim of the study was to determine population dynamics of root lesion nematodes, *P. neglectus, P. penetrans* and *P. thornei,* as well as root penetration rates, and root-feeding behavior on resistant and susceptible chickpea cultivars under laboratory conditions.

## Materials and Methods

### Plant materials

Chickpea cultivars that were determined previously as resistant and susceptible to *P. neglectus, P. penetrans* and *P. thornei* by Behmand (2020) were screened against these root lesion nematodes (Table 1). These cultivars used in an experiment at Plant Protection Department, Çukurova University in 2018 -2019.

Table 1. Chickpea cultivars used for investigation of the population dynamics of *Pratylenchus neglectus*, *P. penetrans* and *P. thornei* in experiments

| Chickpea species | Chickpea cultivars | Reaction* |
|---|---|---|
| *Cicer echinospermum* | Cermi 063 | S |
| *Cicer reticulatum* | Bari3 106 | S |
| *Cicer reticulatum* | Bari2 062 | R |

*R: Resistance S: Susceptible

The chickpea seed was surface sterilized and germinated in an incubator. The seeds were first washed with 30% ethanol for 1 min, rinsed in sterile water and placed in 4% sodium hypochlorite solution for 45 s, and then washed twice with sterile pure water. The seeds were sacrificed by making a small cut on the seed coat to improve water absorption and incubated at 21ºC for 5 d to germinate on moist filter paper in 90-mm Petri dishes. Seeds were sowed singly into sterilized tubes containing 60 g sterilized soil. The tubes were placed in a controlled environment room at 20-25ºC.

**Nematode cultures**

Nematodes used in this study were collected during April and May 2014 to 2016 from a chickpea field (37º08’29" N 38º46’30" E) in Harran District, Şanlıurfa p8province, Turkey (Behmand et al., 2019). The climate is arid to semiarid in that area. The average soil temperature at a depth of 20 cm was 9.4ºC from April to May and average of 460 mm of annual rainfall and average RH of about 49% during April and May in 2014 to 2016. Mass cultures of *P. neglectus, P. penetrans* and *P. thornei* were grown in carrot discs in Plant Protection Department, Çukurova University and used as inoculum for the experiments (Moody et al., 1973).

**Experiment**

The soil was autoclaved at 121ºC for 20 min to kill nematodes and other plant pathogens (Baker, 1962). Autoclaved tubes with a capacity of 60 g (diameter: 2.5 cm, height: 15 cm) were used to grow the plants. Germinated seeds were planted singularly in the tubes supported by a box frame in the controlled environment rooms according to the randomized split-plot design with four replicates. The main plots where the nematodes species, and subplots the wild *Cicer* species [*Cicer echinospermum* P.H.Davis and *Cicer reticulatum* Ladiz (Fabales: Fabaceae)].

One week after sowing, the seedlings were inoculated with number of 225 nematodes consisting of mixed development stages in 1 ml of water (Behmand et al., 2020).

Chickpea cultivars were harvested at 3, 7, 14, 21, 28, 35, 42, 49 and 56 d after planting and all roots from each tube processed. The roots in each tube were carefully washed away and then stained in acid fuchsine to visualize the nematodes (10 ml 1% acid fuchsine, 17.5 ml lactic acid, 12.6 ml glycerin, 12.4 ml pure water) (Moltmann, 1988). At each harvest, population density of nematodes was counted from all stained roots under microscope type Leica 4000B. Reproduction factor (RF) was calculated as Pf/Pi, (the ratio of the final and initial nematode population densities) (Keil et al., 2009). Optimization validation within this study showed that nematodes were entered chickpea roots mostly by day 7. Therefore, Pi values were used from the day 7 assessment.

**Data analysis**

The data at each assessment time were subject to analysis of variance (ANOVA) and means were compared Tukey multiple range test at the $P \leq 0.05$ significance level using SPSS statistical program (Version 25, SPSS, Inc, Chicago, IL, USA). Analyses were performed on nematode density data normalized by using the $\log_{10}(x + 1)$ transformation. Also, a repeated measure analysis of variance (ANOVA) was used to examine interactions of day and nematode species.

## Results and Discussion

An experiment was conducted to study population development and reproduction rates of *P. neglectus, P. penetrans* and *P. thornei* on Bari 2, Bari 3 and Cermi chickpea roots in 56 d the controlled environment room. It was determined that *P. neglectus, P. penetrans* and *P. thornei* entered the roots of Bari 2, Bari 3 and Cermi at 3-7 d after nematode inoculation (Table 2). *Pratylenchus neglectus, P. penetrans* and *P. thornei* were observed feeding at several sites in the roots during all migratory stages of their life cycles.

The highest RF (Table 2) obtained was 44.6 for *P. thornei* in Cermi followed by RF of 36.6 for *P. neglectus* in Bari 3 and 25.4 for *P. penetrans* in Bari 3.

Table 2. Population densities and reproduction factors (RF) for *Pratylenchus neglectus*, *P. penetrans* and *P. thornei* in chickpea roots

| Assessment day | Cultivar | *Pratylenchus thornei* (mean ± SE)[1] | RF | *Pratylenchus neglectus* (mean ± SE) | RF | *Pratylenchus penetrans* (mean ± SE) | RF |
|---|---|---|---|---|---|---|---|
| 3 | Bari3 | 0 ± 0 | 0 | 0 ± 0 | 0 | 0 ± 0 | 0 |
| | Bari2 | 0 ± 0 | 0 | 0 ± 0 | 0 | 0 ± 0 | 0 |
| | Cermi | 0 ± 0 | 0 | 0 ± 0 | 0 | 0 ± 0 | 0 |
| 7 | Bari3 | 11.2 ± 1.4 a | 1 | 5.2 ± 1.2 b | 1 | 6.2 ± 1.3 ab | 1 |
| | Bari2 | 7.5 ± 1.1 a | 1 | 5.5 ± 1.0 b | 1 | 2.7 ± 0.8 b | 1 |
| | Cermi | 8 ± 1.8 a | 1 | 13.2 ± 1.3 a | 1 | 11.5 ± 1.3 a | 1 |
| 14 | Bari3 | 29.7 ± 5.6 a | 2.6 | 32.7 ± 2.6 a | 6.2 | 13 ± 0.7 ab | 2.1 |
| | Bari2 | 34.2 ± 9.9 a | 4.6 | 10.7 ± 0.4 c | 2 | 8.5 ± 1.5 b | 3.1 |
| | Cermi | 56 ± 8.6 a | 7 | 20.2 ± 0.8 b | 1.5 | 19.7 ± 2.9 a | 1.7 |
| 21 | Bari3 | 15.5 ± 7.6 b | 1.4 | 3.7 ± 0.7 b | 0.7 | 7.5 ± 2.1 a | 1.2 |
| | Bari2 | 11.2 ± 6.3 b | 1.5 | 21.7 ± 8.1 a | 4 | 11.2 ± 1.9 a | 4.1 |
| | Cermi | 356.5 ± 20.7 a | 44.6 | 13.7 ± 1.6 a | 1 | 8.7 ± 0.4 a | 0.8 |
| 28 | Bari3 | 27 ± 6.3 b | 2.4 | 192.2 ± 66.5 a | 36.6 | 64 ± 31.8 a | 10.2 |
| | Bari2 | 31.7 ± 5.7 b | 4.2 | 18 ± 2.6 b | 3.3 | 65.5 ± 46.4 a | 23.8 |
| | Cermi | 73.5 ± 1.3 a | 9.2 | 186 ± 79.9 a | 14 | 52.2 ± 25.0 a | 4.5 |
| 35 | Bari3 | 25.2 ± 3.8 b | 2.2 | 73.5 ± 24.7 a | 14 | 71.5 ± 25.3 a | 11.4 |
| | Bari2 | 17.5 ± 1.0 b | 2.3 | 11 ± 4.8 a | 2 | 65.2 ± 17.4 a | 23.7 |
| | Cermi | 69.7 ± 0.8 a | 8.7 | 95 ± 43.0 a | 7.2 | 37.7 ± 1.1a | 3.3 |
| 42 | Bari3 | 57.5 ± 5.5 a | 5.1 | 25 ± 8.6 ab | 4.8 | 94.7 ± 19.3 a | 15.2 |
| | Bari2 | 6.7 ± 1.6 b | 0.9 | 16.7 ± 2.9 b | 3 | 17.5 ± 2.2 b | 6.4 |
| | Cermi | 78.7 ± 0.8 a | 9.8 | 144.7 ± 48.7 a | 10.9 | 80 ± 9.4 a | 7 |
| 49 | Bari3 | 11.2 ± 1.9 b | 1 | 41.5 ± 6.5 a | 7.9 | 158.5 ± 3.1 a | 25.4 |
| | Bari2 | 13.7 ± 1.4 b | 1.8 | 16.5 ± 1.9 b | 3 | 15 ± 2.0 c | 5.5 |
| | Cermi | 100 ± 1.0 a | 12.5 | 51 ± 5.2 a | 3,8 | 45.2 ± 3.3 b | 3.9 |
| 56 | Bari3 | 30.2 ± 1.0 a | 2.7 | 37.7 ± 2.7 b | 7.2 | 53 ± 20.2 a | 8.5 |
| | Bari2 | 17.7 ± 1.1 a | 2.4 | 18.7 ± 1.2 c | 3.4 | 17 ± 0.9 a | 6.2 |
| | Cermi | 50.2 ± 16.7 a | 6.3 | 106.2 ± 3.1 a | 8 | 65 ± 2.8 a | 5.7 |

[1] Data are means of four replicates ± standard errors, and numbers within columns followed by the same letter are not significantly different ($P < 0,05$) according to Tukey multiple range test using transformed data, $\log_{10}(x + 1)$; in each harvest time between cultivars; RF (reproduction factor) = Pf (final population density) / Pi (initial population density).

Over the course of the experiment, the population density of nematodes generally peaked twice. The population density reached its first peak on days 21 to 28, depending on the species and cultivar. *Pratylenchus thornei* reached its highest population density on day 21, and *P. neglectus* and *P. penetrans* on day 28 (Table 2).

Population density of *P. thornei* peaked on day 21 and then again on day 49 in Cermi. It is concluded that *P. thornei* completed its first life cycle in 21 d (Figure 1). *Pratylenchus thornei* reached highest population density on 42 d in Bari 3 whereas low population development was observed in Bari 2. The RF of *P. thornei* in Cermi was higher than in Bari 2 and Bari 3 over the entire experiment (Figure 1). Also, the second highest population density for *P. thornei* was reached after 49 d and the RF decreased to 12.5 in Cermi (Table 2). Based on the population development of *P. thornei* in the roots of chickpea cultivars used in the experiment (Figure 1), it is suggested that Cermi is a susceptible cultivar and the Bari 2 and Bari 3 are resistant or tolerant cultivars.

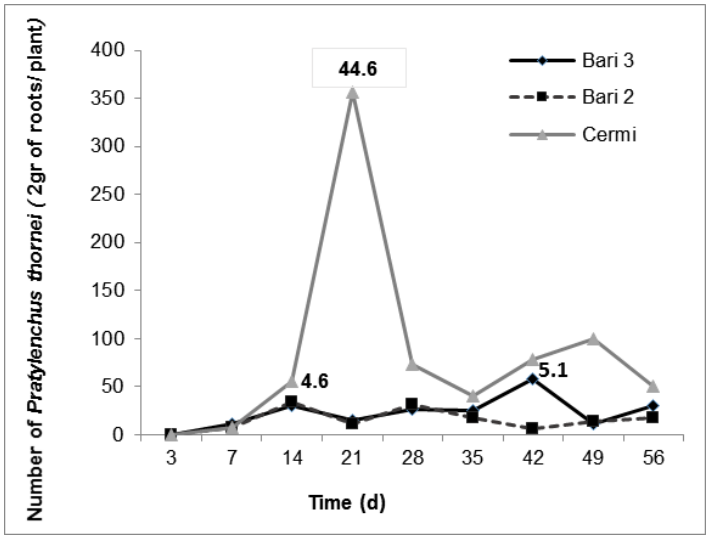


Figure 1. Population changes of *Pratylenchus thornei* in chickpea cultivars, Bari 2, Bari 3 and Cermi.

*Pratylenchus neglectus* reached the highest population density on day 28 in Bari 3 and Cermi but remained at a low density in Bari 2. *Pratylenchus neglectus* had three peaks in Cermi during the its development period whereas there was only one peak for Bari 3. The highest *P. neglectus* population density was on day 28. The RF for *P. neglectus* was 36.6 and 14.0 on day 28 in Bari 3 and Cermi, respectively. Based on the data shown in Figure 2, it is suggested that Bari 2 was the most resistant cultivar with RF of *P. neglectus* not exceeding 4 whereas Bari 3 and Cermi are susceptible to *P. neglectus*. Also, it is concluded that *P. neglectus* completed first life cycle in 28 d in Bari 3 and Cermi.

The population development of *P. penetrans* reached its first peak on day 28 in Bari 2, Bari 3 and Cermi (Figure 3). Also, the RF of *P. penetrans* in Bari 3 was higher than in Cermi and Bari 2 after 49 d whereas *P. neglectus* had two peaks in the three chickpea cultivars during the experiment. Consequently, it was found that the interaction of the day, day by nematode species, day cultivar, day by nematode species by cultivar were significant at $P < 0.0001$, whereas there was no significant difference observed between nematode species. Therefore, all nematode species and day factors were important in this study (Table 3).

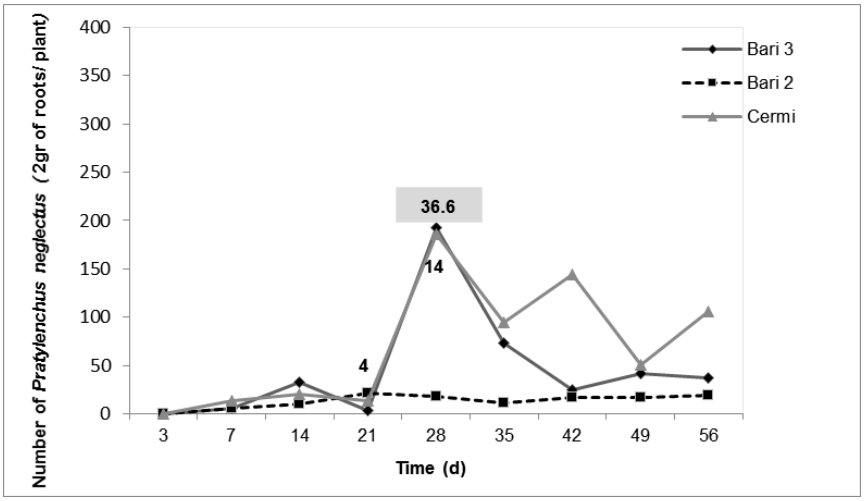


Figure 2. Population changes of *Pratylenchus neglectus* in chickpea cultivars, Bari 2, Bari 3 and Cermi.

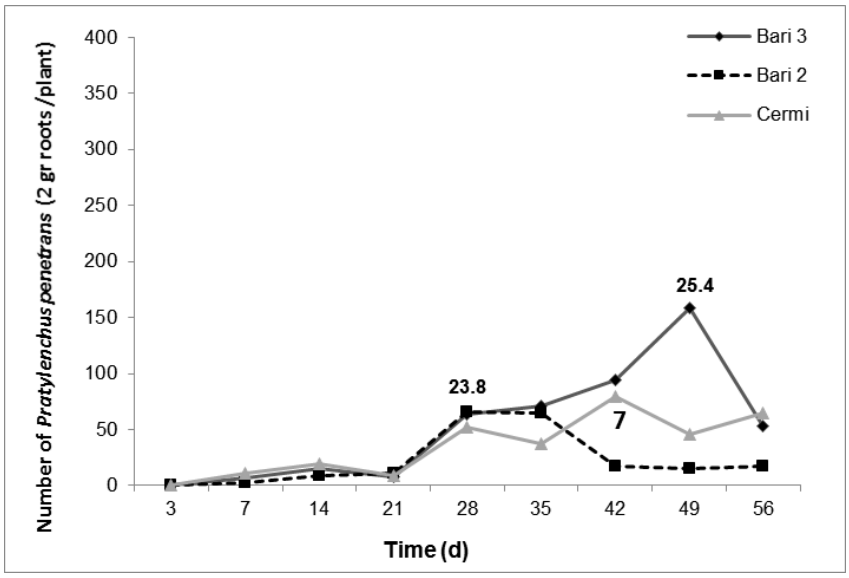


Figure 3. Population changes of *Pratylenchus penetrans* in chickpea cultivars, Bari 2, Bari 3 and Cermi.

Table 3. Variance analysis of the roots of chickpea cultivars (Bari 2, Bari 3 and Cermi) were infected with *Pratylenchus neglectus*, *P. penetrans* and *P. thornei* on different days

| Source of Variation | df | MS | F | P |
|---|---|---|---|---|
| Day | 8 | 22725.693 | 20.577 | <0.0001 |
| Day × nematode species | 16 | 11318.867 | 10.248 | <0.0001 |
| Day × cultivar | 16 | 6924.716 | 6.270 | <0.0001 |
| Day × nematode species × cultivar | 32 | 8896.289 | 8.055 | <0.0001 |
| Error (day) | 216 | 1104.447 | | |
| Intercept | 1 | 535661.346 | 413.591 | <0.0001 |
| Nematode species | 2 | 1271.123 | 0.981 | >0.05 |
| Cultivar | 2 | 60787.077 | 46.935 | <0.0001 |
| Nematode species × cultivar | 4 | 16769.660 | 12.948 | <0.0001 |
| Error | 27 | 1295.146 | | |

df, degree of freedom; MS, mean square; F and P values.

Chickpea is an important legume crop produced in most provinces of Turkey. Economic importance and damage of root lesion nematodes *i*on plants can be measured by the estimation of population density of nematodes per unit of root and/or soil, or by RF (Tiwari et al., 1992; Di Vito et al., 1995; Thompson et

al., 2011; Reen et al., 2019). Ali & Ahmad (2000) demonstrated that the lesions present on infected roots are only symptoms and are not a direct measure of nematode numbers. Factors such as the population density of nematodes and the nematode life cycle have an important effect on the damage to the crop and assessing these will be useful for controlling of nematode population below an economic threshold.

Currently, there are only few studies that have investigated the development of root lesion nematodes on different chickpea cultivars in root and soil. High and low population densities were established to develop an evaluation of nematodes by growing susceptible and resistant chickpea cultivars (Taylor et al., 2000). At planting, the damaging threshold of root lesion nematodes can change the nematode life cycles in chickpea cultivars. This occurs because of the competition between nematode species and the different temperature requirements of the nematode in the soil or root. So, the population density of nematodes in soil may not provide useful information on the number of nematodes in roots. However, if root lesion nematodes are present in the soil then actively managing for root lesion nematodes should be considered. According to the results, there was a significant difference between nematode species in the root sample, and population density of nematodes differed between cultivars of *C. echinospermum* and *C. reticulatum* studied. Behmand et al. (2020) indicated that the population density of root lesion nematodes was differed with species (*P. neglectus* and *P. thornei*) and *Cicer* species. The population density of *P. thornei* in *C. echinospermum* was higher than the population density in *C. reticulatum*. A similar study by Thomson (2011) showed that the RF of root lesion nematodes differed between *C. echinospermum* and *C. reticulatum*, and RF of *P. thornei* in *C. reticulatum* was more than the RF in *C. echinospermum*. Also, the present study found that all root lesion nematodes penetrated roots after the inoculation by days 3 to 7. Peak populations of *P. neglectus, P. penetrans* and *P. thornei* were greatest in Cermi. These findings indicated that Cermi is more susceptible than Bari 2 and Bari 3 to *P. thornei*. Also, the life cycle was completed in different times for nematode species, for *P. thornei* the population density was highest on day 21, and the others on day 28. The development of the population density in to *P. thornei* changed between resistance and susceptible cultivars. A study by Linsell et al. (2014) indicated that the population density of *P. thornei* was reduced in resistance wheat cultivars and Vanstone et al. (2008) showed the full cycle from egg to adult is completed within 45 to 65 d and is greatly influenced by the host, temperature and *Pratylenchus* spp. Hatching activity may decrease with increasing plant age in different plant cultivar roots (Umesh & Ferris, 1992; Pudasaini et al., 2008).

The population density of *P. neglectus* in Bari 3 and Cermi was higher than in Bari 2 on days 28 and 35, however, the population density of *P. penetrans* in Bari 2 on days 42 and 49 was higher. The differences in penetration and population density of these nematodes in similar cultivars at the end of the 56-day experiments might be due plant physiological differences and nematode virulence in these cultivars. Penetration time of nematode species differs between crops. For example, nematodes penetrated *Brassica rapa* L. (Brassicaceae) within the first 6 h after inoculation whereas in *Zea mays* L. (Poaceae) penetration was within 8 to 12 h (Ogiga & Estey, 1975).

Crop loss can be associated with the population density of nematodes in the soil (Olthof & Potter, 1972). However, knowing the number of nematodes in the root is important in order to control root lesion nematodes. The population changes of *Pratylenchus neglectus, P. penetrans* and *P. thornei* were evaluated inside the roots of chickpea to help chickpea breeding programs.

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*Original article (Orijinal araştırma)*

# Impact of maternal age on performance of the progeny in *Galleria mellonella* (L., 1758) (Lepidoptera: Pyralidae)

Ana yaşının *Galleria mellonella* (L., 1758) (Lepidoptera: Pyralidae) oğul döllerinin performansına etkisi

**Yeşim KOÇ**[1] **Evrim SÖNMEZ**[1*]

## Abstract

Maternal age is the age of an insect at the time of depositing an egg and is an important factor impacting on the properties of the progeny. This study determined the influence of maternal age on the preadult total development time, larval development time, pupal development time, pupal weight and adult longevity of *Galleria mellonella* (L., 1758) (Lepidoptera: Pyralidae). This study was conducted in 2018 in the Biology Laboratory of Science Teaching, Sinop University. The insects were grouped into four groups (1, 5, 10 and 15 d-old) and they were kept under laboratory conditions (28 ± 2°C and 65 ± 5% RH and continuous darkness). Larval development time, total preadult development time and longevity decreased with maternal age whereas pupal development time increased. Although the development time of the progeny of younger females lasted longer, total longevity increased. Although, the progeny of younger females had increased longevity, progeny of older females was advantaged in terms of development time.

**Keywords:** *Galleria mellonella*, insect development, longevity, maternal age

## Öz

Ana yaşı, bir böceğin yumurtayı bıraktığı andaki yaşıdır ve oğul dölün özelliklerini etkileyen önemli bir faktördür. Bu çalışma ana yaşının *Galleria mellonella*'nın (L., 1758) (Lepidoptera: Pyralidae) ergin öncesi gelişim süresi, larva gelişim süresi, pupa gelişim süresi, pupa ağırlık ve ergin ömür uzunluğu üzerindeki etkisini belirledi. Bu çalışma 2018 yılında Sinop Üniversitesi, Fen Bilgisi Eğitimi, Biyoloji Laboratuvarı'nda yapıldı. Böcekler dört gruba ayrıldı (1, 5, 10, 15 gün yaşlı ve 28 ± 2 °C, %65 ± 5 bağıl nem ve devamlı karanlık) ve laboratuvar şartları altında tutuldu. Larva gelişim süresi, toplam ergin öncesi gelişim süresi ve ömür uzunluğu yaşla azalırken pupa gelişim süresi arttı. Genç anaların oğul döllerinin gelişim süresi daha uzun sürmesine rağmen, ergin ömür uzunluğu arttı. Genç analardan elde edilen oğul döller ömür uzunluğu bakımından avantajlıyken, yaşlı analardan elde edilen oğul döller gelişim süresi bakımından avantajlı olarak saptanmıştır.

**Anahtar sözcükler:** *Galleria mellonella*, böcek gelişimi, ömür uzunluğu, ana yaşı

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## Introduction

Maternal age is the age of an insect at the time of depositing an egg (Karsavuran & Anaç, 2014). It is known that maternal age has an impact on parameters such as oviposition, egg mass, number of emerged insects, fecundity, survival ratio of the adult insects and longevity (Fiore, 1960; Ambrose et al., 1988; Rossiter, 1991; Gavrilov et al., 1997; Mohaghegh et al., 1998a, 1998b; Fox et al., 2003; Mishra & Omkar, 2004; Tucic et al., 2004). Legaspi & O'Neil (1994) found that the maternal age delayed the nymphal development in *Podisus maculiventris* (Say, 1832) (Hemiptera: Pentatomidae). In some studies, it was found that progeny of young females had higher fecundity than the progeny of older females (Mishra & Omkar, 2004; Zehnder & Hunter, 2007; Montoya & Farfan, 2009).

The greater wax moth, *Galleria mellonella* (L., 1758) (Lepidoptera: Pyralidae*)* can easily be bred under laboratory conditions. For this reason, it is frequently used as a model insect in different fields of biology, such as physiology, molecular biology, microbiology and biochemistry. It is also an important insect used under laboratory conditions as a natural host in rearing parasitoid insects in biocontrol studies, in studies on substances harmful for the environment, e.g., insecticides, and in determining the pathogenicity of microorganisms (Hood et al., 2003; Büyükgüzel & Kalender, 2009; Ciesielczuk et al., 2015; Hosamani et al., 2017; Kecko et al., 2017).

The impact of maternal age on longevity, development time and morphological and physiological traits of progeny is a factor to be taken into consideration in maintaining laboratory cultures of insects, and in studies on such cultures. Mohaghegh et al. (1998a) found that the eggs deposited by old females (young individuals 2-4 weeks-old and old individuals 7 weeks-old) were smaller in *P. maculiventris*, the individuals were lighter and the development was delayed in the generations arising from young females. Development time of insects obtained from the older females was 29.0 ± 0.33 d for females and 28.7 ± 0.26 d for males, whereas these values were 26.7 ± 0.17 and, 26.7 ± 0.21 d, respectively for younger individuals (Mohaghegh et al., 1998a). In the study conducted by Şimşek et al. (2015) on *Rhyzobius lophanthae* (Blaisdell, 1892) (Coleoptera: Coccinellidae), it was found that total development time and survival successes decreased as the maternal age increased and the oviposition time increased as the age increased. In some studies, the progeny of younger females is more fecund as than the progeny of older females (Mishra & Omkar, 2004; Zehnder & Hunter, 2007; Montoya & Farfan, 2009). Therefore, reproduction of young individuals has been suggested as important for continuity in the cultures. Also, the quality of the insects used as hosts is very important in biological control studies.

Accordingly, in this study, the impact of maternal age on the longevity and development time of *G. mellonella* were assessed, comparing the performance of the progeny of 1, 5, 10 and 15 d-old *G. mellonella* adults.

## Materials and Methods

### Setting up the cultures

The study used cultures of successive laboratory stocks of *G. mellonella.* Initial cultures were formed from adults obtained from hives infested with *G. mellonella* obtained from beekeepers in Sinop (Sinop Beekeepers Association, Sinop, Turkey). The cultures were started by placing the adults in 500-ml glass jars (500 × 140 × 84 mm, DTO070, ISOLAB, Istanbul, Turkey) covered with a cloth which did not prevent air flow. Honeycomb without honey was used to feed the insects. The honeycomb was kept in a deep freezer to eliminate parasites and then were sterilized. They were given to insects every day as needed. In order to assist pupation, folded pieces of paper (100 × 100 mm) were put into the jars. Adults reared under laboratory conditions for at least three generations were used in the experiments.

**Forming the age groups**

In order to find out the influence of maternal age on performance of progeny, four groups were formed based on adult age (time since eclosion): 1, 5, 10 and 15 d-old. The adults taken out of stock culture were placed in a separate experimental jar to form the 1 d-old group. For the other age groups, adults were kept for 5, 10 and 15 d in separate jars, and they were put into their own experimental jar, when they reached the required age. The insects in each experiment were kept in separate jars so that they were not allowed to mate until they reached the required age. A total of 20 insects were put in each experimental jar. At this stage, they were allowed to mate and lay eggs. The insects were kept in these jars for 5 d. They were removed from the jars after 5 d. The individuals which came out of these jars were taken in experiments. For the parameters assessed, measurements were made on 15 individuals, i.e., 45 in total. Honeycomb was used as food in all cases.

All experimental jars were monitored daily. For larval development time, the date each larva pupated was recorded. In each experiment, 15 pupae were taken randomly selected for subsequent assessment. Total larval development time was calculated. The 15 pupae were placed in different Petri dishes (90 × 15 mm, ISOLAB). The date the insects emerged as adult was recorded and the pupal development time calculated. They were weighed the first day they reached pupal stage. The time adult insects emerged was used to determine longevity, with each placed in separate jars (250 ml) without allowing them to mate, but allowing them to continue to feed. They were checked every day. Results were recorded for three repeat experiments for each age group with 45 insects in total.

The experiments were conducted under laboratory conditions of 28 ± 2°C, 65 ± 5% RH and continuous darkness. This study investigated the influence of maternal age on *G. mellonella* larval development time, pupal development time, total preadult development time, pupal mass and adult longevity.

**Data analysis**

SPSS 21.0 software (IBM, Armonk, NY, USA) program was used for the statistical assessment of the data. Averages were obtained for the 15 insects in each group of the three experiments (45 insects in total) and then statistical analyses (n = 3 in each age group). Firstly, a normality test was conducted for the groups. According to Kolmogorov-Smirnova and Shapiro-Wilk tests, it was found that the data were not normally distributed. Since the data were not normally distributed and in order to understand whether there were differences between groups, we performed Kruskal-Wallis test for each age group. Differences were found between groups according to Kruskal-Wallis test ($p < 0.05$). Mann-Whitney U test was used to determine if age group differences existed ($p < 0.001$).

## Results

Figure 1 shows the influence of maternal age on *G. mellonella*'s total preadult development time ($H = 109$, $p < 0.001$, $df = 3$), larval development time ($H = 157$, $p < 0.001$, $df = 3$) and pupal development time ($H = 135$, $p < 0.001$, $df = 3$; Kruskal-Wallis test $p < 0.001$). Total development time and larval development time decreased with increase in maternal age whereas pupal development time increased (Man-Whitney U, $p < 0.001$).

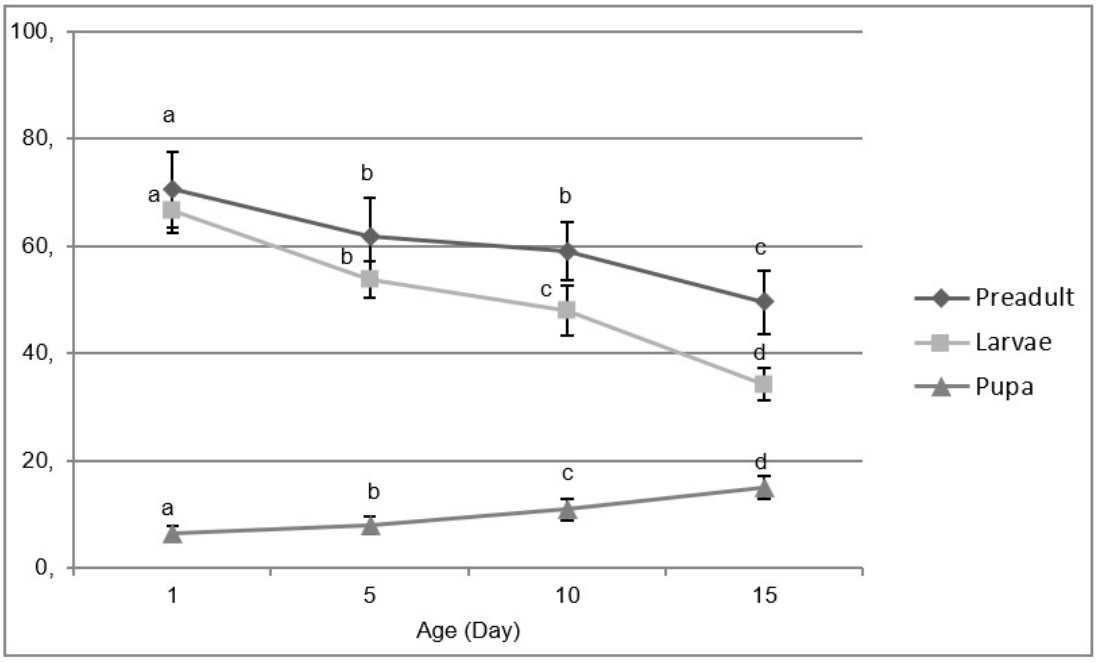


Figure 1. Effect of maternal age on total preadult, larval and pupal development time of the progeny of *Galleria mellonella*. The values with the same letters the same line is not significantly different (Man-Whitney U, $p < 0.001$); n = 3 for each experiment with each an average of 15 individuals.

Figure 2 and Figure 3 show the influence of maternal age on *G. mellonella*'s longevity and pupal mass. Longevity ($H = 40.9$, $p < 0.001$, $df = 3$; Kruskal-Wallis test $p < 0.001$) decreased with the increase in age (Man-Whitney U, $p < 0.001$). While there were significant differences in pupal mass among the age groups, the difference was not consistent ($H = 12.1$, $p < 0.001$, $df = 3$; Kruskal-Wallis test $p < 0.05$; Man-Whitney U, $p < 0.001$, Figure 3).

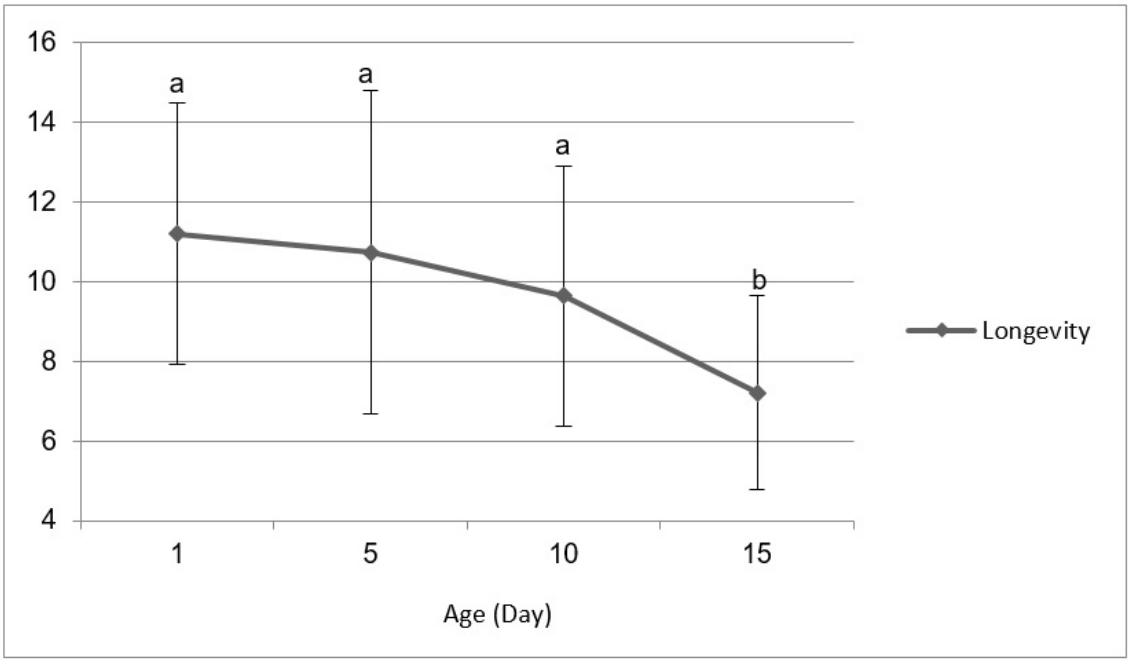


Figure 2. Effect of maternal age on longevity of the progeny in *Galleria mellonella*. The values with the same letters are not significantly different (Man-Whitney U, $p < 0.001$); n = 3 for each experiment with each an average of 15 individuals.

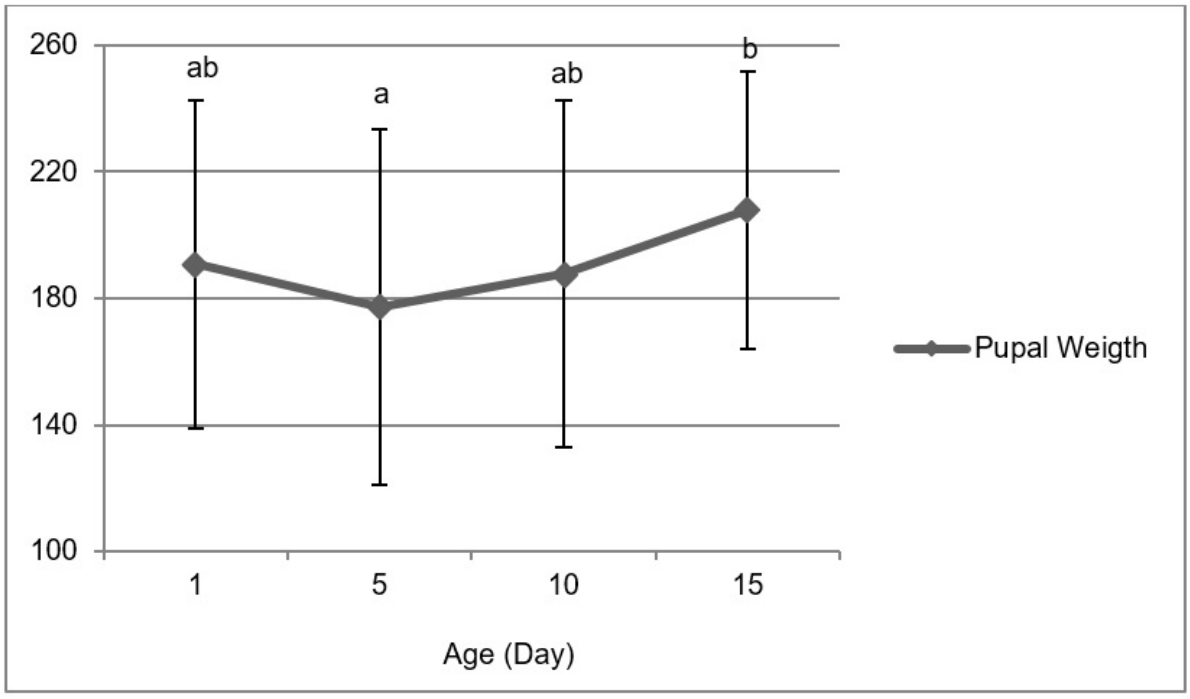


Figure 3. Effect of maternal age on pupal mass of the progeny in *Galleria mellonella*. The values with the same letters are not significantly different (Man-Whitney U, $p<0.001$); n = 3 for each trial with each an average of 15 individuals.

## Discussion

In this study, it was found that larval development time in *G. mellonella* decreased with the increase in maternal age whereas pupal development time increased (Figure 1). The total preadult development time decreased. Adult longevity was found to be shorter in the progeny of older females than younger females (Figure 2). This could result from weaker nutrient composition older egg-laying females passed to its eggs for the next generation. In a study on *Eupelmus vuilleti* (Crawford, 1913) (Hymenoptera: Eupelmidae), Muller et al. (2017) found that eggs and progeny of older females had less glycogen and protein. Consistent with the results of that study, there are some studies which have found that older maternal age reduces total development time, larval development time and longevity (Fiore, 1960; Şimşek et al., 2015). In a study on *Aphis nerii* (Fonscolombe, 1879) (Hemiptera: Aphididae), Parris et al. (2007) observed that the progeny of older females matured more quickly, consistent with the Lansing effect and had shorter longevity. Lansing (1947) stated that longevity could be influenced through a factor that could be transmitted through eggs from the parent female.

Priest et al. (2002), Lind et al. (2015) and Bock et al. (2019) attributed the effects of maternal age on development and longevity to some mutations that occur with aging and stated that genetic factors could influence the following generations. However, they suggested that the maternal effect on longevity mechanism was not fully clarified and that more detailed studies were required. The decreased longevity found in the current study of the progeny of older females could be due to both egg quality and the genetic factors suggested by those authors.

While Ludwig & Fiore (1960), Lints (1978), Phelan & Frumhoff (1991) and Şimşek et al. (2015) found that the development time of progeny of older females was shorter. Muller et al. (2017), Wasserman & Asami (1985), Legaspi & O'Neil (1994) and Mohaghegh et al. (1998a) suggested the opposite. Harvey (1977) could not find any changes on development rates in terms of age. This shows that the development time of species from older females can differ between species.

Correspondingly, probably at the pupal stage, pupae originating from younger females became adults earlier. This could be due to the fact that they complete the required changes and organ formations quicker. The progeny of the oldest egg-laying females of the present study had the shortest lifespan, which implies that individuals from younger females are healthier and can thus have a shortened pupal stage. In the present study, pupal mass was affected less and inconsistently by maternal age (Figure 3). Eggs of old females have reduced hatching, higher death rates and shorter development time than young females (Fox, 1993; Mishra & Omkar, 2004; Karsavuran & Anaç, 2014). According to the "consumption of reproduction sources hypothesis" in the female, these maternal influences can influence egg size, egg survival rate, development time, hatching, pupal development time and adult longevity (Yanagi & Miyatake, 2002). Although development time is often shorter in insects from old females (Ludwig & Fiore, 1960; Phelan & Frumhoff, 1991; Zehnder & Hunter, 2007), in this study pupal time was significantly shorter in insects from young females. As far as we could observe, population density in insects from young females was higher.

In the present study, shorter longevity and longer pupal time of the progeny of older females can be explained by lower quality of their eggs (Mousseau & Dingle, 1991; Phelan & Frumhoff, 1991). This can occur as a result of older egg-laying females rapidly depleting the required resources for egg development. Decreased provisioning of eggs influences nutrient composition of the hatch larvae (Fox & Dingle, 1994). From a study on *Oncopeltus fasciatus* (Dallas, 1852) (Hemiptera: Lygaeidae), Phelan & Frumhoff (1991) suggested that females pass a special biological signal to eggs for a faster embryonic and nymphal development. This in turn can influence the life cycle and performance of hatched insects. In our results, although the development time of the progeny of younger females lasted longer, longevity increased. While the progeny of younger females is advantaged in terms of longevity, progeny of older females are advantaged in terms of development time.

According to these results, more detailed study is needed to determine which age group will be more advantaged the most. A focus on determination of fecundity and larval death rates in future studies would be important.

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*Original article (Orijinal araştırma)*

# Pest status and population dynamics of the lucerne leaf beetle, *Gonioctena fornicata* (Brüggemann, 1873) (Coleoptera: Chrysomelidae), in a lucerne field in Adana Province, Turkey[1]

Adana İli (Türkiye)'nde bir yonca tarlasında Yonca yaprakböceği, *Gonioctena fornicata* (Brüggemann, 1873) (Coleoptera: Chrysomelidae)'nin zarar durumu ve popülasyon dinamiği

**Ekrem ATAKAN[2*]** **Yusuf DAĞ[2]**

## Abstract

The lucerne leaf beetle, *Gonioctena fornicata* (Brüggemann, 1873) (Coleoptera: Chrysomelidae), is an important pest of lucerne in Europe and several parts of Turkey. Its damage and thus the loss of yield due to this pest insect is unknown. This study was conducted at Çukurova University, Faculty of Agriculture Research and Application Farm in Adana Province, Turkey in 2017 and 2018. It was found that the pest had one generation per year. Especially the larvae of *G. fornicata* was found to feed extensively on the leaves, shoots, flowers and seeds of lucerne, almost turning them into leafless. In the untreated plots, there was about 148% loss in both fresh weight and dry weight in 2017 caused by this pest species and up to 27% loss in 2018. *Gonioctena fornicata* negatively affected the fresh and dry weight yields. It is estimated that the yield losses were 3.48 t/ha. Although the yield difference between the treated and untreated plots was lower than expected, the cost of loss of yield was still high. Economic loss in the untreated plot was about 4250 TRY/ha (about 545 USD/ha as of December 2020).

**Keywords:** Adana, alfalfa, damage, *Gonioctena fornicata*, population dynamic

## Öz

Yonca yaprakböceği, *Gonioctena fornicata* (Brüggemann, 1873) (Coleoptera: Chrysomelidae) Avrupa ve Türkiye'nin bazı yerlerinde yoncanın önemli bir zararlısıdır. Buna karşın, bu böcek neden olduğu zarar düzeyi ve verim kayıpları bilinmemektedir. Bu çalışma, Adana ilinde Çukurova Üniversitesi Ziraat Fakültesi Araştırma ve Uygulama Çiftliği'nde 2017 ve 2018 yıllarında yürütülmüştür. Zararlının bir döl verdiği bulunmuştur. Yonca yaprakböceği'nin özellikle larvalarının bitkilerin yaprak, sürgün, çiçek ve tohumlarında obur bir şekilde beslenerek bitkileri adeta yapraksız duruma getirdiği görülmüştür. İlaçsız parsellerde 2017 yılında yaş ağırlıkta %14.7, kuru ağırlıkta, %14.6 kayıp meydana gelmiştir. 2018 yılında ise yaş ağırlıkta %26.3, kuru ağırlıkta %27.4 kayıplar saptanmıştır. *Gonioctena fornicata*'nın yonca deneme alanında mart-mayıs döneminde ana zararlı duruma geldiği belirlenmiştir. *Gonioctena fornicata* kuru ve yaş ağırlığı olumsuz etkilemiştir. Bu böcek nedeniyle hektara verim kaybı 3480 kg'dır. İlaçlı ve ilaçlı parseller arasında verim kayıpları beklenenden daha düşük olmasına karşın, verim kaybının maliyeti yüksek olmuştur. İlaçlanmayan parselde ekonomik kaybın parasal değeri 4250 TL/ha olup, bunun Amerikan doları karşılığı, Aralık 2020 itibarıyla 545 US$/ha'dır.

**Anahtar sözcükler:** Adana, yonca, zarar, *Gonioctena fornicata*, popülasyon dinamiği

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## Introduction

A significant portion of Turkey's population gains a living in agriculture. Turkey is still an agricultural country with its geographical location, climate features and fertile soil suitable for agriculture. Despite this, it is an important problem today that the cultivated areas are gradually decreasing, and now the population reaching 80 million to be fed. In particular, forage crops have an important place in terms of providing sufficient animal protein, and more importance should be attached to the production of forage crops. In this context, lucerne, which is an important perennial legume forage plant, stands out as a quality and high-yielding forage plant (Sumberg et al., 1983; Abd El-Halim et al., 1992). However, lucerne production has been decreasing in Turkey recently. Total production in 2012, 2016, 2017 and 2018 were 676, 652, 659 and 635 kha, respectively (TUİK, 2018).

The most important feature of lucerne is its high nutritional content. Lucerne contains 15-22% crude protein and is an excellent source of minerals and vitamins. As a result of various narrowing and ill-considered destruction of agricultural areas, it has become essential to obtain the highest yield per unit area from lucerne, just as it is for most other crops. One of the biggest factors of low yield in lucerne is disease and pests. Due to these factors, there is a significant loss of production every year and a significant decrease in lucerne yield is observed due to these factors (Yıldırım et al., 1996).

One of the important pests of lucerne is lucerne leaf beetle [*Gonioctena fornicata* (Brüggemann, 1873)] (Coleoptera: Chrysomelidae). Adults and larvae of this pest feed on the leaves, flowers and shoots of lucerne. Kovancı (1982), in the study of the morphology and biology of *G. fornicata* in Ankara Province, reported that this pest occurred in Ayaş, Beypazarı, Nallıhan, Kızılcahamam, Çubuk and Polatlı Districts. Also, Yıldırım et al. (1996) found that *G. fornicata* is an important lucerne pest in Erzurum and Erzincan Provinces in its study on its definition, biology and damage. Aslan & Özbek (1999) also identified *G. fornicata* in their faunistic and systematic studies in Artvin, Erzincan and Erzurum Provinces, Turkey. Life table of the *G. fornicata* has been well studied (Ghavami et al., 1998; Efe & Özgökçe, 2014). It has been reported that *G. fornicata* larvae are sensitive to all *Beauveria bassiana* (Bals.-Criv.) Vuill. isolates used in dose-death studies (Baysal et al., 2019).

Previous research on this pest is available in the world literature but for Turkey information on damage and economic losses caused by this pest is limited. The time of emergence, larvae and adult development of lucerne leaf beetle in the lucerne fields, in other words, the population dynamics are unknown. In several studies conducted in Turkey, population development has been investigated by the sweep net sampling of *G. fornicata* (Anay, 2000; Coşkuncu & Gençer, 2006). In previous studies, damage and economic loss due to infestation of this pest have not been reported for Turkey. The damage and the economic loss caused by the pest in Adana therefore unknown. Population changes of pest species were investigated by plant sampling in this study. For this purpose, as well as the population dynamics in the insecticide-treated and untreated plots, the yield loss caused by it was also revealed. The data obtained can be used for the management of the *G. fornicata* in lucerne fields.

## Materials and Methods

This study was conducted in 2017 and 2018 in the lucerne area of Adana Province, Çukurova University Faculty of Agriculture Research and Application Farm. The experimental was 0.1 ha divided into eight plots each of 100 $m^2$ in a split-plot design. Replicates were created by dividing main plots (with and without treatment). The study was conducted with four replicates, four plots (10 × 10 m) were insecticide free and four plots were treated with insecticides. Two m was left between plots and blocks, and these areas were left bare. Application of insecticides was done according to the program of Research and Application Farm, and when the damage reached 10-20% by counting damaged leaves within 1 $m^2$ area insecticides were applied by field sprayer (Holder) with 1 t capacity and operated with 4 bar pressure. The

insecticides had active ingredients of deltamethrin 20 g/l (EC) (300 ml/ha) in 2017 and chlorpyrifos ethyl 480 g/l (400 ml/ha) in 2018. Lucerne cultivar Nimet was used in the experiment. Two hundred kg/ha of 21% ammonium sulfate and 500 kg/ha of 42% triple super phosphate were applied to the soil before the sowing. Sprinkler irrigation system was operated every 15 d during the experiment.

**Insect sampling**

For this purpose, 1 × 1 m quadrat was arbitrarily thrown twice in each subplot and the plants within the quadrat examined. The adults and larvae of *G. fornicata* were counted in the field and recorded. In order not to prevent the development of the pest population, after the counts, the insects were returned to the plots after counting. Insect sampling was done in the morning between 08:00 and 10:00.

**Lucerne yield**

When flowering of the lucerne crop reached 80% (10 May in both the years), harvesting was done in accordance with the Research and Application Farm management program. Each plot was mowed separately using a disc-formatted machine. To determine the fresh weight, the cut lucerne obtained in the plots were individually bagged and weighed. The lucerne that was left to dry in the field were baled after it dried in the sun. The bales were made separately in each plot and their dry weights were determined with a hand scale. In addition, the economic damage of the lucerne due to the pest feeding was calculate using the market price per kg, the cost of spraying per ha and the loss of yield in the insecticide-free plot. In order to determine the crude protein, ADF (acid detergent cellulose) and NDF (neutral detergent cellulose) values of the dry lucerne hay, 500 g of samples were taken from each plot and numbered on the purse paper (Naidenova & Donschev, 1995). Analysis were done at the Department of Field Crops at the Faculty of Agriculture, Bozok University, Yozgat Province, Turkey.

**Statistical analysis**

Populations of larvae and adults were converted to means for treated and untreated plots recorded weekly. These means were compared by t-test to determine the effect of the treatment. For this purpose, iterative statistical analysis method (repeated measures ANOVA) was used. The effect of sampling date, insecticide treatment and sampling date by treatment interaction were analyzed. The t-test ($P < 0.05$) was again used in the calculation of the mean yield, crude protein, ADF and NDF values for the plots. Yield and crude protein losses (%) were calculated according to Karman (1971). The relationships between the mean number of insects (larvae and adults) and meteorological factors (temperature and RH) were examined by linear regression analysis at $P < 0.05$ significance level. All statistical analyzes were done in SPSS Package Program (Version 15) (SPSS, 2006).

## Results

**Population dynamics of *Gonioctena fornicata***

The sampling date, treatment and sampling date by treatment were significantly affected by the *G. fornicata* population (Table 1).

Population dynamics of adults and larvae of *G. fornicata* in 2017 in the lucerne field in the insecticide-treated and insecticide-free plots are given in Figure 1 and Table 2. The first adults (0.1±0.12 adults/m²) on insecticide-free plots were detected on 18 April. The highest adult density was recorded as 0.6±0.26 adults/$m^2$ on 9 May, when the mean temperature was 20.2ºC, RH was 81.1% and the mean rainfall was 1.62 mm (Figure 2). The first adults in the treated plots were recorded on 18 April, and the highest adult density as found on 9 May (0.9±0.39 adults/$m^2$). No significant differences were found between insecticide-treated and insecticide-free plots in adult population density ($P > 0.05$). This may be related to the low number of adults in both plots.

Table 1. Results of repeated measures ANOVA for *Gonioctena fornicata* in a lucerne field in Adana Province, Turkey during 2017

| Variation source | Df | MS | F | P |
|---|---|---|---|---|
| Date (sampling) | 6 | 2135 | 57.4 | <0.0001 |
| Date x Treatment | 6 | 858 | 23.0 | <0.0001 |
| Error (date) | 168 | 37 | | |
| Treatment | 1 | 6227 | 83.6 | <0.0001 |
| Error | 28 | 75 | | |

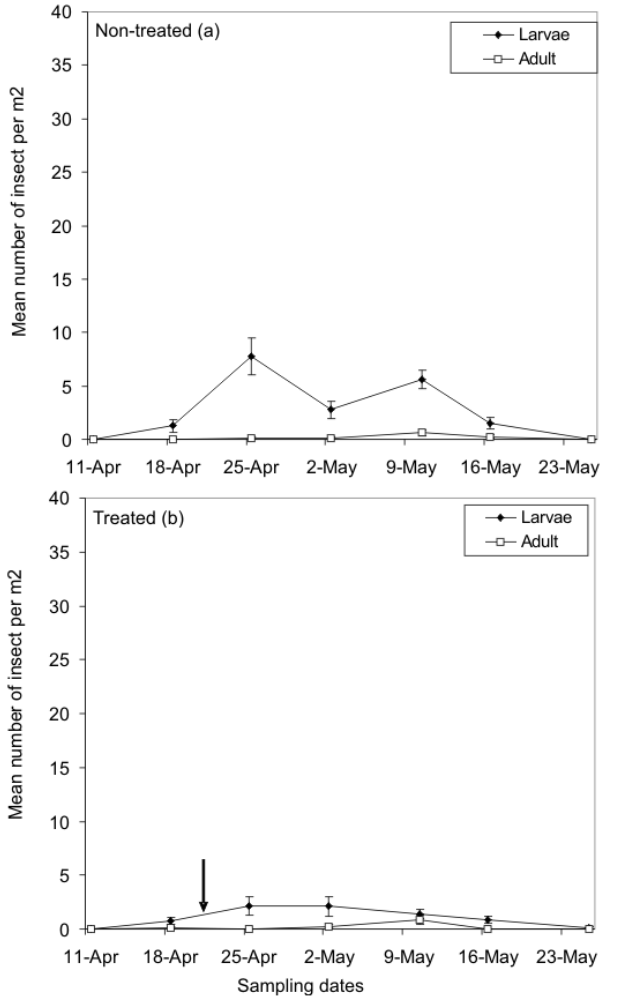


Figure 1. Mean number of *Gonioctena fornicata* per $m^2$ in the untreated (a) and treated plots (b) plots in 2017 by the sampling of lucerne in Balcalı, Adana Province, Turkey. The straight dark arrow sign indicates the date of application (21 April 2017).

Population dynamics of *G. fornicata* larvae in the lucerne field in 2017 on treated and untreated plots are shown in Figure 2 and Table 2. The first larvae were registered on the treated and untreated plots on 18 April. Larvae reached the highest population density in the untreated plot on 25 April (7.8±1.8 larvae/$m^2$). This short-term decrease mean numbers of larvae finished on 9 May (5.6±0.88 larvae/$m^2$). Similar to adults, larval density in untreated plots declined to zero on 23 May. The mean larval density in the treated plot was lower than the untreated plots. When the larval densities are compared in the untreated and treated plots; the difference was significant on 25 April ($F_{1,14}$ = 8.43, t = 2.89, P = 0.012) and 9 May ($F_{1,14}$ = 18.8, t = 4.34, P = 0.001), and the highest larval density was recorded in the untreated plots. No significant correlation was found between plant sampling, larval and adult population densities of pests and meteorological factors (P > 0.05).

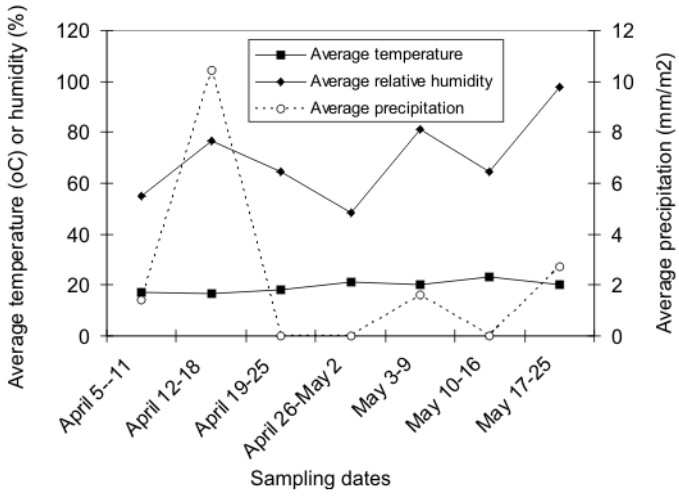


Figure 2. Meteorological data for Adana Province, Turkey in 2017.

Table 2. Mean number of larvae and adults of *Gonioctena fornicata* per $m^2$ in treated and untreated lucerne plot in Balcalı, Adana Province, Turkey during 2017

| Plot type | 11 April | 18 April | 25 April | 2 May | 9 May | 16 May | 23 May |
|---|---|---|---|---|---|---|---|
| | | | | Adult | | | |
| Non-treated | 0.00±0.00 | 0.00±0.00 | 0.12±012 | 0.12±0.12 | 0.62±0.26 | 0.25±0.60 | 0.00±0.00 |
| Treated | 0.00±0.00 | 0.12±0.12 | 0.00±0.00 | 0.25±0.16 | 0.87±0.39 | 0.00±0.00 | 0.00±0.00 |
| | | | | Larvae | | | |
| Non-treated | 0.00±0.00 | 1.25±0.55 | 7.75±1.75*[a] | 2.75±0.79 | 5.62±0.88* | 1.50±0.56 | 0.00±0.00 |
| Treated | 0.00±0.00 | 0.75±0.31 | 2.12±0.85 | 2.12±0.91 | 1.37±0.41 | 0.87±0.35 | 0.12±0.12 |

* Means±SEM within columns followed by an asterisk are statistically different according to the t-test (P < 0.05).

In the statistical analysis for 2018 data, date (sampling) and date × treatment had a significant effect on the population of *G. fornicata* (Table 3). Also, treatment was also found to be significant (Table 3).

Population changes of adults and larvae in 2018 in the lucerne field in Balcalı are shown in Figure 3 and Table 4. Both the treated and untreated plots had higher adult densities than the previous experimental year. The adult density in the untreated plots increased rapidly after 10 April, when the mean temperature was 18.6ºC, RH was 56.1% and the mean rainfall was 11.5 mm. It reached its peak on 17 April (26.3±6.7 adults/$m^2$), when the mean temperature was 19.5ºC, RH was 67.1% and the mean rainfall was 10.1 mm (Figure 4). The insecticide applied at this date probably affected the adult density in the treated plots and reduced the adult population. As seen in Table 4, the adult density in untreated plot increased again on 1 May, when the mean temperature was 23.2ºC, RH was 58.6% and the mean rainfall was 0 mm/$m^2$ (14.25±1.16 adults/$m^2$). After this date, the mean population density decreased to the lowest level (0.12±0.12 adults/$m^2$) on 22 May. Significant differences were found for adult density (24 April, $F_{1,14} = 18.7$, t = 4.31, P = 0.01; 1 May, $F_{1,14} = 53.7$, t = 7.54, P < 0.0001 and 8 May, $F_{1,14} = 56.8$, t = 7.54, P < 0.0001).

Table 3. Results of repeated measures ANOVA for *Gonioctena fornicata* in a lucerne field in Adana Province, Turkey during 2018

| Variation sources | df | MS | F | P |
|---|---|---|---|---|
| Date (sampling) | 6 | 24084 | 96.4 | <0.0001 |
| Date × Treatment | 6 | 1582 | 6.4 | <0.0001 |
| Error (date) | 168 | 249 | | |
| Treatment | 1 | 11861 | 73.6 | <0.0001 |
| Error | 28 | 161 | | |

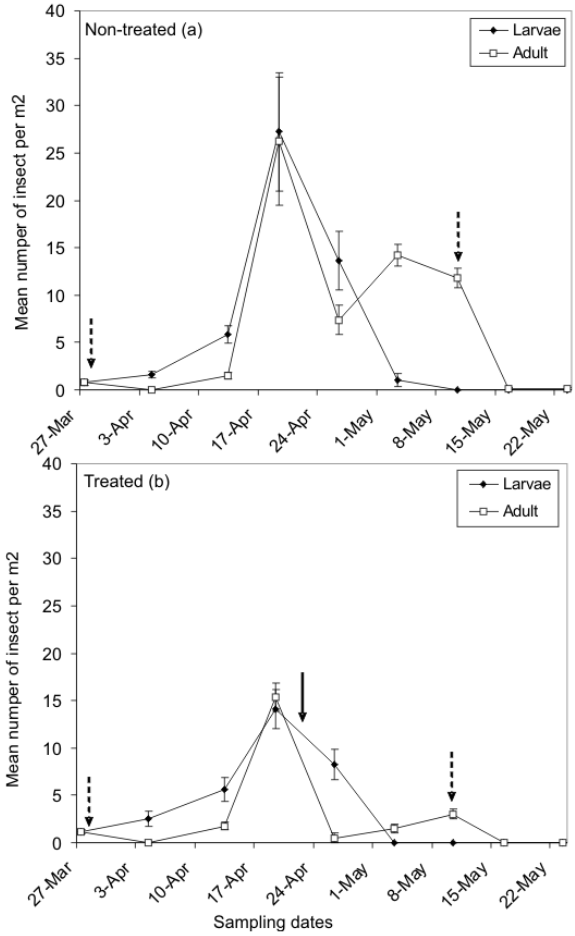


Figure 3. Mean number of *Gonioctena fornicata* per $m^2$ on the untreated (a) and treated (b) plots in 2018 in a lucerne field in Balcalı, Adana Province, Turkey. The straight dark arrow indicates the date of application against *G. fornicata*, (18 April 2018), the dashed arrow lucerne indicates mowing dates of lucerne (27 March 2018 and 10 May 2018).

Table 4. Mean number of larvae and adult of *Gonioctena fornicata* per $m^2$ in treated and untreated lucerne plots in Balcalı, Adana Province, Turkey during 2018

| Treatment | 27 March | 3 April | 10 April | 17 April | 24 April | 1 May | 8 May | 15 May | 22 May |
|---|---|---|---|---|---|---|---|---|---|
| | | | | | Adult | | | | |
| Untreated | 1.8±0.25 | 0.0±0.00 | 1.5±0.32 | 26.3±6.72 | 7.4±1.54* | 14.3±1.16* | 11.8±0.30* | 0.1±0.12 | 0.1±0.12 |
| Treated | 1.1±0.22 | 0.0±0.00 | 1.8±0.41 | 15.4±1.49 | 0.5±0.37 | 1.5±0.50 | 3.0±0.53 | 0.0±0.00 | 0.0±0.00 |
| | | | | | Larvae | | | | |
| Untreated | 0.8±0.25 | 1.6±0.37 | 5.9±0.93 | 27.3±6.24 | 13.6±3.1 | 1.0±0.68 | 0.0±0.00 | 0.0±0.00 | 0.0±0.00 |
| Treated | 0.1±0.22 | 2.5±0.80 | 5.6±1.23 | 14.1±2.09 | 8.3±1.58 | 0.0±0.00 | 0.0±0.00 | 0.0±0.00 | 0.0±0.00 |

* Means±SEM within columns followed by an asterisk are statistically different according to the t-test ($P < 0.05$).

Population changes of larvae in untreated and treated plots in 2018 are given in Figure 3 and Table 4. With the effect of the first mowing on 27 March, the larval density remained low until 10 April. Larvae population reached the highest density on 17 April (27.3±6.2 larvae/$m^2$) in untreated plots. After this date, the larval density declined. After 8 May, larvae could not be found in the untreated plots. Similar to untreated plots, the larval density was highest on 17 April (14.1±2.1 larvae/$m^2$), and no larvae were found in the treated plots after 1 May. Although the larval density was about half of the untreated plots on 17 April, no

significant difference was found (Table 4). The difference between larval densities in the treated and untreated plots was determined on 24 April, and insecticide application significantly reduced the larval density ($F_{1,14}$ = 4.88, t = 2.21, P = 0.044, Table 4). No significant relationship was found between plant sampling, pest larvae and adult population densities and meteorological factors (P > 0.05).

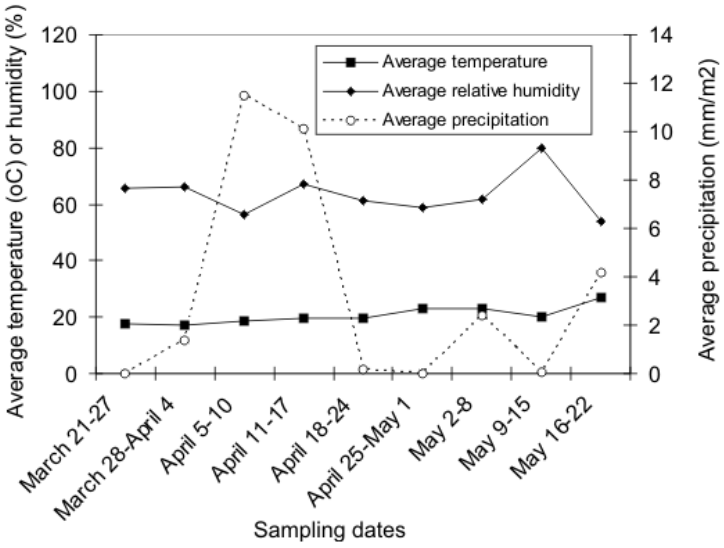


Figure 4. Meteorological data for Adana Province, Turkey in 2018.

Although the mean population density across all the sampling dates was 19.9±1.8 individuals/$m^2$ in the untreated plots in 2017 with plant sampling, 14.62±1.52 individuals/$m^2$ were found in the treated plots and the difference was found to be statistically significant ($F_{1,14}$ = 12.3, t = 3.50, P = 0.004) (Table 5).

Average population density across all the sampling dates in untreated plot was 98.8±11.1 individuals/$m^2$ in 2018 with plant sampling, whereas 50.6±4.0 individuals/$m^2$ were found in treated plot, the difference was statistically significant ($F_{1,14}$ = 16.7, t = 4.08, P = 0.001, Table 5).

Table 5. Total mean numbers of *Gonioctena fornicata* in treated and untreated plots of lucerne in Balcalı, Adana Province, Turkey during 2017 and 2018

| Year | Treatment | Mean insects/$m^2$ |
|---|---|---|
| 2017 | Untreated | 19.9±1.79* |
| | Treated | 14.6±1.52 |
| 2018 | Untreated | 98.8±11.08* |
| | Treated | 50.6±4.02 |

* Means±SEM within columns followed by an asterisk are statistically different according to the t-test (P < 0.05).

**Yield loss due to *Gonioctena fornicata* feeding**

Different factors affected yield and yield components. Among these, the effect of other insects should be considered. However, in the period when this study was conducted (March-May), lucerne leaf beetle was main pest. The fresh and dry weight of the treated and untreated plots in the lucerne field in Balcalı are given in Table 6. Although the fresh weight in the untreated plot was 117±5.6 kg/100 $m^2$ in 2017, it was 138±1.7 kg/100 $m^2$ in the treated plot. The difference was found to be statistically significant ($F_{1,6}$ = 12.0, t = -3.47, P = 0.013). Although the fresh weight was 105±1.8 kg/100 $m^2$ in the untreated plot in 2018, it was 142±5.3 kg/100 $m^2$ in the treated plot. The difference was statistically significant ($F_{1,6}$ = 45.5, t = -6.75, P = 0.001).

Table 6. Mean fresh and dry weights (kg) of lucerne in treated and untreated plots of lucerne in Balcalı, Adana Province, Turkey during 2017 and 2018

| Year | Treatment | Fresh weight | Dry weight |
|---|---|---|---|
| 2017 | Untreated | 117±5.6 | 103±4.8 |
| | Treated | 138±1.7* | 121±1.8* |
| 2018 | Untreated | 105±1.8 | 92±0.4 |
| | Treated | 142±5.3* | 127±5.8* |

* Means±SEM within columns followed by an asterisk are statistically different according to the t-test ($P < 0.05$).

The dry weight in the untreated plot was 103±4.8 kg/100 $m^2$ in 2017, whereas the dry weight in treated plot was 121±1.8 kg/100 $m^2$ (Table 6). The difference was statistically significant ($F_{1,6} = 11.7$, $t = -3.42$, $P = 0.014$). The dry weight in the untreated plot was 92±0.4 kg/100 $m^2$ in 2018, whereas it was 127±5.8 kg/100 $m^2$ in the treated plot (Table 6). The difference was statistically significant ($F_{1,6} = 41.6$, $t = -6.45$, $P = 0.001$).

In 2017, the fresh weight decreased by 14.7% and dry weight by 14.6%. In 2018, fresh weight decreased by 26.3% and dry weight decreased by 27.4%.

For the economic evaluation of the damage of lucerne leaf beetle, the treated and untreated plots were compared and the costs of different applications (insecticide, equipment and workmanship) are shown in Table 7. The dry weight difference was used to find the loss of yield between the two plots. Using the calculation, loss of yield times market price of lucerne minus spraying expense, the financial loss in the untreated plot was determined (Table 7).

Table 7. Economic analysis due to damage of *Gonioctena fornicata* in Turkish Lira (TRY)

| Issues of economic analyses | Cost, price or yield loss |
|---|---|
| Spraying cost (insecticide, equipment and workmanship) | 96.5 TRY/ha |
| Market price of dry lucerne | 1.25 TRY/kg |
| Average yield loss between treated and untreated plots | 3480 kg/ha |
| Economic loss in the untreated plot | 4250 TRY/ha |

**Loss of crude protein, acid detergent fiber and neutral detergent fiber in harvested lucerne**

No statistically significant difference was found between ADF and NDF ($P > 0.05$, Table 8). Although crude protein was 25.6±0.91% in the treated plot, it was 22.70±0.81% in the non-treated plot. The crude protein value was slightly higher in the treated plots, and the difference between the treated and untreated plots was statistically significant ($F_{1,6} = 8.75$, $t = 2.96$, $P = 0.025$).

Table 8. Mean (±SEM) crude protein, ADF and NDF values of lucerne in Balcalı, Adana Province in 2018

| Treatment | Crude protein (%) | ADF (acid detergent fiber) (%) | NDF (neutral detergent fiber) (%) |
|---|---|---|---|
| Untreated | 22.0±0.81 | 24.2±1.35 | 38.1±1.81 |
| Treated | 25.6±0.91* | 29.0±1.66 | 42.8±2.09 |

* Means±SEM within columns followed by an asterisk are statistically different according to the t-test ($P < 0.05$).

## Discussion

Although the first adults were seen in the lucerne plots in early April in 2017, the larvae appeared in late April. In 2018, the pest infestation started earlier (March). The first adults and eggs were detected in mid-March before the plots were established in 2018. The differences in the appearance of the first adults and larvae by sampling years may be related to plant phenology. Given that of lucerne field was planted in 2017,

this may be related to the fact that lucerne establishment takes a long time and plant growth is not suitable for larvae and adults to feed. The first sampling in 2017 could only be made in the second week of April, since the plant growth was quite short. The higher density of pests in 2018 may attributable to meteorological factors (relatively high temperature) being more favorable for pest population development in 2017. Anay (2000) found the first adults in Balcalı (Adana Province) in late February and their larvae in mid-March. In our study, the first adults and larvae appeared in late-March, especially in 2018; this may be related to sampling starting later. However, Çoşkuncu & Gençer (2006) found the first adults in late-March to early-April under natural conditions. Kovancı (1982) reported that the first appearance of the overwintering adults was in late-March to early-April according to climatic conditions. In Plovdiv (Bulgaria), *G. fornicata* first adults appeared in April and their larvae later in May (Atanasova & Semerdjieva, 2009). These differences may be related to the fact that Adana, located in the Mediterranean climate zone, has a warmer and humid climate.

The larvae were actively feeding on leaves, shoots and flower organs of lucerne plants for 5 weeks in 2017 and for 6 weeks in 2018. It was observed that the larvae and adults of the pest were feeding in the cool time of the day, and they were withdrawing to the soil during the hot time of the day. Brovdii (1977) reported that larvae fed in the clover field in Ukraine for 19-27 d, the pupa period lasted 5-9 d, and young adults (new generation adults) appeared in the period between late-June and mid-July. The differences between these findings and the current study may be related to ecological factors.

Although the peak number of adults in the lucerne plots appeared 2 weeks after the first detection, the larvae reached the highest population density 1 week after their first detection (25 April). In 2018, larvae and adults were recorded at the highest densities in mid-April, about 3 weeks later after they were first detected. This may be related to plant phenology, as mentioned earlier. However, in general, it can be emphasized that adults and larvae reached their highest population density in mid- to late-April. After April, larval and adult densities declined. New generation adults were recorded in the first week of May. Adults and larvae were not found until after mid-May. Çoşkuncu & Gençer (2006) reported that populations of *G. fornicata* were the highest in late-May to early-June with a sweep net sampling performed lucerne field in Bursa Province, Turkey. The later emergence of adults and larvae in that study may be related to the colder conditions in Bursa than in Adana. Brovdii (1977) reported that *G. fornicata* is an important pest in Ukraine, overwintering as an adult 20 cm deep in the soil, and adults are seen in the Transcarpathian Region in mid-April to early-May. Lustun & Panu (1968) found that adults of *G. fornicata* overwinter 10-15 cm depth of the soil, and Kovancı (1982) found that *G. fornicata* adults overwintered in the soil at a depth of 1-20 cm in Ankara. Yıldırım et al. (1996) reported that *G. fornicata* overwintered 10-25 cm deep in a field of lucerne in Erzurum Province, and when the lucerne reached 10 cm in early April, they left the overwintering area and moved to the lucerne. It was found that this harmful pest species had one generation a year. Coşkuncu & Gençer (2006) also reported that *G. fornicata* had one generation per year under conditions of Bursa Province. Kovancı (1982) reported that *G. fornicata* had obligatory diapause and was a species that has only one generation per year, Keresi & Sekulic (2005) reported that *G. fornicata* overwintered as adult and had one generation per year. Bronskikh (1987) reported that *G. fornicata* has one generation per year, and for this pest in Kishinev (Russia), Moldavia and Ukraine, larvae and adult damage are important, primarily feeding on the tips of leaves, buds and young shoots.

Insecticide application decreased the pest population by 26.4% in 2017 and 48.7% in 2018. In 2017, insecticide deltamethrin 20 g/l was not sufficiently effective, and in 2018 a relatively higher effect was observed with chlorpyrifos ethyl 480 g/l, and it reduced the harmful population by only about 50%. Bronskikh (1987) reported that *G. fornicata* is an important pest in the lucerne fields in Kishinev, and endosulfan (2.5 kg/ha) was highly effective, reducing pest populations by 95, 98 and 85% after 4, 7 and 13 d from application, respectively. Atanasova & Andreev (2012) found that pyrethrum FSEC (pyrethrin, sesame oil and soft potassium soap), Neem Azal T/S (azadirachtin) were effective against *G. fornicata* adults and larvae. They also reported that the preparation of *Bacillus thuringiensis* subsp. *kurstaki* de Barjac &Lemille, 1970 gave highly positive results against adults and larvae of the pest.

*Gonioctena fornicata* negatively impact fresh and dry weight yields. It is estimated that the actual yield loss (greater than the measured 3.40 t/ha) will be higher because the insecticides used were not sufficiently effective. Although the yield difference between the treated and non-treated plots was lower than expected, the cost of the loss was still high (Table 7). The financial loss in the untreated plots was about 4,250 TRY/ha (about 545 USD/ha as of December 2020). Grigorov (1976) reported that fresh weight decreased by over 60% and seed yield decreased by around 100% as a result of the feeding of adult and larvae of *G. fornicata* in Central and Southwest Europe. Naidenova & Donschev (1995) reported that *Hypera postica* (Gyllenhal, 1813) and *G. fornicata* were important pests in lucerne in Pleven (Bulgaria), and that the dry weight loss varied between 48 and 67%, and the yield of the shoots decreased by 32-45%.

Lucerne leaf beetle did not affect ADF and NDF values. In contrast, the crude protein value was relatively lower in the insecticide-free plots (Table 8). In other words, this pest significantly affected crude protein. Naidenova & Donschev (1995) found that loss of crude protein due to leaf beetles feeding ranged from 49-70%.

This study reveals that the *G. fornicata* is the main pest and the needs insecticides application to minimize economic damage in large production areas. There are no registered insecticides to control *G. fornicata* in Turkey. The mowing done in March 2018 delayed larval population development of this pest by about 2 weeks, and the population density of larvae remained low. It is recommended as a precautionary measure to make the spring mowing earlier (e.g., early- to mid-March) to reduce the population of this pest. The economic threshold for the *G. fornicata* is unknown in lucerne in Turkey. Therefore, further study is needed to determine its economic threshold in lucerne in different ecological regions of Turkey. In Tokat Province, Turkey, the two-parasitoid species of *G. fornicata* in lucerne fields were identified as *Macquartia tenebricosa* (Meigen, 1824) and *Meigenia mutabilis* (Fallen, 1810) (Diptera: Tachinidae) (Atay, 2018). According to that study, larval parasitization rates varied between 0.5% and 3.7%. *Meigenia mutabilis* was found to be the more potent parasitoid of *G. fornicata*. In this present study, although the larvae were cultured, no parasitoid species could be detected in the larvae. More detailed studies are needed on this issue.

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*Original article (Orijinal araştırma)*

# Distribution and population density of plant parasitic nematodes on cereal production areas of Isparta and Burdur Provinces of Turkey[1]

## Türkiye Isparta ve Burdur illeri tahıl üretim alanlarında bitki paraziti nematodların dağılımı ve popülasyon yoğunluğu

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## Abstract

Plant parasitic nematodes were systematically surveyed in cereal production areas of Isparta and Burdur Provinces of Turkey in 2016-2017. Nine plant parasitic nematode genera were identified in Isparta and Burdur [*Ditylenchus* (23%)*, Geocenamus* (20%), *Helicotylenchus* (33%)*, Heterodera* (<1%), *Meloidogyne* (3%)*, Pratylenchus* (76%)*, Pratylenchoides* (52%), *Paratylenchus* (41%) and *Tylenchus* (18%)]. *Pratylenchus* spp. was found in 82% and 68% of samples, and *Pratylenchoides* spp. in 55% and 63% samples in Isparta and Burdur Province, respectively. The densities of *Pratylenchus* and *Pratylenchoides* species were higher in Isparta than in Burdur, and were often over the threshold for economic damage. As a result of morphological diagnostic studies, *Pratylenchus crenatus* Loof, 1960, *Pratylenchus neglectus* (Rensch, 1924) Filipjev & Schuurmans-Stekhoven, 1941, *Pratylenchus penetrans* (Cobb, 1917) Filipjev & Schuurmans-Stekhoven, 1941*, Pratylenchus thornei* Sher & Allen, 1953 (Tylenchida: Pratylenchidae)*, Pratylenchoides alkani* Yüksel, 1977, *Pratylenchoides crenicauda* Winslow, 1958, *Pratylenchoides erzurumensis* Yüksel, 1977, *Pratylenchoides leiocauda* Sher, 1970, *Pratylenchoides ritteri* Sher, 1970 and *Pratylenchoides variabilis* Sher, 1970 (Tylenchida: Merliniidae) were identified. *Pratylenchoides* alkani, *P. erzurumensis*, *P. neglectus* and *P. thornei* were the most common species in wheat and barley fields in Burdur and Isparta Provinces.

**Keywords:** Cereal, population density, *Pratylenchoides* spp., root lesion nematode, survey

## Öz

Türkiye'nin Isparta ve Burdur illerinde 2016 ve 2017 yıllarında tahıl üretim alanlarında bitki paraziti nematodların sürveyi sistematik olarak gerçekleştirilmiştir. Burdur ve Isparta illerinde dokuz bitki paraziti nematod cinsi [*Ditylenchus* (%23)*, Geocenamus* (%20), *Helicotylenchus* (%33)*, Heterodera* (<%1), *Meloidogyne* (%3)*, Pratylenchus* (%75,5)*, Pratylenchoides* (52,1%), *Paratylenchus* (41%) and *Tylenchus* (18%)] tespit edilmiştir. *Pratylenchus* spp. Isparta ve Burdur illerinde sırasıyla %82 ve %68 olarak tespit edilirken, *Pratylenchoides* spp. Isparta'da %55 ve Burdur'da %63 olarak bulunmuştur. *Pratylenchus* ve *Pratylenchoides* türleri Isparta İli'nde Burdur İli'nden daha yüksek belirlenmiş ve çoğu tahıl alanında ekonomik zarar seviyesinin üzerinde tespit edilmiştir. Morfolojik teşhis çalışmaları sonucunda, *Pratylenchus crenatus* Loof, 1960, *Pratylenchus neglectus* (Rensch, 1924) Filipjev & Schuurmans-Stekhoven, 1941, *Pratylenchus penetrans* (Cobb, 1917) Filipjev & Schuurmans-Stekhoven, 1941*, Pratylenchus thornei* Sher & Allen, 1953 (Tylenchida: Pratylenchidae)*, Pratylenchoides alkani* Yüksel, 1977, *Pratylenchoides crenicauda* Winslow, 1958, *Pratylenchoides erzurumensis* Yüksel, 1977, *Pratylenchoides leiocauda* Sher, 1970, *Pratylenchoides ritteri* Sher, 1970 ve *Pratylenchoides variabilis* Sher, 1970 (Tylenchida: Merliniidae) tespit edilmiştir. *Pratylenchoides* alkani, *P. erzurumensis*, *P. neglectus* ve *P. thornei* Burdur ve Isparta illerinde buğday ve arpa alanlarında en yaygın türler olarak saptanmıştır.

**Anahtar sözcükler:** Tahıl, popülasyon yoğunluğu, *Pratylenchoides* spp., kök lezyon nematodu, sürvey

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## Introduction

Turkey has approximately 11 Mha under cereal production and the major cereal, wheat, yielding about 20 Mt production annually, followed by barley, maize and rice. Durum wheat production has been reported 73 and 48 Mt in Burdur and Isparta Provinces, respectively (TÜİK, 2020). In Burdur and Isparta Provinces, wheat production excluding durum wheat, was 42 and 32 Mt, respectively (TÜİK, 2020). Additionally, barley production is higher in Burdur Province (112 kt) than Isparta (86 kt) (TÜİK, 2020). Wheat and barley are commonly grown in all agricultural areas of Burdur Province. However, Yalvaç and Şarkikaraağaç Districts of Isparta Province are the prominent wheat and barley production areas.

*Heterodera avenae* Wollenweber, 1924, *Heterodera filipjevi* (Madzhidov, 1981) Stelter, 1984, *Heterodera latipons* Franklin, 1969 (Tylenchida: Heteroderidae), *Pratylenchus thornei* Sher & Allen, 1953 and *Pratylenchus neglectus* (Rensch, 1924) Filipjev & Schuurmans-Stekhoven, 1941 (Tylenchida: Pratylenchidae) are reported as important parasitic nematodes of cereals worldwide (Nicol et al., 2003; Smiley & Nicol, 2009). These nematodes have also been reported on cereal fields in Turkey (Mısırlıoğlu & Pehlivan, 2007; Yavuzaslanoglu et al., 2012, 2020; Dababat et al., 2015; Toktay et al., 2020). Particularly, root lesion nematodes were surveyed and identified on wheat cultivation by researchers in different regions of Turkey (Yıldız, 2007; Yavuzaslanoğlu et al., 2012, 2020; Kasapoğlu et al., 2014; Kasapoğlu Uludamar et al., 2018). İmren et al. (2015) and Yavuzaslanoğlu et al. (2012, 2020), reported that *P. thornei* and *P. neglectus* were found in different densities and mixed population in wheat fields in Turkey. Another migratory endo-ecto parasitic nematode genera, *Pratylenchoides* spp., have been reported in wheat and other plants in Turkey (Yüksel, 1977; Elekcioglu, 1992, 1996; Evlice & Ökten, 2008; İmren & Elekçioğlu, 2008; Yavuzaslanoğlu et al., 2012; Söğüt et al., 2014). Yavuzaslanoğlu et al. (2012), determined 36% prevalence of *Pratylenchoides* spp. in wheat in the Central Anatolia Region in Turkey, and their population density was high in all provinces in this region.

*Pratylenchus* and *Pratylenchoides* spp. have a migratory endoparasitic feeding behavior (Yeates et al., 1993) and cause brown lesions on the plant roots and loss of root function, and consequently, reduce in plant vigor and yield (Townshend et al., 1989; Agrios, 1997; Jones & Fosu-Nyarko, 2014). Also, root lesion nematodes assist the invasion of soilborne pathogens into plant root tissue and, this interaction increases the importance for such infections (Smiley & Nicol, 2009). It was estimated that *P. thornei* causes up to 62% wheat yield loss in the northern grain region of Australia (Owen et al., 2014). Nicol & Ortiz-Monastterio (2004) reported that cereal cyst and root lesion nematodes yield losses in wheat were 50% on the Central Anatolian Plateau of Turkey.

Research on plant parasitic nematodes in cereal culture of the Lakes Region including Isparta and Burdur Provinces were limited. However, cereal nematodes were surveyed at a limited number of locations in the previous studies (Yavuzaslanoğlu et al., 2012, 2020; Söğüt et al., 2014; Toktay et al., 2020). The objectives of this study are to investigate the distribution and density of plant parasitic nematodes in detail and to determine if they occur as mixed populations in cereal fields in Isparta and Burdur Provinces in Lakes Region of Turkey.

## Materials and Methods

### Nematode sampling locations

Cereal production fields in Isparta and Burdur Provinces were surveyed in the study. A total of 441 soil and root samples were collected systematically during June-August in the years of 2016 and 2017 from wheat, barley, oat and rye crops. Two hundred and thirty-five samples (124 wheat, 95 barley, 13 oat and 3 rye) were taken from Isparta and 206 samples (111 wheat, 71 barley, 19 oat and 5 rye) from Burdur Province. Samples were collected from the fields adjacent to the roadside with intervals of about 2-5 km (Figure 1). A 5-kg bulk sample consisting of 10-15 subsamples were taken from each field by shovel to 30 cm deep in

a zigzag pattern. The elevation, latitude and longitude for each sampling site were recorded by using the global positioning system.

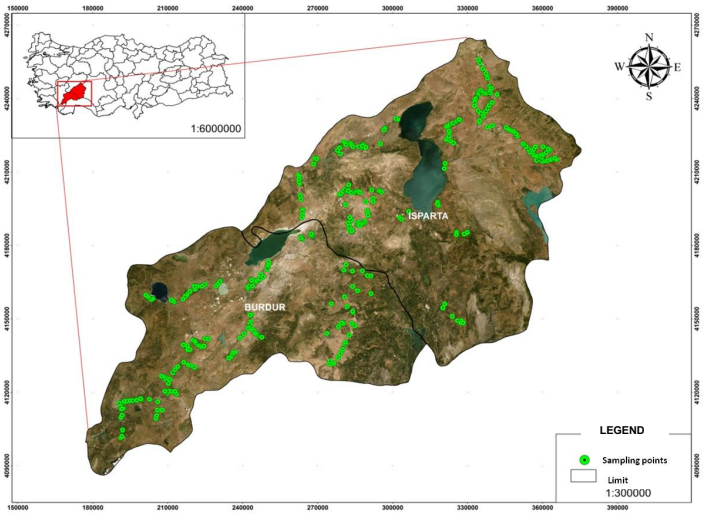


Figure 1. Sampling points in districts of Isparta and Burdur Provinces of Turkey.

**Nematode extraction, population estimation and species identification**

Migratory nematodes were extracted from 100 g dry soil and 10 g fresh root from each sample using a modified Baermann funnel technique (Whitehead & Hemming, 1965; Hooper, 1986a). Soil moisture content was measured by drying 10 g of soil from each sample in oven at 90°C for 2 d. Roots removed from sample placed in a separate dish and soil adhering to the roots gently removed. Each root sample was examined under a stereomicroscope for evidence of root galls (*Meloidogyne* spp.) or cyst nematodes (*Heterodera* spp.). The roots were then finely chopped with a scissors in a dish. All chopped roots were mixed thoroughly and 10 g placed on a labeled sieve and water added. After an extraction period of 48 h, the sieve was removed and roots tissue discarded. The same process was repeated for soil samples. The resultant nematode suspensions were placed in measuring cylinders for 8 h to settle, the supernatant discarded, and the concentrated nematodes transferred to 15-ml tubes. Nematodes were counted to genera under the light microscope at 100x magnification. The nematode counts from the soil and root samples were converted to the number of nematodes 100 g of dry soil and 10 g fresh root.

*Heterodera* cysts were extracted by using the modified Fenwick can method from 250 g dry soil under constant water flow (Fenwick, 1940; Stirling et al., 1999). The numbers of cyst, with or without eggs, were counted under a dissecting microscope at 20x magnification. Permanent slides were prepared according to published procedures (Hooper, 1986b). Nematode species were morphologically identified using morphology and morphometric characters according to Baldwin et al. (1983) and Castillo & Vovlas (2007) under the light microscope. Identification of the specimens was performed by the senior author.

**Visualization of migratory endoparasitic nematodes**

Inverse distance weighting method with ArcGIS 10.2 software was used for mapping distribution and population densities of *Pratylenchus* and *Pratylenchoides* spp. in 23 districts of Isparta and Burdur Provinces.

Population densities (number/100 g of dry soil and 10 g fresh root) of *Pratylenchus* and *Pratylenchoides* spp. were analyzed the SPSS (version 20.0) program. The Kruskal-Wallis test was used because the number of samples taken on district was not homogeneous and the data obtained were non-parametric. For statistical lettering, Tamhane's T2 multiple comparison test was applied in ANOVA analysis.

## Results

### Incidence of plant parasitic nematodes

Nine plant parasitic nematode genera were recorded, *Ditylenchus* (23% of samples)*, Geocenamus* (20%), *Helicotylenchus* (33%)*, Heterodera* (<1%), *Meloidogyne* (3%)*, Pratylenchus* (76%)*, Pratylenchoides* (52%), *Paratylenchus* (41%) and *Tylenchus* (18%). *Pratylenchus* and *Pratylenchoides* spp. were found in the study as important plant parasitic nematodes in cereal fields of Isparta and Burdur Provinces. *Pratylenchus* spp. was found to be 82% and 68% of samples, and *Pratylenchoides* spp. in 55% and 63% of samples from Isparta and Burdur Provinces, respectively.

In a few soil samples, second stage juveniles (J2s) of cyst and root-knot nematodes were found and these samples were examined under a stereomicroscope, however, no galls, egg masses and cysts were found in the roots. Cyst nematode larvae were found in one wheat soil sample from Isparta Central District (20 J2s/100 g soil) and two barley soil samples (20-40 J2s/100 g soil) from Keçiborlu District. In addition, J2s of *Meloidogyne* spp. were found in seven wheat root samples (4 in Burdur Province and 3 in Isparta Province), two oat (Burdur Province) and one barley (Burdur Province) by the modified Baermann funnel technique. The soil density of these samples varied between 20 and 240 J2s/100 g soil.

### *Pratylenchus* species by morphological identification

*Pratylenchus crenatus* Loof, 1960, *P. neglectus*, *Pratylenchus penetrans* (Cobb, 1917) Filipjev & Schuurmans-Stekhoven, 1941 and *P. thornei* (Tylenchida: Pratylenchidae), root lesion nematodes species, were identified morphologically in samples from cereal fields of Burdur and Isparta Provinces. *Pratylenchus thornei* and *P. neglectus* were found 35% and 35% of all samples from Burdur Province, followed by *P. penetrans* at 10% and *P. crenatus* at 8%. Root lesion nematode species were found in mixed populations in 11% of samples from Burdur Province, with 4% being mixtures of *P. penetrans* and *P. thornei*. *Pratylenchus thornei* was the most common species in the cereal fields in Isparta Province being found in 63% of samples and the least common species was *P. crenatus* at 3%. *Pratylenchus neglectus* and *P. penetrans* were found in 13% and 12% samples from Isparta Province. Mixed populations of *Pratylenchus* spp. occurred at 9% in Isparta, which was less than in Burdur.

### *Pratylenchoides* species by morphological identification

*Pratylenchoides alkani* Yüksel 1977, *Pratylenchoides crenicauda* Winslow, 1958, *Pratylenchoides erzurumensis* Yüksel, 1977, *Pratylenchoides leiocauda* Sher, 1970, *Pratylenchoides ritteri* Sher, 1970 and *Pratylenchoides variabilis* Sher, 1970 (Tylenchida: Merliniidae) were identified from the samples from both Burdur and Isparta Provinces. The most common species were *P. alkani* (40% of samples) and *P. erzurumensis* (31%) in Burdur Province, followed by *P. variabilis* at 18% and *P. crenicauda* at 5.4%. *Pratylenchoides alkani* was the most common species in Isparta Province at 45% and the least common species was *P. leiocauda* at 6%. *Pratylenchoides erzurumensis, P. variabilis* and *P. ritteri* were found at 19%, 12% and 14%, respectively in Isparta Province. Mixed *Pratylenchoides* populations were found 6% and 5% of samples from in Burdur and Isparta Provinces, respectively.

### Incidence of *Pratylenchus* and *Pratylenchoides* species in cereal species in Isparta and Burdur Provinces

The incidence of *Pratylenchus* and *Pratylenchoides* spp. in districts of Burdur and Isparta Provinces are given in Tables 1 and 2. *Pratylenchus neglectus* and *P. thornei* was more common in wheat in both Burdur and Isparta Provinces than other root lesion nematode species (Table 1; 2). *Pratylenchus thornei* was more common in barley in the two provinces. *Pratylenchus penetrans* was found at low frequency in seven districts (Bucak, Burdur Central, Ağlasun, Tefenni, Karamanlı, Yeşilova and Kemer) of Burdur Province and nine districts (Gönen, Atabey, Isparta Central, Gelendost, Uluborlu, Senirkent, Şarkikaraağaç, Yalvaç and Eğirdir) of Isparta Province (Table 1;2). *Pratylenchus crenatus* was found in Gölhisar, Central, Tefenni,

Yeşilova and Kemer Districts of Burdur Province (Table 1) and Yalvaç, Şarkikaraağaç, Gelendost, Gönen and Central Districts of Isparta Province (Table 2). *Pratylenchus thornei* was more common in oat in two Provinces (Tables 1 & 2).

Table 1. Incidence (%) of *Pratylenchus* and *Pratylenchoides* species in cereal fields in districts of Burdur Province

| District | Cereal | Samples No | *Pratylenchus* | | | | *Pratylenchoides* | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | Pc | Pn | Pp | Pt | Pa | Prc | Pe | Pl | Pr | Pv |
| Ağlasun | Wheat | 7 | - | 71 | 14 | - | 40 | 20 | - | - | - | 40 |
| | Barley | 2 | - | - | 50 | 50 | - | - | 100 | - | - | - |
| | Rye | 2 | - | - | - | 100 | - | - | 100 | - | - | - |
| Bucak | Wheat | 16 | - | 50 | 6 | 12 | 67 | - | - | - | - | 33 |
| | Barley | 4 | - | 50 | 25 | 25 | 50 | - | - | - | - | - |
| | Oat | 4 | - | - | - | 100 | - | - | 75 | - | - | - |
| Central (Burdur) | Wheat | 14 | 14 | 50 | 14 | 29 | - | 57 | - | - | - | 43 |
| | Barley | 13 | - | 7 | 33 | 60 | - | - | 77 | - | - | - |
| | Oat | 5 | 20 | 20 | - | 60 | 80 | - | - | - | - | - |
| Çavdır | Wheat | 4 | - | 100 | - | - | 100 | - | - | - | - | - |
| | Barley | 2 | - | - | - | 100 | 100 | - | - | - | - | - |
| | Oat | 1 | - | - | - | 100 | 100 | - | - | - | - | - |
| Çeltikçi | Wheat | 3 | - | - | - | 67 | 67 | - | - | - | - | - |
| | Barley | 4 | - | - | - | 100 | 100 | - | - | - | - | - |
| | Oat | 1 | - | - | - | - | - | - | - | - | - | - |
| Dirmil | Wheat | 5 | - | 80 | - | - | 60 | - | 20 | - | - | - |
| | Barley | 1 | - | - | - | 100 | - | - | 100 | - | - | - |
| | Oat | 1 | - | 100 | - | - | - | - | 100 | - | - | - |
| | Rye | 1 | - | 100 | - | - | 100 | - | - | - | - | - |
| Gölhisar | Wheat | 14 | - | 79 | - | 21 | 36 | - | 64 | - | - | - |
| | Barley | 5 | 20 | - | - | 80 | - | 50 | - | - | - | 50 |
| | Oat | 1 | - | - | - | - | - | - | - | - | - | - |
| Karamanlı | Wheat | 10 | - | 100 | - | - | 50 | - | - | - | - | - |
| | Barley | 7 | - | - | 38 | 63 | - | - | - | - | - | 57 |
| | Oat | 1 | - | - | - | 100 | 100 | - | - | - | - | - |
| Kemer | Wheat | 13 | 29 | 57 | 7 | 7 | - | - | 31 | - | - | - |
| | Barley | 11 | 45 | - | 18 | 27 | - | - | 46 | - | - | - |
| Tefenni | Wheat | 12 | 12 | 44 | 6 | 38 | - | - | 58 | - | - | - |
| | Barley | 11 | 14 | - | 14 | 71 | 57 | 14 | - | - | - | 29 |
| | Oat | 3 | 33 | - | - | 67 | - | - | - | - | - | 67 |
| | Rye | 2 | - | - | - | 100 | 50 | - | - | - | - | - |
| Yeşilova | Wheat | 14 | - | 71 | - | 29 | 43 | - | - | - | - | 57 |
| | Barley | 11 | 15 | 8 | 23 | 54 | 82 | - | - | - | - | - |
| | Oat | 2 | - | - | 50 | 50 | - | - | 100 | - | - | - |

Pc, *Pratylenchus crenatus*; Pn, *Pratylenchus neglectus*; Pp, *Pratylenchus penetrans*; Pt, *Pratylenchus thornei*; Pa, *Pratylenchoides alkani*; Pcr, *Pratylenchoides crenicauda;* Pe, *Pratylenchoides erzurumensis;* Pl, *Pratylenchoides leiocauda;* Pr, *Pratylenchoides ritteri*; and Pv, *Pratylenchoides variabilis*.

*Pratylenchoides* alkani and *P. erzurumensis* were more common in wheat and barley in both Burdur and Isparta Provinces than other *Pratylenchoides* spp. (Tables 1 and 2). *Pratylenchoides leiocauda* and *P. ritteri* were not found in then samples from Burdur Province (Table 1). *Pratylenchoides leiocauda* was found in barley samples from Gelendost and Sütçüler Districts, and wheat and barley samples from Atabey Districts of Isparta Province (Table 2). *Pratylenchoides ritteri* was found wheat and barley samples from four districts (Central, Uluborlu, Yalvaç and Şarkikaraağaç) (Table 2). *Pratylenchoides crenicauda* was found in two wheat (Tefenni, Gölhisar) and two barley samples (Ağlasun and Central) in Burdur Province (Table 1) and in only one district (Yalvaç) in Isparta Province (Table 2). Rye samples only had *P. alkani,* oat samples had *P.* alkani and *P. erzurumensis* (Tables 1 & 2), but *P. variabilis* was only found in oat sample from Tefenni (Table 1).

Table 2. Incidence (%) of *Pratylenchus* and *Pratylenchoides* species in cereal fields in districts of Isparta Province

| District | Cereal | Samples No | *Pratylenchus* | | | | *Pratylenchoides* | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | Pc | Pn | Pp | Pt | Pa | Prc | Pe | Pl | Pr | Pv |
| Aksu | Wheat | 5 | - | - | - | 80 | 40 | - | - | - | - | - |
| | Barley | 1 | - | - | - | 100 | - | - | - | - | - | - |
| Atabey | Wheat | 1 | - | - | 100 | - | - | - | - | 100 | - | - |
| | Barley | 6 | - | 17 | - | 83 | - | - | - | 66 | - | - |
| | Oat | 1 | - | - | - | 100 | 100 | - | - | - | - | - |
| | Rye | 1 | - | - | - | 100 | 100 | - | - | - | - | - |
| Central (Isparta) | Wheat | 15 | - | 6 | 24 | 71 | - | - | - | - | 44 | 56 |
| | Barley | 8 | 12 | 12 | - | 75 | - | - | - | - | - | 62 |
| | Oat | 1 | - | - | - | - | 100 | - | - | - | - | - |
| Eğirdir | Wheat | 5 | - | - | 20 | 80 | - | - | 60 | - | - | 40 |
| | Barley | 1 | - | - | - | 100 | - | - | - | - | - | - |
| Gelendost | Wheat | 14 | 7 | 14 | 14 | 57 | 50 | - | - | - | - | - |
| | Barley | 5 | - | - | 40 | 60 | - | - | - | 40 | - | - |
| | Oat | 1 | - | - | - | 100 | 100 | - | - | - | - | - |
| Gönen | Wheat | 5 | - | - | - | 100 | 100 | - | - | - | - | - |
| | Barley | 9 | 11 | - | - | 89 | 88 | - | - | - | - | - |
| | Oat | 3 | - | - | 33 | 67 | 100 | - | - | - | - | - |
| Keçiborlu | Wheat | 6 | - | 33 | - | 67 | 100 | - | - | - | - | - |
| | Barley | 7 | - | 14 | - | 86 | 43 | - | - | - | - | - |
| Senirkent | Wheat | 9 | - | - | - | 22 | 40 | - | - | - | - | - |
| | Barley | 7 | - | - | 14 | 57 | 60 | - | - | - | - | - |
| Sütçüler | Wheat | 5 | - | 20 | - | 40 | 60 | - | - | - | - | - |
| | Barley | 2 | - | 50 | - | 50 | 50 | - | - | 50 | - | - |
| Şarkikaraağaç | Wheat | 29 | 3 | 17 | 14 | 48 | 50 | - | 19 | - | 23 | 8 |
| | Barley | 14 | - | 27 | 13 | 60 | 36 | - | 64 | - | - | - |
| | Rye | 2 | - | - | - | 50 | - | - | 50 | - | - | - |
| Uluborlu | Wheat | 7 | - | - | 14 | 57 | - | - | - | - | 42 | - |
| | Barley | 5 | - | - | 20 | 60 | - | - | - | - | 40 | - |
| Yalvaç | Wheat | 23 | 4 | 19 | 19 | 58 | - | 12 | 75 | - | 6 | 6 |
| | Barley | 30 | 12 | 24 | 9 | 55 | 56 | - | 33 | - | 11 | - |
| | Oat | 7 | - | - | 14 | 71 | - | - | 71 | - | - | - |

Pc, *Pratylenchus crenatus*; Pn, *Pratylenchus neglectus*; Pp, *Pratylenchus penetrans*; Pt, *Pratylenchus thornei*; Pa, *Pratylenchoides alkani*; Pcr, *Pratylenchoides crenicauda;* Pe, *Pratylenchoides erzurumensis;* Pl, *Pratylenchoides leiocauda;* Pr, *Pratylenchoides ritteri*; and Pv, *Pratylenchoides variabilis*.

**Population density of *Pratylenchus* and *Pratylenchoides* spp. in cereal fields in Burdur and Isparta Provinces**

The lowest *Pratylenchus* spp. root densities were found in Dirmil, Gölhisar, Çeltikçi, Ağlasun, Bucak and Kemer Districts and the highest was found in Çavdar and Central District of Burdur Province (Table 3). The differences between the *Pratylenchus* spp. soil densities were not statistically significant in districts of Burdur Province ($p \geq 0.05$). There was no statistically significant difference between districts of Burdur Provinces in terms of root and soil density of *Pratylenchoides* spp. (Table 3).

In Isparta Province, lower *Pratylenchus* spp. root densities were found in Sütçüler, Senirkent, Gelendost, Gönen and Keçiborlu Districts and higher densities in Şarkikaraağaç, Eğirdir, Yalvaç and Uluborlu Districts (Table 4). Lower *Pratylenchus* spp. soil densities were found in Eğirdir, Aksu and Senirkent Districts and higher densities in Şarkikaraağaç, Yalvaç, Isparta Central, Gelendost, Gönen, Atabey and Keçiborlu Districts. The lower *Pratylenchoides* spp. root densities were found in Uluborlu and Gelendost District but these were not found significantly different to Isparta Central and Senirkent Districts. The average of *Pratylenchoides* spp. soil densities was lower in Eğirdir, Aksu, Sütçüler, and Senirkent Districts than in Isparta central, Uluborlu, Gelendost, Gönen and Atabey Districts but these were not significantly different (Table 4).

Table 3. Soil and root density of *Pratylenchus* and *Pratylenchoides* species in cereal samples from districts of Burdur Province

| Districts | Samples No | *Pratylenchus* | | *Pratylenchoides* | |
|---|---|---|---|---|---|
| | | Mean rank | | | |
| | | Root density (10 g fresh root) | Soil density (100 g dry soil) | Root density (10 g fresh root) | Soil density (100 g dry soil) |
| Ağlasun | 11 | 76,4 b* | 72,1 a | 114,5 a | 138,5 a |
| Bucak | 24 | 55,9 b | 70,0 a | 118,5 a | 132,7 a |
| Central (Burdur) | 32 | 138,6 a | 106,7 a | 113,2 a | 94,7 a |
| Çavdır | 7 | 146,5 a | 127,5 a | 148,5 a | 98,7 a |
| Çeltikçi | 8 | 100,6 b | 90,0 a | 139,1 a | 145,2 a |
| Dirmil | 11 | 76,4 b | 72,1 a | 114,5 a | 138,5 a |
| Gölhisar | 19 | 97,9 b | 87,0 a | 110,6 a | 84,1 a |
| Karamanlı | 18 | 133,2 ab | 148,2 a | 92,1 a | 97,6 a |
| Kemer | 24 | 53,9 b | 81,7 a | 73,3 a | 84,5 a |
| Tefenni | 28 | 134,3 ab | 123,0 a | 96,5 a | 99,6 a |
| Yeşilova | 27 | 123,6 ab | 145,1 a | 95,5 a | 98,0 a |

* There is no significant difference between the means followed by the same letter with a column based on Tamhane's T2 multiple comparison test.

Table 4. Soil and root density of *Pratylenchus* and *Pratylenchoides* species cereal samples from districts of Isparta Province

| Districts | Samples No | *Pratylenchus* | | *Pratylenchoides* | |
|---|---|---|---|---|---|
| | | Mean rank | | | |
| | | Root density (10 g fresh root) | Soil density (100 g dry soil) | Root density (10 g fresh root) | Soil density (100 g dry soil) |
| Aksu | 6 | 118,1 ab* | 56,5 b | 108,0 ab | 62,5 b |
| Atabey | 9 | 115,3 ab | 125,5 a | 151,2 a | 109,4 ab |
| Central (Isparta) | 24 | 122,3 ab | 128,5 a | 99,5 ab | 112,9 ab |
| Eğirdir | 6 | 140,7 a | 56,5 b | 141,4 a | 92,0 b |
| Gelendost | 20 | 68,7 b | 136,3 a | 96,4 b | 118,1 ab |
| Gönen | 17 | 109,4 b | 155,8 a | 167,4 a | 118,2 ab |
| Keçiborlu | 13 | 101,5 b | 159,6 a | 121,6 a | 142,9 a |
| Senirkent | 16 | 85,4 b | 45,2 b | 103,4 ab | 85,8 b |
| Sütçüler | 7 | 76,2 b | 89,8 ab | 121,9 a | 91,7 b |
| Şarkikaraağaç | 43 | 140,3 a | 115,3 a | 139,1 a | 129,7 a |
| Uluborlu | 12 | 130,0 a | 103,2 ab | 68,4 b | 107,0 ab |
| Yalvaç | 60 | 128,0 a | 122,7 a | 105,6 a | 125,4 a |

* There is no significant difference between the means followed by the same letter with a column based on Tamhane's T2 multiple comparison test.

Root population densities of *Pratylenchus* spp. ranged between 1,000 and 2,000 nematodes/10 g fresh root in 19 samples and between 2,000 and 3,000 nematodes/10 g root in 12 samples in Burdur Province. *Pratylenchus* spp. had over 3,000 nematodes/10 g root in Central, Tefenni and Yeşilova Districts. *Pratylenchus* spp. had higher root densities in Isparta than Burdur Province. Root densities were over the 70,000 nematodes/10 g fresh root in two samples in Central District of Isparta. Also, 19 samples in Isparta had *Pratylenchus* spp. between 40,000 and 60,000 nematodes/ 10 g fresh roots. The root density of *Pratylenchus* spp. were ranged between 1,000 and 2,000 individuals/10 g fresh root in 29 samples, 2,000 and 3,000 nematodes/10 g fresh root in 24 samples, 4,000 and 5,000 nematodes/10 g fresh root in 17 samples, 6,000 and 7,000 nematodes/10 g fresh root in 12 samples and 8,000 and 9,000 nematodes/10 g fresh root at five samples from Isparta (Figure 2).

Soil densities of *Pratylenchus* spp. were generally between 0 and 100 nematodes/100 g dry soil in Burdur and Isparta Province. *Pratylenchus* spp. densities where higher in Tefenni District with 3,000 and Karamanlı District with 2,800 nematodes/100 g dry soil in Burdur Province. The densities were between 1,000 and 2,000 nematodes/100 g dry soil in nine samples in Tefenni, Karamanlı, Yeşilova and Kemer Districts in Burdur Province. *Pratylenchus* spp. had over 3,000 nematodes/100 dry g soil in three samples from Isparta Province. Four samples contained 2,480, 2,000, 1,000 and 1,100 nematodes in 100 g dry soil in Central, Keçiborlu, Yalvaç and Şarkikaraağaç Districts, respectively (Figure 3).

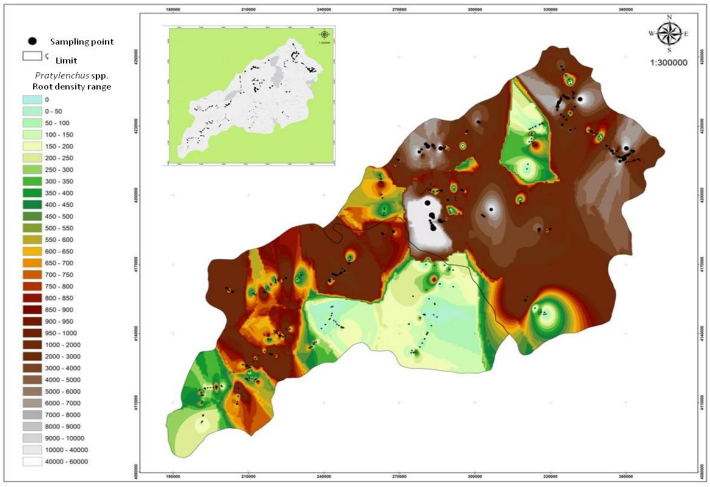


Figure 2. Root population density of *Pratylenchus* spp. at the sampling points in Burdur and Isparta Provinces.

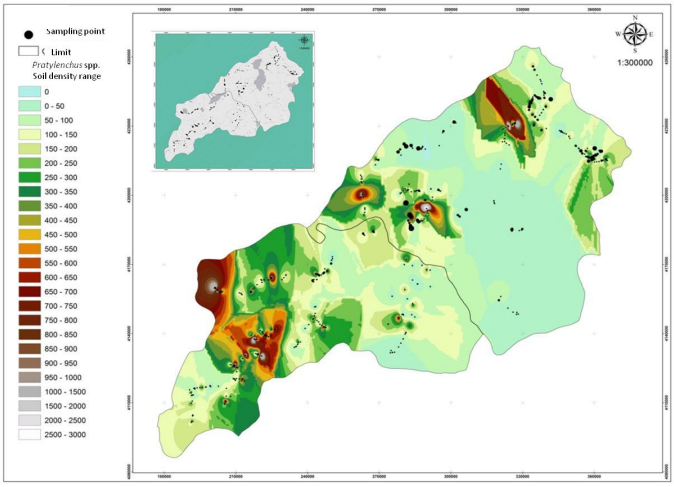


Figure 3. Soil population density of *Pratylenchus* spp. at the sampling points in Burdur and Isparta Provinces.

Root density of *Pratylenchoides* spp. ranged between 600 and 6,000 nematodes/10 g fresh root in Isparta Province. However, root density was over 10,000 nematodes/10 g fresh root in several locations. *Pratylenchoides* spp. had between 6,000 and 10,000 nematodes/10 g fresh root in one sample in Senirkent, two samples in Yalvaç and four samples in Şarkikaraağaç Districts in Isparta Province. Density of *Pratylenchoides* was lower in Burdur Province than Isparta. The density ranged between 1,000 and 2,000 nematodes/10 g fresh root at nine samples and between 2,000 and 3,000 nematodes/10 g fresh root densities at six samples in Burdur Province. The highest root densities were found in two samples with 3,580 and 3,060 nematodes/10 g fresh root in Bucak and Central Districts, respectively.

*Pratylenchoides* spp. had lower densities in soil than roots in all samples. The higher population densities of *Pratylenchoides* spp. in Isparta were in one sample from Gelendost, one sample from Central and two samples from Keçiborlu Districts with 1600, 1800, 2200 and 2860 nematodes/100 g dry soil, respectively. In Burdur, *Pratylenchoides* spp. had highest population density in soil in four samples in Karamanlı, Tefenni, Kemer and Bucak Districts with 2,580, 1,200, 1,200 and 1,060 nematodes/100 g dry soil, respectively (Figure 4).

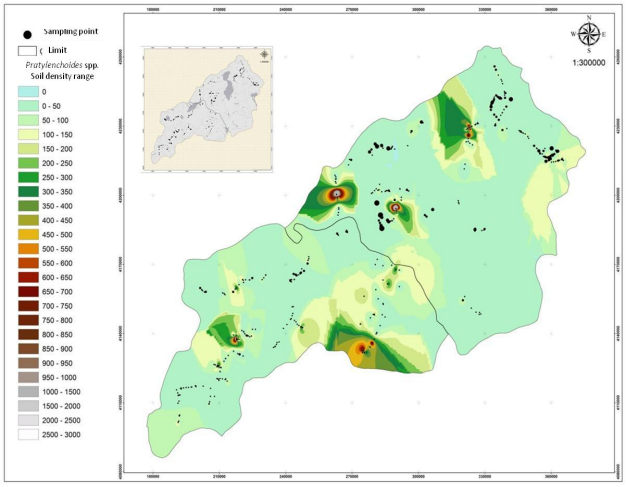


Figure 4. Soil population density of *Pratylenchoides* spp. at the sampling points in Burdur and Isparta Provinces.

## Discussion

In this study, nine genera of plant parasitic nematode species were found in cereal fields in Burdur and Isparta Provinces of Turkey. *Pratylenchus* and *Pratylenchoides* were found to be the two most common plant parasitic genera. *Pratylenchus thornei* and *P. neglectus* were common; however, *P. penetrans* and *P. crenatus* were also found in Isparta and Burdur. Some lesion nematode species were found in mixed populations. The other important finding was that *Pratylenchoides* spp. occurred at high density in some cereal sample roots and soils. In addition, *P. alkani* and *P. erzurumensis* were common in cereal fields in Isparta and Burdur Provinces. While *P. leiocauda* and *P. ritteri* were found in Isparta, they were not found in Burdur Province. *Pratylenchoides* spp. was found mixed populations with *Pratylenchus* spp. in both Provinces. *Pratylenchus thornei*, *Pratylenchus fallax* Seinhorst, 1968*, P. crenatus, P. neglectus* and *P. penetrans* have been reported in cereal fields in the Eastern Mediterranean region, Central Anatolia Region and Southeastern Anatolia of Turkey (Elekcioglu & Gözel, 1998; Yıldırım et al., 2007; Yavuzaslanoğlu et al., 2012; Öcal & Elekcioğlu, 2015). Kasapoğlu Uludamar et al. (2018) identified *P. neglectus, P. thornei* and *P. alkani* in soil from barley and wheat fields in Adıyaman. While *P. neglectus* has been reported to be widely distributed in Bolu Province (Dababat et al., 2019), *P. thornei* was more common in Konya and Karaman Provinces (Yavuzaslanoğlu et al., 2020). Yavuzaslanoğlu et al. (2012) identified *P. alkani*, *P. erzurumensis*, *P. variabilis*, *P. crenicauda* and *P. ritteri* from soil samples from Central Anatolian Plateau. As a result, *Pratylenchus* and *Pratylenchoides* spp. identified in the present study are potentially of economic importance for cereal production in Isparta and Burdur Provinces.

The results of this study showed that distribution, incidence and population density of *Pratylenchus* and *Pratylenchoides* spp. varied. In previous studies, researchers noted that there are several factors thought to contribute to this variation including cereal species, cultivar, soil type, pH, organic matter, fallow, planting times and tillage practices (Sundararaju & Jeyabaskaran, 2003; Castillo & Vovlas, 2007; Govaerts et al., 2008; Thompson et al., 2008; Collins et al., 2011). In our study, *Pratylenchus* and *Pratylenchoides* spp. had high densities in root and soil in both Burdur and Isparta Provinces. Also, *Pratylenchoides* spp. densities were higher than *Pratylenchus* spp. in some samples and these two migratory endoparasitic nematodes were found mixed population in many samples. *Pratylenchus* spp. densities were found at over 1,000 nematodes/100 g soil in 11 samples from Burdur and in seven samples from Isparta, which is over the economic threshold level proposed by Dickerson et al. (2000). Dickerson et al. (2000) calculated economic damage threshold level for control of root lesion nematodes at 250 nematodes/100 g of soil in wheat fields. Moreover, Van Gundy et al. (1974) reported that the threshold level of lesion nematode was

42 nematodes/100 g soil. Yavuzaslanoğlu et al. (2012) reported that *Pratylenchus* spp. densities of at most 274, 140, 119, 113, 69 and 52 nematodes/100 g soils, Konya, Niğde, Kırşehir, Sivas, Denizli and Eskişehir Provinces, respectively, and also found *Pratylenchoides* spp. at high density of soil from cereal fields of Central Anatolia where it ranged between 133 and 749 nematodes/100 g soil. In addition, Dababat et al. (2019) found for 12% of samples collected from five districts in Bolu Province that, on average, *P. neglectus* were at 155 nematodes/100 g soil, while 12% of samples had more than 250 nematodes/100 g soil. Yavuzaslanoğlu et al. (2020) found that a higher density of nematodes in Karapınar, Kadınhanı, Selçuklu and Cihanbeyli Districts of Konya Province; a mean of 14 ± 14, 13 ± 8, 16 ± 16 and 9 ± 4 nematodes/100 g dry soil, respectively while *Pratylenchoides* spp. was found only one district at a low density with a mean of 5 ± 5 nematodes/100 g dry soil in Konya Province. The reason for higher density of *Pratylenchus* spp. in present study than in others studies in Turkey could be due to sampling time. In previous studies, the sampling time was between March and April (Yavuzaslanoğlu et al., 2012, 2020). Sampling times were delayed due to late cereal planting and a particularly wet spring in Isparta and Burdur Provinces in 2016 and 2017. Also, Söğüt et al. (2011) collected from 198 samples in 15 districts in the Lakes Region between May and June in 2008-2010 and *P. thornei, P. neglectus, P. crenatus* and *P. alkani* were determined in the region. It was found that *Pratylenchus* spp. had over the economic threshold levels in soil and roots in June-August in 2016 and 2017, and caused serious yield loss in Burdur and Isparta Provinces.

Cereal cyst nematodes were not found in the roots in present study. This may be because with high densities of *Pratylenchu*s and *Pratylenchoides* spp. cyst nematodes may not be able to compete effectively. It might also have been a result of the ecological conditions of the Isparta and Burdur Provinces. In addition, crop rotation is successfully practicing in cereal fields in Isparta and Burdur Provinces. The changing soil conditions and host plant are effective in reducing the incidence and population densities of the main damaging plant parasitic nematode species (Sundararaju & Jeyabaskaran, 2003; Collins et al., 2011). Toktay et al. (2020) reported that the fields where cyst nematodes were not found were generally rotated with other crops. Also, climate change and global warming affect spatial distribution and damage potential of pathogens and pests (Morgan & Wall, 2009).

In conclusion, it appears necessary to work on control strategies for *Pratylenchus* and *Pratylenchoides* species in order to increase yields in Isparta and Burdur Provinces. In addition, the incidence of these nematodes in other host plants involving crop rotation should be investigated. Several researchers and CIMMYT in Turkey have been focusing on host reactions of wheat lines and cultivars to improve resistance. Also, some future studies should focus on the economic significance of *Pratylenchoides* in cereals as there have been no studies of host reactions to *Pratylenchoides* spp. This is needed to improve integrated control strategies in cereal fields.

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*Original article (Orijinal araştırma)*

# Toxic efficacy of *Cuscuta campestris* Yunck. (Solanales: Convolvulaceae) and *Lupinus albus* L. (Fabales: Fabaceae) plant crude extracts against nymphs and adults of *Orosanga japonica* (Melichar, 1898) (Hemiptera: Ricaniidae) under laboratory conditions[1]

*Orosanga japonica* (Melichar, 1898) (Hemiptera: Ricaniidae)'nın nimf ve erginlerine karşı *Cuscuta campestris* Yunck. (Solanales: Convolvulaceae) ve *Lupinus albus* L. (Fabales: Fabaceae) bitki ham özütlerinin laboratuvar koşulları altında toksik etkinliği

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## Abstract

The planthopper, *Orosanga japonica* (Melichar, 1898) (Hemiptera: Ricaniidae), is an important agricultural pest of grapevine, kiwifruit and tea in Asia and in some countries of Eastern Europe. The efficacy of the crude extracts of *Cuscuta campestris* Yunck. (Solanales: Convolvulaceae) and *Lupinus albus* L. (Fabales: Fabaceae) plants was evaluated under laboratory conditions for control of *O. japonica* nymphs and adults collected in 2019 from Rize (Turkey). Their toxic efficacies were investigated by two different methods. Fixed-dose death rates were used for $LT_{50}$ calculation and dosage test results were used for $LC_{50}$ calculation. Also, the phenolic constituents of active plant extracts were examined using HPLC-DAD. Generally, the $LT_{50}$ values obtained using ethyl acetate extracts were lower than those with methanol extracts. $LT_{50}$ values of adults were found lower than in nymphs. The test plants crude extracts had high activity at and below 2 g/L ($LC_{90}$) for two different plants. HPLC-DAD results showed that the high concentration of kaempferol and quercetin for each extract. Extracts of both plants gave promising results for use in *O. japonica* control, but more detailed studies on the active constituents of these candidate plants need to be undertaken.

**Keywords:** Biocontrol, field dodder, insecticidal activity, *Orosanga japonica,* white lupin

## Öz

Yalancı kelebek, *Orosanga japonica* (Melichar, 1898) (Hemiptera: Ricaniidae), Asya'da ve bazı Doğu Avrupa ülkelerinde üzüm, çay ve kivide önemli bir tarımsal zararlıdır. 2019 yılında Rize (Türkiye)'den toplanan *O. japonica* nimfleri ve erginlerinin kontrolüne karşı *Cuscuta campestris* Yunck. (Solanales: Convolvulaceae) ve *Lupinus albus* L. (Fabales: Fabaceae)'un ham özütlerinin laboratuvar koşullarında etkinliği değerlendirilmiştir. Bunların toksik etkileri iki farklı yöntemle araştırılmıştır. Sabit doz ölüm oranları $LT_{50}$ ve dozaj test sonuçları $LC_{50}$ hesaplamaları için kullanılmıştır. Ayrıca aktif bitki özütlerinin fenolik bileşenleri HPLC-DAD kullanılarak incelenmiştir. Genellikle, etil asetat özütleri kullanılırken elde edilen $LT_{50}$ değerleri, metil alkol özütlerinden düşük olmuştur. Erginlerin $LT_{50}$ değerleri nimflere göre daha düşük bulunmuştur. Deneme bitkilerinin ham özütleri, iki farklı bitki için 2 g/L ($LC_{90}$) civarında ve altında yüksek aktivite göstermiştir. HPLC-DAD sonuçları, her bir özüt için yüksek kaempferol ve quercetin konsantrasyonunu göstermiştir. Her iki bitkinin özütleri, *O. japonica*'nın kontrolünde kullanımı için umut vericidir, ancak bu aday bitkilerin aktif bileşenleri üzerinde daha ayrıntılı çalışmaların yapılması gerekmektedir.

**Anahtar sözcükler:** Biyolojik mücadele, tarla küskütü, insektisidal aktivite, *Orosanga japonica,* beyaz acı bakla

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## Introduction

Planthoppers are a large group of more than 9,000 species within the Fulgoromorpha suborder of the Hemiptera (O'Brien & Wilson, 1985). Most of the species are of little economic importance, but a few are major agricultural pests, including *Pochazia sublimate* (Schumacher, 1915), *Ricania speculum* (Walker, 1851) and *Scolypopa australis* (Walker, 1851) (Hemiptera: Ricaniidae), which can transmit plant pathogens by means of absorbent mouth parts or cause significant damage to plant tissues. They are among the most destructive pests of major agricultural products worldwide (Fletcher, 2008; Choi et al., 2012; Jeon et al., 2017). The family Ricaniidae is known as broad-winged planthoppers. Many species in this family have been recorded in tropical areas and some in the Palearctic regions (Demir, 2009). *Orosanga japonica* (syn. *Ricania japonica*) (Melichar, 1898) (Hemiptera: Ricaniidae), has become an important destructive species on agricultural products in the coastal regions of the Eastern Black Sea in Turkey (Demir, 2009; Ak et al., 2015; Akıner et al., 2019).

*Orosanga japonica* is a common species in Eastern Asian countries, including Korea, China and Japan. This species was first recorded in Russia, near Ukraine in the western Palearctic region. It is believed that during the transport of seedlings and plants purchased for botanical gardens in the 1900s, these insects were transported to Russia from their natural habitat. Then in the 1950s, this species was recorded in Georgia (EPPO, 2016). *Orosanga japonica* was first recorded in Turkey by Demir (2009). Since then, it has rapidly spread westward (Akcakoca/Düzce, Turkey) along the Black Sea coastline and in the European part of İstanbul (Demir, 2009, 2018; Öztemiz & Doğanlar, 2015; Arslangündoğdu & Hizal, 2019). The insect has become an economically important pest of some agricultural plants in the Eastern Black Sea Region, including bean, cucumber, fig, grapevine, hazelnut, kiwifruit, tea and tomato (Ak et al., 2015; Öztemiz & Doğanlar, 2015; Göktürk et al., 2017; Jeon et al., 2017; Akıner et al., 2019). This is particularly important as it reduces the production of nuts and tea, which comprise the livelihood of local people. In addition to the limited use of chemical fertilizers in the cultivation of agriculture products under natural conditions, the use of chemical pesticides is also restricted in this region by the General Directorate of Tea Enterprises in Turkey. For this purpose, different mechanical and biological control methods have been tested to reduce the population of *O. japonica*. However, neither of these types of control is sufficient for long-term population control (Güçlü et al., 2010; Ak et al., 2015; Öztemiz & Doğanlar, 2015; Göktürk et al., 2017).

Insect pest control using chemical insecticides can be a substantial problem due to insecticide resistance, effects on nontarget organisms and environmental pollution (Lichtfouse et al., 2009). Many plants produce biologically active metabolites, some of which are useful for insect control. Applying these natural products should decrease the use of chemical insecticides. The use of botanical based insecticides is becoming increasingly common, and the use of environmentally friendly pest control agents has become an international success (Lichtfouse et al., 2009). Plant-derived materials are nontoxic, biodegradable, safer for nontarget organisms such as humans or animals, and they are safer for the environment than chemical insecticides. Therefore, it would be ideal to use botanical insecticides for insect pest control. Three main classes of chemical compounds from plants with insecticidal activity are cited as having higher biological activity than others. These classes are terpenoids (37%), alkaloids (30%), and phenolic compounds (20%) (Boulogne et al., 2012). Although the effects of a large number of plant essential oil and crude extracts against different insect pest species have been investigated, few studies are available on the use of plant materials for the control of *Ricania* spp. (Singh & Upadhyay, 1993; Shin et al., 2010; Boulogne et al., 2012; Choi et al., 2012; Kim et al., 2013; Jeon et al., 2016; Göktürk et al., 2017; Jeon et al., 2017; Lee et al., 2018).

Turkey has a high diversity of natural aromatic and medicinal plant species. Previous studies for *Lupinus* and *Cuscuta* spp. have shown that phenolic and alkaloid contents of this plants are high. In addition, it has been observed that their potential to be natural alternative insecticides against many pests

has been investigated (Thackray et al., 2000; Torres et al., 2009; Shekarchi et al., 2014; Selvi et al., 2017; Karamac et al., 2018; Hassan et al., 2019). However, no study has found effectiveness of the same plants against *O. japonica*. Therefore, the main objective of this research was to determine the toxic efficacy of crude extracts from *Cuscuta campestris* Yunck. (Solanales: Convolvulaceae) and *Lupinus albus* L. (Fabales: Fabaceae) plants naturally growing in Turkey against nymphs and adults of *O. japonica* related to the high phenolic contents and possible bio insecticidal properties (Yorgancılar, 2013). The second objective of this study was to use HPLC-DAD to identify and quantify the phenolic compounds that are potentially the active compounds related to their insecticidal (on the nymph and adults) activities under laboratory conditions.

## Materials and Methods

### Chemicals and solvents

Phenolic standards (analytical grade) were obtained from Sigma-Aldrich (St. Louis, MO, USA). HPLC (high pressure liquid chromatography) syringe filters (RC-membrane, 0.25 µm) were purchased from Sartorius Minisart RC 15, Sartorius (Goettingen, Germany). Other chemicals and solvents such as n-hexane, methanol and ethyl acetate were sourced from Merck (Darmstadt, Germany).

### Plant materials

*Cuscuta campestris* was collected from Talas, Kayseri during the vegetative growing stage (1-3 August), and seeds of *L. albus* were collected from Doğanhisar, Konya 14-15 August 2018. Depending on the results of the preliminary laboratory studies, seed (*L. albus*) and vegetative growing stage (*C. campestris*) of the plants to be studied were determined. This choice is supported by the literature (Shekarchi et al., 2014; Hassan et al., 2019). The specimen identification was performed by Prof. Vagif Atamov from the Faculty of Science and Arts, Recep Tayyip Erdogan University in Rize, Turkey. A sample of each plant was prepared and deposited at mentioned above university in the Herbarium of the Biology Department, Rize, Turkey.

### Biological material

Nymphs (third stage) and adults of *O. japonica* were collected with an electric aspirator around Rize City (Alipasa, 41º1’31.66” N, 40º28’58.09” E and Recep Tayyip Erdoğan University Campus, 41º2’11.28”’ N, 40º29’36.50” E) from early June to late August 2019. About 1000 *O. japonica* nymphs and adults collected from region were placed into the different cages (20 x 20 x 20 cm, maximum of 50 nymphs or 50 adults in each cage) and transferred to the laboratory. The samples brought to the insectarium were taken into cages of 50x50x50 cm for ease of feeding (maximum of 100 nymphs in each cage) during the acclimatization period. The cages were previously sterilized to avoid contamination. Samples were held at 25 ± 2ºC with 65 ± 10% RH and 12:12 h L:D photoperiod. Sufficient fresh blackberry branches were put into the cages for feeding and replaced daily for ensuring freshness. *Orosanga japonica* individuals were kept for 24 h in the insectarium to acclimatize them to the environment before testing.

### Extraction of plant materials

Following specimen identification, the stems (*C. campestris*) and seeds (*L. albus*) were cleaned and thoroughly washed with distilled water and ethyl alcohol (1:1 v/v) to prevent fungal contamination. The fresh materials of *C. campestris* were then dried for 1 week at room temperature. Dried plant (*C. campestris*) and seed (*L. albus*) samples were ground with a blender and divided into 150 g portions for solvent extraction. Each sample was cleaned with 100 mL of chloroform at 30ºC for 30 min to remove waxy parts. Samples were extracted separately with ethyl acetate (3 x 50 mL) and methanol (3 x 50 mL) at room temperature for 1.5 h in an ultrasonic bath (Bandelin, Germany). The crude extracts were filtered with black ribbon filter paper (pore size 20-25 µm), evaporated to dryness and lyophilized at -80ºC and 0.5 kP to completely

remove the solvent used in the extraction (Freezone® 2.5 L Freeze Dry System, Labconco USA). The crude oil obtained was weighed. The amount of crude oil was from *C. campestris* was 2.35 g (ethyl acetate) and 5.76 g (methanol) per 100 g of plant tissue, and for *L. albus,* 3.86 and 3.46 g was obtained per 100 g of seed. After extraction, 25 mg/L of crude plant extract was reserved for HPLC-DAD analysis. A stock solution (0.1 g/mL) of each crude extract was prepared in dimethyl sulfoxide (DMSO) for the bioassays and stored at -4ºC until tested (Selvi et al., 2017).

**Bioassays of plant extracts**

Laboratory assays were conducted to assess the efficacy of the two plant extracts by two methods on *O. japonica* nymphs and adults. A leaf dipping bioassay (Güven et al., 2015) and spray bioassay with slight modifications (Choi et al., 2012) were used to evaluate the efficacy of treatments at different concentrations.

**Residual bioassay with leaf dipping method**

Blackberry branches with leaves without any insecticide application, collected from the field and washed with distilled water, were used. Branches (10 cm) with four to five leaves were selected. Branches were dipped in fixed concentrations of extract solution (in 0.1% v/v Tween 80) for 5 s and then kept at room temperature for 15-20 min to dry. Cotton wool moistened with 5 mL of distilled water was placed on the bottom of a glass jar to prevent drying, and the branches were placed on top. Tween 80 solution (0.1% v/v) was used as a negative control with the same procedure. Twenty acclimatized individuals (nymphs and adults, separately) were then placed in every glass jar for testing.

**Residual bioassay with indirect spraying method**

Filter paper (10 x 20 cm) was sprayed used for contact toxicity. DMSO impregnated filter papers were used as a negative control. The filter paper was placed on the inner surface of the 250 mL glass jar. Cotton wool moistened with 5 mL of distilled water was placed on the bottom of the glass jar. Fresh blackberry branches for feeding were placed on top of the cotton to prevent drying. Twenty acclimatized individuals (nymphs and adults, separately) were then placed in the glass jars for testing. Jars were covered with a cotton cloth held in place by a rubber band.

The bioassays were maintained at 25 ± 2ºC, 65 ± 10% RH and 12:12 h L:D photoperiod. All tests were done with three replicates over two consecutive weeks (3 x 2 replicates). Neem Azal® (0.5 g/L dose) was used as a positive control. Same application methods were used for two test type. A fixed dose was used for lethal time 50 ($LT_{50}$) calculation. Death rates were counted after 12, 24, 48 and 72 h for $LT_{50}$. 1 g/L was used as a highest dose for lethal concentrations 50 and 90 ($LC_{50}$ and $LC_{90}$) calculations. Five-consecutive concentrations were used in the experiments (1, 0.5, 0.25, 0.125 and 0.0675 g/L). Death rates were determined after 24 h for $LC_{50}$ and $LC_{90}$ calculations.

**Statistical analysis**

The percent mortality was calculated and corrected using Abbott's formula (Abbott, 1925) with negative control results. Lethal concentrations and times ($LC_{50}$, $LC_{90}$ and $LT_{50}$) for two tests condition were evaluated by probit analysis. Differences between groups were analyzed with an independent t test probit analysis and independent t-test were performed by using the IBM® SPSS® statistics program version 22.

**HPLC-DAD procedure**

This study measured 20 phenolic compounds: lapigenin, caffeic acid, chlorogenic acid, ellagic acid, ferulic acid, fisetin, gallic acid, isorhamnetin, kaempferol, myricetin, o-coumaric acid, p-coumaric acid, p-OH benzoic acid, paeonol, protocatechuic acid, quercetin, rutin, syringic acid, thymol and vanillic acid by HPLC-DAD.

HPLC-DAD analyses were performed using a Thermo Dionex Ultimate 3000 HPLC-DAD system. An Agilent reverse phase C18 column (150 mm x 4.6 mm i.d., 5 µm particle, 100 A°; Agilent) with a guard column (3 mm i.d., Macherey-Nagel, Düren, Germany) was used. The mobile phase was (A) 2% acetic acid in water and (B) 70:30 acetonitrile: water. The programmed solvent used began with a linear gradient held at 94.5% A:5.5% B for 3 min; decreasing to 77% A:23% B at 10 min; 69% A:31% B at 20 min; 44% A:56% B at 30 min; 15% A:85% B at 40 min; and finally, 9.45% A:90.55 % B at 60 min. The injection volume was 10 µL, the column temperature was 30°C, and the flow rate was 1.0 mL/min. Chromatograms were obtained at 254, 280, 315 and 370 nm (Selvi et al., 2017).

**Determination of total phenolic content in extracts**

Total phenolic compounds were analyzed with Folin-Ciocalteu phenol reagent (Singleton & Rossi, 1965). Gallic acid and quercetin were used to generate standard curves in a range from 0.015 and 0.5 mg/mL ($R^2_{\text{gallic acid}}$ = 0.999, $R^2_{\text{quercetin}}$ = 0.998). First, 20 µL of methanolic plant extract, 400 µL of 0.5 N Folin-Ciocalteu reagent, and 680 µL of distilled water were mixed. This mixture was well vortexed. Then 400 µL of $Na_2CO_3$ (10%) was added and incubated for 2 h at room temperature. The absorbance of the mixture was measured at 760 nm in HPLC (Thermo Dionex Ultimate 3000 HPLC-DAD). The concentration of total phenolic compounds was calculated as mg of gallic acid equivalent (GAE) per g dry weight (DW) and as mg of quercetin equivalent (QE) per g DW. All measurements were performed in triplicate.

## Results and Discussion

Crude plant extracts are known to include complex mixtures of biologically active secondary metabolites. Two different crude extracts of *C. campestris* and *L. albus* were tested at a fixed concentration of 0.5 g/L with two different methods. Using the indirect spraying method, $LT_{50}$ values varied between 12.2 h (*C. campestris* ethyl acetate extract on adults) and 24.5 h (*C. campestris* ethyl acetate extract on nymphs) (Table 1). Generally, the $LT_{50}$ values of compounds extracted with ethyl acetate values were lower than those extracted with methanol in the same plant and life stage. The $LT_{50}$ values of the adult stage were lower than for the nymphs. Statistical analysis indicated significant differences between $LT_{50}$ values for nymphs and adults ($p < 0.05$, $F = 10.9$, p-value = 0.002). Although $LT_{50}$ values against nymphs and adults for the different plant extracts varied widely, there was no significant difference in plant species efficacy ($p > 0.05$, $F = 0.709$, p-value = 0.403). Similarly, no difference was detected for extraction type ($p > 0.05$, $F = 0.009$, p-value = 0 926).

Using the leaf dipping method, $LT_{50}$ values varied between 14.5 h (*C. campestris* methanol on adults) and 32.0 h (*C. campestris* methanol on nymphs). Results of the leaf dipping method were similar to the results found using the indirect spraying method. $LT_{50}$ values calculated from using the leaf dipping method were higher than those found using the indirect spraying method, except for *C. campestris* ethyl acetate and *L. albus* methanol on nymphs. Although the leaf dipping method results were higher than those found using the indirect spraying method, no significant difference between test techniques was detected ($p > 0.05$, $F = 0.188$, p-value = 0.666). Similar results were found for Neem Azal® for two test techniques ($p > 0.05$, $F = 0.001$, p-value = 0.980).

$LC_{50}$ and $LC_{90}$ experiments were performed according to the promising $LT_{50}$ results. Two different crude extracts of *C. campestris* and *L. albus* were tested at five-consecutive concentrations by with two different methods. The results are shown Table 2. $LC_{50}$ results varied between 0.96 g/L (*C. campestris* ethyl acetate extraction against adult) and 1.32 g/L (*L. albus* ethyl acetate extraction against nymph) for residual indirect spraying method. $LC_{50}$ results for leaf dipping method were varied between 1.05 g/L (*C. campestris* methanol extraction against adult) and 1.58 g/L (*L. albus* ethyl acetate extraction against nymph). The highest $LC_{50}$ results for two plant species were found with ethyl acetate extraction against nymph except *C. campestris* methanol extraction for residual indirect spraying method. Although the results varied widely

between the two test and extraction methods, there is no significant differences between test and extraction method ($p < 0.05$, $F = 0.056$, p-value = 0.819 for test types, $F = 0.074$, p-value = 0.792 for extraction methods). Generally, nymph $LC_{50}$ results were found higher than adults and showed significant differences between adults and nymphs ($p < 0.05$, $F = 9.77$, p-value = 0.003).

Table 1. $LT_{50}$ values of *Orosanga japonica* nymphs and adults exposed to crude extracts (500 mg/L) of *Cuscuta campestris* (whole plant) and *Lupinus albus* (seed) using residual leaf dipping and residual indirect spraying methods*

| Plant | Stage | Solvent | Residual Indirect spraying* | | | | | Residual leaf dipping | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | $LT_{50}$ | LCL | UCL | $\chi^2$ | CM | $LT_{50}$ | LCL | UCL | $\chi^2$ | CM |
| *Cuscuta campestris* | nymph | EtOAc | 24.5 | 10.6 | 71.4 | 13.7 | 9.16 | 22.5 | 4.0 | 46.3 | 10.6 | 8.3 |
| | | MeOH | 30.2 | 10.6 | 71.1 | 11.5 | 7.83 | 32.0 | 11.2 | 88.6 | 9.0 | 6.8 |
| | adult | EtOAc | 12.2 | 10.9 | 13.4 | 0.082 | 8.16 | 15.3 | 4.6 | 23.5 | 4.4 | 6.0 |
| | | MeOH | 13.5 | 11.8 | 15.0 | 2.71 | 6.66 | 14.5 | 11.9 | 16.9 | 1.4 | 5.8 |
| *Lupinus albus* | nymph | EtOAc | 23.3 | 6.2 | 43.7 | 8.59 | 7.50 | 24.8 | 21.9 | 27.9 | 0.4 | 4.8 |
| | | MeOH | 23.9 | 21.3 | 26.6 | 0.024 | 6.83 | 23.7 | 20.9 | 26.5 | 1.3 | 4.7 |
| | adult | EtOAc | 12.6 | nd | nd | 8.57 | 9.00 | 14.7 | 12.5 | 16.7 | 1.8 | 6.2 |
| | | MeOH | 15.7 | 0.8 | 27.1 | 5.19 | 7.66 | 18.8 | 3.6 | 32.0 | 6.7 | 5.5 |
| Neem Azal | nymph | | 40.9 | 27.4 | 56.3 | 4.08 | 3.50 | 40.1 | 14.4 | 70.3 | 8.1 | 2.7 |
| | adult | | 54.7 | nd | nd | 14.7 | 3.66 | 53.9 | nd | nd | 14.0 | 2.8 |

* Each test methods include 120 individuals for each extraction solvents, plants and life stages. $LT_{50}$, lethal time 50; LCL, lower confidence limit (95% fiducial limit); UCL, upper confidence limit (95% fiducial limit); $\chi^2$, chi-squared; CM, percent control mortality; EtOAc, ethyl acetate; MeOH, methanol; and nd, not determined.

$LC_{50}$ and $LC_{90}$ experiments were performed according to the promising $LT_{50}$ results. Two different crude extracts of *C. campestris* and *L. albus* were tested at five-consecutive concentrations by with two different methods. The results are shown Table 2. $LC_{50}$ results varied between 0.96 g/L (*C. campestris* ethyl acetate extraction against adult) and 1.32 g/L (*L. albus* ethyl acetate extraction against nymph) for residual indirect spraying method. $LC_{50}$ results for leaf dipping method were varied between 1.05 g/L (*C. campestris* methanol extraction against adult) and 1.58 g/L (*L. albus* ethyl acetate extraction against nymph). The highest $LC_{50}$ results for two plant species were found with ethyl acetate extraction against nymph except *C. campestris* methanol extraction for residual indirect spraying method. Although the results varied widely between the two test and extraction methods, there is no significant differences between test and extraction method ($p < 0.05$, $F = 0.056$, p-value = 0.819 for test types, $F = 0.074$, p-value = 0.792 for extraction methods). Generally, nymph $LC_{50}$ results were found higher than adults and showed significant differences between adults and nymphs ($p < 0.05$, $F = 9.77$, p-value = 0.003).

In addition to the efficacy and $LC_{50}$ results, HPLC-DAD analyses were performed for the determination of primary components of active extracts. As a result, the primary components of *L. albus* extracted by using ethyl acetate were kaempferol (15.0 mg/g), vanillic acid (8.58 mg/g), and *o*-coumaric acid (3.08 mg/g). The primary components in the methanol extract were almost the same, but their concentration was lower. Ferulic acid (2.59 mg/g) was the most abundant phenolic compound by using the methanol extract of the same plant. HPLC-DAD analysis of the *C. campestris* plant was done in the previous study (Selvi et al., 2017) and the primary component ratios included in this plant are given in Table 3 for comparison.

Table 2. $LC_{50}$ and $LC_{90}$ values (g/L) of *Orosanga japonica* nymphs and adults exposed to crude extracts of *Cuscuta campestris* (whole plant) and *Lupinus albus* (seed) using residual leaf dipping and residual indirect spraying methods

| Plant | Stage | Solvent | Residual indirect spraying* | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | | | $LC_{50}$ | LCL | UCL | $LC_{90}$ | LCL | UCL | $\chi^2$ |
| *Cuscuta campestris* | nymph | EtOAc | 1.11 | 0.75 | 1.86 | 1.84 | 1.36 | 3.76 | 19.30 |
| | | MeOH | 1.26 | 1.02 | 1.71 | 1.90 | 1.52 | 2.87 | 8.91 |
| | adult | EtOAc | 0.96 | 0.61 | 1.75 | 1.69 | 1.21 | 3.99 | 20.00 |
| | | MeOH | 1.01 | 0.61 | 1.76 | 1.97 | 1.41 | 4.24 | 17.80 |
| *Lupinus albus* | nymph | EtOAc | 1.32 | 0.96 | 1.98 | 2.10 | 1.60 | 2.87 | 15.60 |
| | | MeOH | 1.27 | 0.81 | 2.22 | 2.15 | 1.55 | 4.62 | 23.00 |
| | adult | EtOAc | 1.22 | 1.02 | 1.52 | 1.90 | 1.58 | 2.54 | 5.95 |
| | | MeOH | 1.12 | 0.74 | 1.92 | 1.92 | 1.40 | 4.02 | 19.70 |
| Neem Azal | nymph | | 0.90 | 0.70 | 1.20 | 1.47 | 1.18 | 2.13 | 10.30 |
| | adult | | 0.99 | 0.79 | 1.29 | 1.62 | 1.31 | 2.25 | 8.52 |
| | | | Residual leaf dipping | | | | | | |
| | | | $LC_{50}$ | LCL | UCL | $LC_{90}$ | LCL | UCL | $\chi^2$ |
| *Cuscuta campestris* | nymph | EtOAc | 1.14 | 0.76 | 1.81 | 1.99 | 1.47 | 3.74 | 17.30 |
| | | MeOH | 1.13 | 0.56 | 3.74 | 1.91 | 1.28 | 10.26 | 36.40 |
| | adult | EtOAc | 1.11 | 0.75 | 1.74 | 1.99 | 1.48 | 3.68 | 15.40 |
| | | MeOH | 1.05 | 0.68 | 2.21 | 1.74 | 1.23 | 5.03 | 22.60 |
| *Lupinus albus* | nymph | EtOAc | 1.58 | 1.48 | 1.70 | 2.31 | 2.14 | 2.51 | 3.15 |
| | | MeOH | 1.48 | 1.37 | 1.60 | 2.25 | 2.08 | 2.46 | 4.78 |
| | adult | EtOAc | 1.18 | 0.97 | 1.55 | 1.78 | 1.45 | 2.59 | 7.58 |
| | | MeOH | 1.19 | 0.86 | 1.80 | 1.94 | 1.47 | 3.39 | 15.30 |
| Neem Azal | nymph | | 0.86 | 0.65 | 1.20 | 1.46 | 1.14 | 2.24 | 11.60 |
| | adult | | 1.05 | 0.87 | 1.29 | 1.71 | 1.43 | 2.23 | 5.61 |

* Each test methods include 120 individuals for each extraction solvents, plants and life stages. $LC_{50}$, lethal concentration 50; $LC_{90}$, lethal concentration 90; LCL, lower confidence limit (95% fiducial limit); UCL, upper confidence limit (95% fiducial limit); $\chi^2$, ºhi-squared; EtOAc, ethyl acetate; and MeOH, methanol.

Table 3. Phenolic compounds present in *Cuscuta campestris* and *Lupinus albus* extracts after extraction with methanol and ethyl acetate identified by HPLC-DAD

| Compounds | Retention Time (min) | *Lupinus albus* | | *Cuscuta campestris* [a] | |
|---|---|---|---|---|---|
| | | EtOAc* | MeOH | EtOAc[a] | MeOH[a] |
| *p*-OH Benzoic acid | 11.8 | nd | 0.4 | 0.8 | 0.7 |
| Vanillic acid | 13.3 | 8.6 | 1.1 | 0.4 | 0.8 |
| Rutin | 14.8 | nd | 1.5 | 0.2 | 3.4 |
| *p*-Coumaric acid | 16.5 | 0.4 | 0.1 | 1.2 | 2.3 |
| Ferulic acid | 18.9 | 0.7 | 2.6 | 1.5 | 4.4 |
| *o*-Coumaric acid | 20.4 | 3.1 | 0.1 | nd | nd |
| Quercetin | 28.5 | nd | 0.4 | 5.6 | 12.5 |
| Kaempferol | 32.3 | 15.0 | 0.6 | 5.0 | 10.7 |
| Isorhamnetin | 33.1 | - | - | 6.6 | 17.0 |
| Total | | 27.7 | 6.8 | 21.3 | 51.8 |
| Total flavanol | | 15.0 | 2.5 | 17.4 | 43.6 |

* EtOAc, ethyl acetate; MeOH, methanol; nd, not detected. [a]HPLC-DAD analysis results of *C. campestris* in Selvi et al. (2017).

In the present study, the total phenolic content (TPC) determination of four extracts from two different plants and two extraction solvents was performed spectroscopically. Gallic acid and quercetin were used as standards for TPC with a linear calibration curve at $R^2_{\text{gallic acid}} = 0.999$ and $R^2_{\text{quercetin}} = 0.998$. The level of phenolic compounds ranged from 17.1 to 250 mg GAE/g, and from 8.00 to 140 mg QE/g, respectively. The highest TPC was obtained from the ethyl acetate extract of *C. campestris* while the lowest was obtained from the methanol extract of *L. albus* (Table 4).

Table 4. Total phenolic content (TPC) of *Cuscuta campestris* and *Lupinus albus* extracts

| Sample | Extract | TPC | |
|---|---|---|---|
| | | mgGAE/g* | mgQE/g* |
| *Cuscuta campestris* | MeOH | 50.5 ± 0.03 | 38.4 ± 0.05 |
| | EtOAc | 250.5 ± 0.02 | 140.2 ± 0.01 |
| *Lupinus albus* | MeOH | 17.1 ± 0.02 | 8.0 ± 0.01 |
| | EtOAc | 20.4 ± 0.03 | 11.2 ± 0.02 |

* GAE, gallic acid equivalents; QE, quercetin equivalents; EtOAc: Ethyl acetate; MeOH: methanol.

Previous studies have reported that the differences in sensitivity of *Ricania* nymphs and adults to essential oils are related to variation in biological factors, such as detoxified glutathione S-transferase and hydrolase levels in nymphs and adults (Lee & Lee, 2015). In addition, several studies have reported the insecticidal activity of different plant essential oils on both nymphs and adults of a *Ricania* sp. and *O. japonica* (Choi et al., 2012; Lee et al., 2016, 2018; Jeon et al., 2016, 2017; Göktürk et al., 2017). Jeon et al. (2016) tested the possible activity of essential oils from seven different plants on nymphs and adults of *Ricania* sp. This study results showed that the high insecticidal toxicity of *Tagetes erecta* L. (Asterales: Asteraceae) essential oils, after exposure for 72 h. The primary constituents in the essential oil of this plant were identified as caryophyllene, terpinolene, (*E*)-ocimenone, ocimene, piperitenone, and limonene (Sefidkon et al., 2004). In another study, it was suggested that the essential oils of *Salvia officinalis* Spenn. (Lamiales: Lamiaceae) killed 74.1% of *O. japonica* insects after 96 h of exposure and could be used as an effective means of control (Göktürk et al., 2017). In contrast, our tested plants (*C. campestris* and *L. albus*) showed a higher efficacy against nymphs (highest $LT_{50}$ value 32.0 h with methanol in *C. campestris)* and adults (highest $LT_{50}$ value 18.8 h with methanol in *L. albus*) after 72 h of exposure. In a study by Jeon et al. (2017), results showed that high insecticidal toxicity of *Cinnamomum cassia* L. (Laurales: Lauraceae) ($LC_{50}$ value of nymph was 37.7 mg/L and of adult 77.4 mg/L) and *Cinnamomum verum* J. Presl (Laurales: Lauraceae) ($LC_{50}$ value of nymph 72.6 mg/L and on adult 135 mg/L) essential oils on a *Ricania* sp. The GC-MS analysis of the plants showed that cinnamaldehyde contained in *C. cassia* and *C. zeylanicum* oils (80.2% and 46.3%, respectively) were effective against a *Ricania* sp. nymphs and adults. $LC_{50}$ values for nymphs and adults were 31.3 and 62.4 mg/L, respectively, in another study by the same group. Lee et al. (2018) tested the insecticidal effects of *Valeriana officinalis* L. (Dipsacales: Caprifoliaceae) essential oils extracted by steam distillation, solvent, and supercritical extraction on a *Ricania* sp. As a result, they observed that the extract obtained by steam distillation had the highest mortality for a *Ricania* sp. adults (1.04 µg/mL) and nymphs (2.37 µg/mL). Our results showed high mortality of nymphs and adults after 72 h of exposure to a fixed dose (500 ppm) of crude extracts and LC tests results showed high activity at and below 2 g/L ($LC_{90}$) crude extracts for both plants. Previous studies on *Ricania* or *Orosanga* species control have generally included essential oils from different genera and native plant species *a*. Our study included two plant species grown in Turkey using crude extracts. Therefore, more detailed study of different distillation methods and essential oils is needed.

In general, when plants exhibiting insecticidal activity are examined, the concentration of alkaloids, phenolic and terpenoids compounds is high. Therefore, in the second part of our study, the phenolic contents

of *C. campestris* and *L. albus*, which showed insecticidal activity, were determined by HPLC-DAD. In the previous study of Selvi et al. (2017) on *C. campestris* extracts extracted by using ethyl acetate and methanol, they reported isorhamnetin (6.61 and 17.0 mg/g), quercetin (5.60 and 12.5 mg/g), and kaempferol (5.00 and 10.7 mg/g) as major phenolic compounds (Table 2). Similarly, ferulic acid, p-coumaric acid, p-OH benzoic acid, rutin and vanillic acid have been detected in the two extracts of *C. campestris* (Selvi et al., 2017).

Our results were similar to the results for *Cuscuta* sp. and *Lupinus* sp. reported in the literature. Previous studies with methanol extracts of *Cuscuta* spp. have commonly identified astragaline, chlorogenic acid, hyperoside, isorhamnetin, kaempferol, quercetin, and rutin as phenolic compounds within the extracts (Ye et al., 2005). Król et al. (2018) reported that ferulic acid, p-coumaric acid and sinapic acid are present in *Cuscuta* sp. In another study, *p*-coumaric acid derivatives and apigenin-6,8-di-*C*-glucoside were detected in the same plant (Siger et al., 2012). In a study by Karamac et al. (2018), apigenin, coumarin derivatives, ferulic acid, gallic acid, hesperidin, kaempferol, quercetin and vanillic acid were detected in HPLC-DAD analysis of *Lupinus s*p. The importance of kaempferol, quercetin and their derivatives for insecticidal activity has been reported by other authors (Upasani et al., 2003; Mendki et al., 2005). Upasani et al. (2003) reported excellent insecticidal activity of aqueous leaf extracts of *Ricinus communis* L. (Malpighiales: Euphorbiaceae) against *Callosobruchus chinensis* (L., 1758) (Coleoptera: Bruchidae). They concluded that the quercetin and kaempferol constituents are important for insecticidal activity. Similarly, Mendki et al. (2005) reported that the alcoholic foliar extract of *Calotropis procera* Aiton (Gentianales: Apocyneceae) gave a mixture of flavonoids including quercetin-3-O-gal (hyperoside) and kaempferol-3-O-rha or quercetin-3-O-ara and exhibited excellent insecticidal activity on *C. chinensis*. Huang et al. (2013) reported that ferulic acid and derivatives have potential because of their insecticidal activities. Hussain et al. (2018) reported that different applications of coumarin affect agricultural pests and concluded that coumarin and its derivatives are highly phytotoxic but that their performance in controlling insects has shown very promising results. Our HPLC-DAD results show a high concentration of kaempferol and quercetin after using two different extraction solvents, as well as different concentrations of coumarin and ferulic acid. The high efficacy of crude extracts that we observed may be explained by these constituents.

This research is the first examination of the insecticidal activities of *C. campestris* and *L. albus* crude extracts against *O. japonica* nymphs and adults. The results showed that extracts from both plants may be candidates for use as botanical-based insecticides. This study is also important for its evaluation of bioactive compounds from naturally growing plants in long-term control, as other Ricaniidae family species are seen as invasive species in Western Palearctic.

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***Original article*** (***Orijinal araştırma***)

# Susceptibility of different plant species to two populations of *Ditylenchus dipsaci* Kühn, 1857 (Tylenchida: Anguinidae) from Turkey[1]

## Farklı bitki türlerinin Türkiye'den iki *Ditylenchus dipsaci* Kühn, 1857 (Tylenchida: Anguinidae) popülasyonuna hassasiyetleri

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## Abstract

Stem and bulb nematode, *Ditylenchus dipsaci* Kühn, 1857 (Tylenchida: Anguinidae), is one of the most important plant parasitic nematodes worldwide. The host range of local populations needs to be determined for control using crop rotation. Susceptibility of 29 plant species was tested for onion and garlic populations of *D. dipsaci* from Central Anatolian Plateau in Turkey under growth chamber conditions in Karaman in 2019. Based on the reproduction factor of the nematodes, garlic, onion and tomato were excellent hosts for both populations of *D. dipsaci.* Pea and spinach were excellent hosts for the onion population, while good host for the garlic population with cucumber a good host for both populations. Eggplant, pepper and zucchini were identified as poor hosts for both nematode populations. Bean and potato were poor hosts for the onion population, and were non-hosts for the garlic population. Alfalfa, barley, carrot, chickpea, daffodil, hyacinth, kale, leek, lettuce, maize, melon, oat, rye, strawberry, sugar beet, tobacco, tulip and wheat were non-hosts for both populations of *D. dipsaci*. Infection with *D. dipsaci* significantly reduced plant weight of onion and tomato, and plant height of pepper. Rotational crops for use in areas infected with *D. dipsaci* in Central Anatolian Plateau in Turkey were indicated by the current study.

**Keywords:** Host range, plant parasitic nematode, race, rotation, stem and bulb nematode

## Öz

Soğan sak nematodu *Ditylenchus dipsaci* Kühn, 1857 (Tylenchida: Anguinidae) dünyanın her yerine yayılmış önemli bitki paraziti nematodlardan biridir. Nematodun kontrolü için yerel popülasyonların konukçu spektrumunun bilinmesi gerekmektedir. Yirmi dokuz bitki türünün hassasiyet durumları Türkiye'de Orta Anadolu Bölgesi'nden elde edilen *D. dipsaci*'nin soğan ve sarımsak izolatları için Karaman'da 2019 yılında büyütme dolabı koşullarında test edilmiştir. Soğan, sarımsak ve domates, her iki *D. dipsaci* popülasyonu için de mükemmel konukçu olarak belirlenmiştir. Bezelye ve ıspanak, soğan popülasyonu için mükemmel bir konukçu iken, sarımsak popülasyonu için iyi konukçudur. Salatalık her iki popülasyon için de iyi konukçudur. Biber, kabak ve patlıcan her iki nematod popülasyonu için zayıf konukçu olarak tanımlanmıştır. Fasulye ve patates bitkileri soğan popülasyonu için zayıf konukçu iken, sarımsak popülasyonu için konukçu değildir. Arpa, buğday, çavdar, çilek, havuç, kara lahana, kavun, lale, marul, mısır, nergis, nohut, pırasa, sümbül, şeker pancarı tütün, yonca ve yulaf, her iki nematod popülasyonuna da konukçu değildir. *Ditylenchus dipsaci* ile enfeksiyon, soğan ve domatesin bitki ağırlığını ve biberin bitki boyunu önemli ölçüde azaltmıştır. Gerçekleştirilen çalışma ile Türkiye'de Orta Anadolu Bölgesi'nde *D. dipsaci* ile bulaşık alanlarda kullanılabilecek rotasyon bitkileri belirlenmiştir.

**Anahtar sözcükler:** Konukçu spektrumu, bitki paraziti nematod, ırk, ekim nöbeti, soğan sak nematodu

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## Introduction

Stem and bulb nematode, *Ditylenchus dipsaci* Kühn, 1857 (Tylenchida, Anguinidae), is a migratory endoparasitic nematode damaging more than 500 plant species (Brzeski, 1991). Stem and bulb nematode comprise a species complex which includes individuals with diploid features known as *D. dipsaci* sensu stricto or “normal-sized species" and individuals having polyploid features (Subbotin et al., 2005). The nematodes in *D. dipsaci* sensu stricto are phylogenetically very close to each other and cannot be distinguished as subspecies (Subbotin et al., 2005). The nematodes in this group are described as races according to their reproduction in different plant species. Sturhan & Brzeski (1991) reported more than 30 races for *D. dipsaci*. The races are different for economically and globally important plant species. The race isolated originally from onion is known as the onion race.

In Turkey, the stem and bulb nematode is found in onion fields in Aksaray, Ankara, Eskişehir, Karaman and Konya Provinces in the Central Anatolia Region, İstanbul and Tekirdağ Provinces in the Marmara Region, Amasya, Çorum, Kastamonu and Tokat Provinces in the Black Sea Region and Adana, Hatay and Mersin Provinces in the Mediterranean Region (Yavuzaslanoglu et al., 2019). Onion yield losses due to the stem and bulb nematode have been reported as 41.5% (Yavuzaslanoglu et al., 2015) to 65% (Mennan & Ecevit, 2002) in Turkey.

Since stem and bulb nematode is a quarantine nematode for onion in Turkey (EPPO, 2020), quarantine measures are made to prevent its spread. In addition, since the host spectrum is quite wide, in order to maintain economic production in the areas where it is infected, it is necessary to apply integrated pest management practices to keep the population level below the economic damage threshold in field. Rotation with non-host plants is a promising control method. It has been found that 3-4 years of rotation with non-host plants significantly reduces *D. dipsaci* populations (Roberts & Grathead, 1986). As host range of the races of *D. dipsaci* can vary in local populations (Viglierchio, 1971), determination of the biological race and the host spectrum of the local nematode population are very important for the implementation of rotation practices. Damage caused by *D. dipsaci* from onion from Amasya Province, Suluova District in Black Sea Region in Turkey was investigated on different plant species (Mennan, 2001). Of the investigated plant species, nematode symptoms were seen on bean, garlic, onion and tomato, and no damage was observed in cucumber, kale, pepper, spinach, wheat and zucchini (Mennan, 2001). However, susceptibility of the plant species to the stem and bulb nematode populations from other locations of onion and garlic production in Turkey have not investigated.

The aim of the study was to investigate the susceptibility of the 29-plant species which could be used in rotation with onion and garlic in field to two populations of *D. dipsaci* from Central Anatolian Plateau in Turkey.

## Materials and Methods

### Plant materials

Totally 29 plant species from 12 families were investigated for their susceptibility against *D. dipsaci*. Taxonomic classification and denomination of plant species are given according to The Plant List (Anonymous, 2020). Standard cultivars of plant species were obtained from commercial seed firms, Agricultural Research Institutes and growers (Table 1).

### Nematode populations

Two populations of *D. dipsaci* were used in the study. Nematode populations were originally isolated from Karaman Province, Central District in Central Anatolian Plateau from onion (37º11'01.7" N, 33º11'23.9" E) and garlic plants (37º10'35.1" N, 33º11'70.0" E).

Nematodes were identified morphologically and with species specific PCR technique using the primers of PF1-PR1, PF2-PR2, DdpS1-rDNA2, DİTNF1-rDNA2, H05-H06, DipU F-DipU R and 18S-26S (Subbotin et al., 2005; Esquibet et al., 2003; Marek et al., 2010). Isolation of DNA and PCR conditions were as same as described by Yavuzaslanoglu et al. (2018). Morphological measurements were made according

to literature for this nematode (Öztürk, 1990; Sturhan & Brzeski, 1991; Kepenekçi, 1999; Mennan, 2001; Chizhov et al., 2010).

Table 1. Family, cultivar and source of plant species used in this study from Turkey

| Family | Plant species | Cultivar | Source |
|---|---|---|---|
| Amaranthaceae | Spinach (*Spinacia oleracea* L.) | Matador | Arzuman Seed, Konya |
| | Sugar beet (*Beta vulgaris* L.) | Standard | Arzuman Seed, Konya |
| Amaryllidaceae | Daffodil (*Narcissus* spp.) | Carlton | Asya tulip, Konya |
| | Garlic (*Allium sativa* L.) | Standard | Karaman |
| | Leek (*Allium ampeloprasum* L.) | İnegöl | İntfa Seed, Konya |
| | Onion (*Allium cepa* L.) | Banko | İntfa Seed, Konya |
| Apiaceae | Carrot (*Daucus carota* L.) | Nantes | Paşa Seed, Balıkesir |
| Asparagaceae | Hyacinth (*Hyacinthus orientalis* L.) | Fondant | Asya tulip, Konya |
| Brassicaceae | Kale (*Brassica oleracea* L.) | Morris | İntfa Seed, Konya |
| Asteraceae | Lettuce (*Lactuca sativa* L.) | Yedikule 5701 | Arzuman Seed, Konya |
| Cucurbitaceae | Cucumber (*Cucumis sativus* L.) | Beith Alpha | Arzuman Seed, Konya |
| | Melon (*Cucumis melo* L.) | Ananas | Arzuman Seed, Konya |
| | Zucchini (*Cucurbita pepo* L.) | Pelin | Arzuman Seed, Konya |
| Fabaceae | Alfalfa (*Medicago sativa* L.) | Bilensoy 80 | Beyza Seed, Konya |
| | Bean (*Phaseolus vulgaris* L.) | Dermason | Karaman |
| | Chickpea (*Cicer arietinum* L.) | Sarı 98 | Karaman |
| | Pea (*Pisum sativum* L.) | Utrillo | Arzuman Seed, Konya |
| Liliaceae | Tulip (*Tulipa gesneriana* L.) | Negrita | Asya tulip, Konya |
| Poaceae | Barley (*Hordeum vulgare* L.) | Kral 97 | Bahri Dağdaş International Agricultural Research Institute, Konya |
| | Maize (*Zea mays* L.) | Standard | Dekalb, Konya |
| | Oat (*Avena sativa* L.) | Standard | Karaman |
| | Rye (*Secale cereale* L.) | Standard | Karaman |
| | Wheat (*Triticum aestivum* L.) | Çeşit 1252 | Karaman |
| Rosaceae | Strawberry (*Fragaria vesca* L.) | Standard | Karaman |
| Solanaceae | Eggplant (*Solanum melongena* L.) | Balıkesir 76 | EkoherbAsgen Seed, İstanbul |
| | Pepper (*Capsicum annuum* L.) | Çetinel 150 | Arzuman Seed, Konya |
| | Potato (*Solanum tuberosum* L.) | Standard | Karaman |
| | Tobacco (*Nicotiana tabacum* L.) | Basma | Aegean Agricultural Research Institute, İzmir |
| | Tomato (*Solanum lycopersicum* L.) | Kokteyl | Ekoherb Asgen Seed, İstanbul |

**Preparation of inoculum**

Nematodes were cultured on sterile carrot discs (Behmand et al., 2017). Large carrots without any damage or decay were peeled and surface sterilized for 10 min using 95% ethanol and flamed. After a second peeling, sterile carrots were sliced into discs 1 cm thick and placed into 6-cm diameter sterile plastic Petri dishes. Nematode cultures were started from one female and one male nematode. Nematode cultures were incubated at 20ºC in dark. Cultures were subcultured every eight weeks to obtain enough inoculum for the study.

Nematodes applied as inoculum in susceptibility tests were extracted from 2-month-old sterile carrot cultures by washing the surface of the carrot discs with sterile tap water. The nematode suspension was then concentrated to about 200 nematodes per 10 µl for application to plants.

**Experimental design**

Plastic square pots at 7x7x8 cm dimensions were filled with 300 ml sand:field soil:organic matter mixture (70:29:1) sterilized in an autoclave at 121ºC for 120 min. One seed was planted per pot for each plant species with 10 replicate pots. The experiment was laid out in a completely randomized plot design. Experiment included plants inoculated with the two populations of *D. dipsaci* and uninoculated control. Two hundred nematodes including all stages in 10 µl carboxymethyl cellulose solution (1%) from each nematode

population was inoculated between first two leaves of plants at the third- to fourth-leaf stage (Kühnhold et al., 2006). The experiment was conducted under growth chamber conditions at 20ºC, 70% RH and 16:8 h L:D photoperiod.

Plants were harvested 6 weeks after inoculation. Plant height, fresh plant weight and symptoms of nematode damage were recorded. Nematodes were extracted from the whole plant and soil in the pots for 24 h using a modified Baermann funnel technique (Hallmann & Subbotin, 2005). The nematode suspensions were then concentrated to 1 ml. The nematode numbers were counted in 50 µl subsample and multiplied by 20 to give the total number of nematodes per sample. The results were expressed as the total number of nematodes for plant and soil per pot. The RF value was calculated by dividing final number of nematodes per pot by the initial nematode number applied (i.e., 200 nematodes/plant). Plant species were categorized as non-host for RF < 1, poor host for 1 < RF < 2, good host for 2 < RF < 4 and excellent host for 4 < RF (Hajihassani et al., 2016).

### Statistical analysis

Data of total number of nematodes per pot was analyzed to determine whether the data comes from the normal distribution using a goodness-of-fit test. According to the test result, the data was transformed to ln(x + 1) values to provide normality. Levene's test was used to test homogeneity of variance of the transformed data.

Analysis of variance (ANOVA) and Tukey HSD test of transformed total numbers of nematodes among plant species were performed for evaluation of the susceptibility of the plant species. Differences between the transformed total numbers and reproduction factors of two nematode populations were examined using t-tests.

Statistically significant difference in plant weight and height of each plant species among nematode treatments was investigated with ANOVA and Tukey HSD test. Statistical analyses were performed using JMP© 5.0 software (JMP, 2020).

## Results and Discussion

### Species identification of nematode populations

Morphological measurements of nematodes from carrot cultures were in agreement with previous records (Öztürk, 1990; Sturhan & Brzeski, 1991; Kepenekçi, 1999; Mennan, 2001; Chizhov et al., 2010) (Table 2).

Table 2. Morphological and morphometric measurements of *Ditylenchus dipsaci* onion and garlic populations and from the literature

| Characteristics* | Onion population | Garlic population | Öztürk, 1990 | Sturhan & Brzeski, 1991 | Kepenekçi, 1999 | Mennan, 2001 |
|---|---|---|---|---|---|---|
| n | 4 | 5 | 20 | | 12 | 200 |
| L (mm) | 1.11-1.18 | 1.00-1.09 | 0.53-1.10 | 1.00-2.20 | 0.65-0.87 | 1.25-1.71 |
| Stylet length (µm) | 11.70-11.97 | 11.26-12.08 | 6.00-11.80 | 10.00-13.00 | 10.00-13.00 | 12.00-14.00 |
| Tail length (µm) | 66.79-78.19 | 67.81-75.00 | 48.00-66.20 | | 45.00-53.00 | |
| a | 42.49-47.23 | 37.32-42-60 | 34.70-44.20 | 36.00-64.00 | 39.50-52.20 | 39.00-51.00 |
| b | 6.33-7.22 | 5.83-7.02 | 4.80-6.80 | 6.50-12.00 | 5.60-7.20 | 8.41-8.70 |
| c | 14.69-16.99 | 14.13-16.00 | 9.90-12.60 | 11.00-20.00 | 11.20-17.30 | 11.42-21.60 |
| c' | 4.72-6.19 | 4.12-5.45 | 5.60-6.90 | 3.00-6.00 | 4.09-4.81 | |
| V (%) | 78.75-80.48 | 80.03-81.95 | 73.40-81.20 | 76.00-86.00 | 80.20-81.10 | 76.00-73.00 |

* n, number of specimens; L, total body length; a, body length/the largest width part of body; b, body length/distance from esophagus intestine overlapping part to anterior end of body; c, body length/tail length; c', tail length/body width at anus; and V (%), distance from anterior end of body to vulva/body length x 100.

The primers; PF1-PR1, PF2-PR2, DdpS1-rDNA2, DİTNF1-rDNA2, H05-H06, DipU F-DipU R and 18S-26S provided specific bands at 327, 396, 517, 263, 242, 333, 967 bp for both populations, respectively (Figure 1).

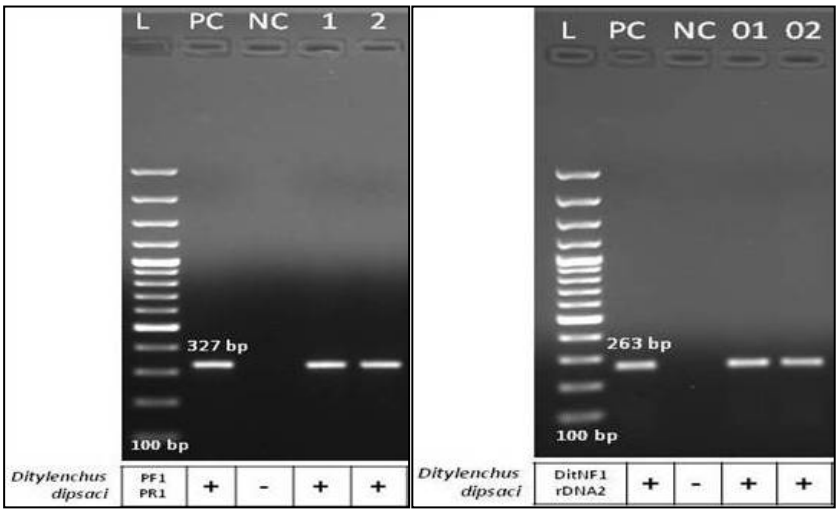


Figure 1. Agarose gels for molecular characterization of onion (1) and garlic (2) populations of *Ditylenchus dipsaci* with PF1-PR1 primer amplified a 327 bp for *D. dipsaci* and DitNF1-rDNA2 primer amplified 263 bp products for *D. dipsaci.* (L, 100 bp ladder; PC, positive control for *D. dipsaci*; and NC,distilled water negative control).

Both nematode populations were identified with all primers used in the study. Additionally, DITNF1-rDNA2, H05-H06, DipU F-DipU R and 18S-26S primers showed both populations were belonging to *D. dipsaci* sensu stricto (Subbotin et al., 2005; Esquibet et al., 2003; Marek et al., 2010). Considering complex nature of *D. dipsaci* species, identification of nematode populations using species specific primers provides useful information. However, host reactions of different plant species potentially grown in the area need to be tested against the local nematode populations for implementation of crop rotation strategies for control of the nematode. Additionally, use of genetically pure nematode cultures for susceptibility tests of plant species is important to eliminate variability in nematode virulence. Therefore, nematode cultures established from one female and male nematode were used in the current study.

**Susceptibility of plant species**

Typical symptoms of *D. dipsaci,* curling of leaves and stunting of pants, were observed on nematode inoculated bean, cucumber, eggplant, garlic, onion, pepper, potato, spinach, tomato and zucchini in the experiment. Mennan (2001) reported that damage symptoms were seen on bean, garlic, onion and tomato, but not on kale and wheat, supporting our results.

The highest nematode reproduction was obtained on garlic (RFs 12.8 and 5.64), tomato (RFs 8.72, and 6.98) and onion (RFs 7.35 and 5.46) for the onion and garlic populations of *D. dipsaci*, respectively. Those plants were determined to be excellent hosts for the two populations of *D. dipsaci*. The transformed total number of nematodes of garlic, tomato and onion was significantly different from alfalfa, daffodil, hyacinth, kale, leek, maize, oat, rye, strawberry and wheat with the onion population, and daffodil, alfalfa, barley, carrot, chickpea, hyacinth, kale, lettuce, maize, melon, oat, rye, sugar beet and tobacco with the garlic population.

The reproduction of the onion population of *D. dipsaci* was generally higher than of the garlic population. However, there was no significant difference between two nematode populations. Pea (RF 4.68) and spinach (RF 5.82) were excellent hosts for the onion population, and they were good hosts for the garlic population with RF 2.81 and 2.60, respectively. Cucumber was good host for both nematode

populations (RFs 2.84 and 2.70) (Table 3). Reactions of pepper (RFs 1.87 and 1.33), zucchini (RFs 1.40 and 1.96) and eggplant (RFs 1.42 and 1.46) were poor host with 1 < RF < 2 for both populations (Table 3). Potato (RFs 1.16 and 0.61) and bean (RFs 1.46 and 0.91) were poor host for the onion population but they were non-hosts for the garlic population with RF < 1 (Table 3). Alfalfa, barley, carrot, chickpea, daffodil, hyacinth, kale, leek, lettuce, maize, melon, oat, rye, strawberry, sugar beet, tobacco, tulip and wheat were considered as non-hosts with RF < 1 for both populations (Table 3).

Table 3. Untransformed mean numbers and reproduction factor (RF) values of *Ditylenchus dipsaci* onion and garlic populations per pot

| Plant species | Nematod number (mean ± SED) | | RF | |
|---|---|---|---|---|
| | Onion population | Garlic population | Onion population | Garlic population |
| Alfalfa (*Medicago sativa*) | 47 ± 27 d-g | 114 ± 60 d-g | 0.23 | 0.57 |
| Barley (*Hordeum vulgare*) | 165 ± 132 b-g | 20 ± 20 f-h | 0.83 | 0.10 |
| Bean (*Phaseolus vulgaris*) | 291 ± 142 a-g | 182 ± 64 c-g | 1.46 | 0.91 |
| Carrot (*Daucus carota*) | 53 ± 33 c-g | 20 ± 0 d-h | 0.27 | 0.10 |
| Chickpea (*Cicer arietinum*) | 72 ± 19 c-g | 60 ± 30 d-h | 0.36 | 0.30 |
| Cucumber (*Cucumis sativus*) | 569 ± 217 a-f | 540 ± 460 a-g | 2.84 | 2.70 |
| Daffodil (*Narcissus* spp.) | 111 ± 25 d-g | 160 ± 160 d-h | 0.56 | 0.80 |
| Eggplant (*Solanum melongena*) | 284 ± 112 a-g | 291 ± 129 a-f | 1.42 | 1.46 |
| Garlic (*Allium sativa*) | 2555 ± 1370 a | 1128 ± 260 ab | 12.8 | 5.64 |
| Hyacinth (*Hyacinthus orientalis*) | 33 ± 13 d-g | 28 ± 8 d-h | 0.17 | 0.14 |
| Kale (*Brassica olerac*) | 70 ± 21 e-g | 45 ± 19 d-h | 0.35 | 0.23 |
| Leek (*Allium ampeloprasum*) | 20 ± 0 d-g | 67 ± 29 a-h | 0.10 | 0.33 |
| Lettuce (*Lactuca sativa*) | 53 ± 18 b-g | 40 ± 12 d-h | 0.27 | 0.20 |
| Maize (*Zea mays*) | 54 ± 20 e-g | 10 ± 10 g-h | 0.27 | 0.05 |
| Melon (*Cucumis melo*) | 47 ± 18 b-g | 57 ± 26 d-g | 0.23 | 0.28 |
| Oat (*Avena sativa*) | 33 ± 6.7 e-g | 27 ± 4 e-h | 0.17 | 0.13 |
| Onion (*Allium cepa*) | 1470 ± 668 a-c | 1093 ± 411 a-c | 7.35 | 5.46 |
| Pea (*Pisum sativum*) | 936 ± 48 a-d | 562 ± 210 a-e | 4.68 | 2.81 |
| Pepper (*Capsicum annuum*) | 374 ± 102 a-f | 266 ± 98 a-g | 1.87 | 1.33 |
| Potato (*Solanum tuberosum*) | 231 ± 25 a-f | 122 ± 23 b-g | 1.16 | 0.61 |
| Rye (*Secale cereale*) | 108 ± 73 d-g | 0 ± 0 h | 0.54 | 0.00 |
| Spinach (*Spinacia oleracea*) | 1164 ± 559 a-e | 520 ± 169 a-d | 5.82 | 2.60 |
| Strawberry (*Fragaria vesca*) | 33 ± 10 f-g | 30 ± 10 b-h | 0.17 | 0.15 |
| Sugar beet (*Beta vulgaris*) | 60 ± 31 b-g | 20 ± 0 d-h | 0.30 | 0.10 |
| Tobacco (*Nicotiana tabacum*) | 50 ± 30 a-g | 80 ± 80 d-h | 0.25 | 0.40 |
| Tomato (*Solanum lycopersicum*) | 1745 ± 809 ab | 1396 ± 352 a | 8.72 | 6.98 |
| Tulip (*Tulipa gesneriana*) | 30 ± 10 b-g | 127 ± 107 a-h | 0.15 | 0.63 |
| Wheat (*Triticum aestivum*) | 20 ± 20 g | 30 ± 10 b-h | 0.10 | 0.15 |
| Zucchini (*Cucurbita pepo*) | 280 ± 46 a-f | 392 ± 297 a-g | 1.40 | 1.96 |

There was no distinct pattern for the botanical families for susceptibility to *D. dipsaci*. Although, some species were excellent and good hosts, other species from the same family were poor host; for example, pea in the Fabaceae is excellent and good host, whereas alfalfa, bean and chickpea in the same family were poor or non-hosts. However, all plant species in the Poaceae were non-hosts.

Seinhorst (1957) developed a host differential set to differentiate 11 races of *D. dipsaci*. This set includes alfalfa, daffodil, pea, potato, red clover, teasel, tulip and white clover. Edwards & Taylor (1963) investigated reaction of this host differential set to an onion population of *D. dipsaci* from Illinois, USA. They reported pea and potatoes as hosts, and alfalfa, clovers (both), daffodil, hyacinth, teasel and tulip as non-hosts. In our study, nematodes reproduced well on pea, but reproduction was lower on potatoes. We also found alfalfa, daffodil, hyacinth and tulip to be non-hosts for both populations of *D. dipsaci*. Hajihassani et al. (2016) investigated the susceptibility of different bean and pea cultivars to *D. dipsaci* garlic population from Canada and the results were in agreement with our results with pea cultivars excellent host, and bean cultivars poor hosts. Consistent with our results, reproduction factor of garlic as control plant was higher than 6 (excellent host) and wheat was less than 1 (non-host). Recently, Poirier et al. (2019) reported barley, carrot, lettuce and maize as non-hosts for a garlic population of *D. dipsaci* from Canada, and therefore

useful for rotation in *D. dipsaci*-infested garlic production areas in Canada, these results are similar to our results. They also found the nematode reproduced well on garlic and onion, and less well on potatoes, as we found in our study. In addition, Viglierchio (1971) and Janssen (1994) reported different host reactions for the same race of *D. dipsaci* from different locations. Therefore, it is essential to investigate host reactions of plant species to local *D. dipsaci* populations when planning crop rotations. Results from the current study will contribute to integrated pest management that use crop rotation in the nematode-infested plant production areas. Alfalfa, barley, carrot, chickpea, kale, leek, lettuce, maize, melon, oat, rye, sugar beet and wheat were found to be non-hosts in the current study, could be used as rotational crops with onion and garlic to reduce *D. dipsaci* population densities.

**Fresh weight and height of plant species**

A statistically significant decreases were recorded in plant weight of onion and tomato plants with the nematode inoculation with both nematode populations ($P < 0.05$). Fresh weights of onion plants inoculated with both nematode populations were 95% lower than the uninoculated control. Fresh weights of tomato plants were lower 56% and 81% lower in the treatments nematode inoculated with the onion and garlic populations than the uninoculated control, respectively (Table 4). Fresh weight of bean and cucumber were higher with the garlic population than onion nematode population and uninoculated control.

Table 4. Plant fresh weight of plant species in the onion and garlic population of *D. dipsaci* inoculated and uninoculated treatments and statistical differences among nematode treatments according to Tukey HSD test

| Plant species | Plant fresh weight (g ± SEM) and Tukey HSD group | | |
|---|---|---|---|
| | Onion population | Garlic population | Non-inoculated |
| Alfalfa (*Medicago sativa*) | 0.32 ± 0.12 a | 0.30 ± 0.08 a | 0.35 ± 0.18 a |
| Barley (*Hordeum vulgare*) | 1.41 ± 0.22 a | 1.43 ± 0.49 a | 1.77 ± 0.27 a |
| Bean (*Phaseolus vulgaris*) | 3.62 ± 0.45 b | 6.46 ± 0.84 a | 3.01 ± 0.67 b |
| Carrot (*Daucus carota*) | 0.10 ± 0.03 a | 0.19 ± 0.07 a | 0.13 ± 0.06 a |
| Chickpea (*Cicer arietinum*) | 3.44 ± 0.50 a | 4.50 ± 0.82 a | 4.31 ± 0.29 a |
| Cucumber (*Cucumis sativus*) | 1.43 ± 0.19 b | 2.86 ± 0.15 a | 1.36 ± 0.37 b |
| Daffodil (*Narcissus* spp.) | 37.5 ± 1.44 a | 43.1 ± 1.72 a | 38.4 ± 3.39 a |
| Eggplant (*Solanum melongena*) | 0.12 ± 0.01 a | 0.14 ± 0.02 a | 0.17 ± 0.01 a |
| Garlic (*Allium sativa*) | 4.16 ± 0.77 a | 2.69 ± 0.71 a | 3.13 ± 1.24 a |
| Hyacinth (*Hyacinthus orientalis*) | 66.8 ± 7.70 a | 70.7 ± 4.67 a | 70.6 ± 1.43 a |
| Kale (*Brassica oleracea*) | 3.01 ± 0.39 a | 2.84 ± 0.53 a | 3.42 ± 0.45 a |
| Leek (*Allium ampeloprasum*) | 0.04 ± 0.03 a | 0.38 ± 0.37 a | 0.04 ± 0.01 a |
| Lettuce (*Lactuca sativa*) | 1.19 ± 0.12 a | 1.84 ± 0.39 a | 1.88 ± 0.64 a |
| Melon (*Cucumis melo*) | 2.04 ± 0.17 a | 1.72 ± 0.29 a | 1.07 ± 0.23 a |
| Maize (*Zea mays*) | 4.37 ± 0.59 a | 3.74 ± 0.10 a | 4.27 ± 0.32 a |
| Oat (*Avena sativa*) | 0.89 ± 0.31 a | 1.11 ± 0.28 a | 1.77 ± 0.63 a |
| Onion (*Allium cepa*) | 0.01 ± 0.00 b | 0.01 ± 0.00 b | 0.19 ± 0.06 a |
| Pea (*Pisum sativum*) | 4.85 ± 0.45 a | 4.27 ± 0.55 a | 4.20 ± 0.63 a |
| Pepper (*Capsicum annuum*) | 0.14 ± 0.02 a | 0.22 ± 0.05 a | 0.28 ± 0.07 a |
| Potato (*Solanum tuberosum*) | 20.8 ± 2.66 a | 27.0 ± 1.82 a | 17.2 ± 1.83 a |
| Rye (*Secale cereale*) | 0.86 ± 0.14 a | 0.67 ± 0.28 a | 0.83 ± 0.06 a |
| Spinach (*Spinacia oleracea*) | 2.62 ± 0.18 a | 2.16 ± 0.30 a | 3.30 ± 0.89 a |
| Sugar Beet (*Beta vulgaris*) | 1.84 ± 0.30 a | 0.76 ± 0.50 a | 1.53 ± 0.54 a |
| Strawberry (*Fragaria vesca*) | 5.37 ± 0.84 a | 2.21 ± 0.61 a | 4.74 ± 0.95 a |
| Tobacco (*Nicotiana tabacum*) | 0.98 ± 0.12 a | 1.45 ± 0.31 a | 1.77 ± 0.74 a |
| Tomato (*Solanum lycopersicum*) | 0.86 ± 0.23 b | 0.35 ± 0.11 b | 1.94 ± 0.55 a |
| Tulip (*Tulipa gesneriana*) | 31.3 ± 1.07 a | 22.3 ± 5.58 a | 26.5 ± 2.85 a |
| Wheat (*Triticum aestivum*) | 2.18 ± 0.01 a | 1.29 ± 0.43 a | 1.43 ± 0.35 a |
| Zucchini (*Cucurbita pepo*) | 2.83 ± 0.72 a | 3.39 ± 1.34 a | 4.74 ± 1.56 a |

The height of pepper was significantly lower by 29% and 30% with the onion and garlic populations than the uninoculated plants, respectively ($P < 0.05$). The height of maize was higher in both nematode population than uninoculated control. The height of daffodil with the garlic population was higher than with then onion population and uninoculated control (Table 5).

Table 5. Height of plant species in the onion and garlic population of *D. dipsaci* inoculation and uninoculated treatments and statistical differences among nematode treatments according to Tukey HSD test

| Plant species | Plant height (cm ± SEM) and Tukey HSD group | | |
|---|---|---|---|
| | Onion population | Garlic population | Non-inoculated |
| Alfalfa (*Medicago sativa*) | 13.7 ± 3.8 a | 8.9 ± 2.0 a | 9.7 ± 1.7 a |
| Barley (*Hordeum vulgare*) | 39.3 ± 3.1 a | 42.5 ± 0.5 a | 37.3 ± 3.2 a |
| Bean (*Phaseolus vulgaris*) | 34.3 ± 6.4 a | 41.6 ± 6.4 a | 19.7 ± 0.3 a |
| Carrot (*Daucus carota*) | 3.5 ± 0.8 a | 5.7 ± 09 a | 6.0 ± 1.5 a |
| Chickpea (*Cicer arietinum*) | 24.0 ± 2.7 a | 32.0 ± 2.4 a | 28.7 ± 1.3 a |
| Cucumber (*Cucumis sativus*) | 6.4 ± 0.4 a | 7.8 ± 0.3 a | 5.8 ± 1.4 a |
| Daffodil (*Narcissus* spp.) | 19.0 ± 1.2 b | 36.5 ± 0.5 a | 19.0 ± 3.8 b |
| Eggplant (*Solanum melongena*) | 3.1 ± 0.2 a | 3.2 ± 0.2 a | 4.0 ± 0.0 a |
| Garlic (*Allium sativa*) | 30.4 ± 3.4 a | 33.0 ± 2.2 a | 32.8 ± 2.4 a |
| Hyacinth (*Hyacinthus orientalis*) | 11.3 ± 0.7 a | 17.0 ± 2.0 a | 19.3± 2.9 a |
| Kale (*Brassica oleracea*) | 10.3 ± 0.7 a | 12.3 ± 0.5 a | 11.2 ± 0.9 a |
| Leek (*Allium ampeloprasum*) | 6.0 ± 5.0 a | 6.0 ± 3.5 a | 11.3 ± 0.8 a |
| Lettuce (*Lactuca sativa*) | 4.8 ± 0.4 a | 5.6 ± 0.5 a | 5.0 ± 0.6 a |
| Maize (*Zea mays*) | 37.0 ± 1.9 a | 44.5 ± 0.5 a | 28.3 ± 0.9 b |
| Melon (*Cucumis melo*) | 14.0 ± 0.6 a | 12.5 ± 1.0 a | 9.0 ± 2.1 a |
| Oat (*Avena sativa*) | 40.0 ± 5.9 a | 44.3 ± 5.5 a | 42.3 ± 2.9 a |
| Onion (*Allium cepa*) | 6.4 ± 1.3 a | 9.9 ± 1.4 a | 11.2 ± 0.4 a |
| Pea (*Pisum sativum*) | 29.3 ± 1.6 a | 26.4 ± 1.8 a | 27.0 ± 4.2 a |
| Pepper (*Capsicum annuum*) | 4.1 ± 0.3 b | 4.1 ± 0.3 b | 5.8 ± 0.4 a |
| Potato (*Solanum tuberosum*) | 12.7 ± 1.2 a | 22.1 ± 3.7 a | 17.0 ± 6.0 a |
| Rye (*Secale cereale*) | 30.6 ± 4.3 a | 35.5 ± 4.5 a | 35.3 ± 4.9 a |
| Spinach (*Spinacia oleracea*) | 9.4 ± 0.9 a | 12.2 ± 2.5 a | 16.0 ± 6.1 a |
| Strawberry (*Fragaria vesca*) | 8.6 ± 0.9 a | 5.5 ± 1.5 a | 9.0 ± 1.2 a |
| Sugar beet (*Beta vulgaris*) | 11.0 ± 1.0 a | 8.8 ± 2.3 a | 8.5 ± 1.5 a |
| Tobacco (*Nicotiana tabacum*) | 9.0 ± 1.0 a | 9.3 ± 1.3 a | 9.2 ± 2.9 a |
| Tomato (*Solanum lycopersicum*) | 10.9 ± 1.7 a | 12.7 ± 4.9 a | 15.3 ± 3.2 a |
| Tulip (*Tulipa gesneriana*) | 25.0 ± 5.0 a | 9.7 ± 3.3 a | 13.0 ± 3.5 a |
| Wheat (*Triticum aestivum*) | 40.0 ± 0.0 a | 35.0 ± 2.0 a | 35.7 ± 1.8 a |
| Zucchini (*Cucurbita pepo*) | 10.6 ± 1.1 a | 9.8 ± 1.5 a | 12.0 ± 1.5 a |

Wright & Perry (2006) reported stunting and lose of weight duo to the tissue decay with *D. dipsaci* infection of plants. Plant weight and height was used successfully for interpretation of susceptibility of onion, pepper and tomato to *D. dipsaci* in the current study in consistent with those results. Significantly higher height of daffodil and maize and weight of bean and cucumber were recorded with nematode inoculated treatments were probably related to plant genetic and physiological properties and environmental conditions.

In conclusion, results of this study can be applied by growing of the poor and non-host plant species, such as alfalfa, barley, bean, carrot, chickpea, kale, leek, maize, oat, potato, rye, sugar beet and wheat in rotation with main hosts, onion and garlic, in areas in Central Anatolian Plateau that are infested with stem and bulb nematode. Future research should development of integrated control strategies including host rotation for areas in Central Anatolian Plateau in Turkey with *D. dipsaci* infestations.

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*Original article (Orijinal araştırma)*

# Effect of spinosad, azadirachtin and kaolin on *Stephanitis pyri* (Fabricius, 1775) (Hemiptera: Tingidae) under laboratory conditions

Laboratuvar koşullarında spinosad, azadirachtin ve kaolinin *Stephanitis pyri* (Fabricius, 1775) (Hemiptera: Tingidae) üzerine etkileri

**Hasan MARAL**[1*]

## Abstract

This study was conducted in Diyarbakır (Turkey) in 2019 to determine the effects of natural insecticides, spinosad, azadirachtin and kaolin, on the fourth instar nymphs and adults of *Stephanitis pyri* (Fabricius, 1775) (Hemiptera: Tingidae). The study was conducted under the laboratory conditions; 25 ± 1ºC, 65 ± 5% humidity and 16:8 h L:D photoperiod. The mortality of nymphs 3 and 6 d after treatment with spinosad, azadirachtin and kaolin was 49, 44 and 11%, and 54, 51 and 47%, respectively. The mortality of adults 3 and 6 d after treatment with spinosad, azadirachtin and kaolin was 75, 11 and 14%, and 89, 18 and 12%, respectively. The damage index, feeding damage ratio on the upper surface of the leaf, after spinosad, azadirachtin and kaolin treatment of nymphs was 1.25, 1.50 and 1.50, and adults was 1.00, 2.00 and 2.00, respectively. After the application of spinosad, azadirachtin and kaolin, the number of droppings left by nymphs was 55, 98 and 75, and adults was 15, 98 and 93, respectively. Based these data, spinosad was determined as the most effective product in all parameters except for the fourth instar nymph damage index. However, azadirachtin and kaolin were somewhat inhibitory to nymphs.

**Keywords:** Control, damage index, Diyarbakır, mortality ratio, natural insecticides

## Öz

Bu çalışma doğal insektisitlerden spinosad, azadirachtin ve kaolinin *Stephanitis pyri* (Fabricius, 1775) (Hemiptera: Tingidae)'nin dördüncü dönem nimfleri ve erginler üzerine olan etkilerini tespit etmek amacıyla 2019 yılında Diyarbakır (Türkiye)'da yürütülmüştür. Çalışma 25 ± 1ºC sıcaklık, %65 ± 5 orantılı nem ve 16:8 (A:K) aydınlatmalı laboratuvar koşullarında yürütülmüştür. Çalışma sonucunda spinosad, azadirachtin ve kaolin uygulaması sonrası toplam ölü nimf oranı üçüncü günde sırasıyla %49, %44 ve %11; altıncı günde sırasıyla %54, %51 ve %47 olarak gerçekleşmiştir. Spinosad, azadirachtin ve kaolin uygulaması sonrası toplam ölü ergin oranı üçüncü günde sırasıyla %75, %11 ve %14; altıncı günde sırasıyla %89, %18 ve %12 olarak gerçekleşmiştir. Yaprak yüzeyinde beslenme sonrası oluşan zarar oranını ifade eden zarar indeksi nimflerde spinosad, azadirachtin ve kaolinde sırasıyla 1.25, 1.50 ve 1.50; erginlerde sırasıyla 1.00, 2.00 ve 2.00 olarak tespit edilmiştir. Beslenme sonucu bıraktıkları dışkı sayısı spinosad, azadirachtin ve kaolinde sırasıyla nimflerde 55, 98 ve 75; erginlerde 15, 98 ve 93 olarak tespit edilmiştir. Bu veriler ışığında nimf zarar indeksi hariç çalışmada incelenen bütün parametrelerde spinosad en etkili ürün olarak tespit edilmiştir. Azadirachtin ve kaolin ise nimfleri baskı altına alma açısından ön plana çıkmıştır.

**Anahtar sözcükler:** Kontrol, zarar indeksi, Diyarbakır, ölüm oranı, doğal insektisitler

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## Introduction

*Stephanitis pyri* (Fabricius, 1775) (Hemiptera: Tingidae) is one of the most important species causing economic damage in many fruit trees, such as apple, apricot, cherry, hawthorn, pear, peach, walnut, in Turkey including almond [*Prunus amygdalus* (Linnaeus, 1758) (Rosales: Rosaceae)] (Lodos, 1982; Çam, 1993; Gülperçin & Önder, 1999; Schaefer & Panizzi, 2000; Çınar et al., 2004; Aysal & Kıvan, 2008; Maral et al., 2013). The nymphs and adults of *S. pyri* are phytophagous. They live on the undersides of leaves and feed by sucking plant sap after piercing the parenchyma tissue with their stylet. Whitish spots appear at the feeding sites. The damaged leaves fall prematurely. Thus, they weaken the plants and cause losses both in quality and yield (Bodenheimer, 1958; Nizamlıoğlu, 1961; Göksu, 1964; Drake & Ruhoff, 1965; Lodos, 1982; Péricart, 1983; Lodos & Önder, 1983; Gülperçin & Önder, 1999; Schaefer & Panizzi, 2000; Guilbert, 2001; Demirsoy, 2006). A study was conducted on the density of tingids on almond trees in Diyarbakır, Elazığ and Mardin Provinces and found that *S. pyri* is among the top three tingid species occurring at high densities (Bolu, 2007).

Organophosphate insecticides, such as malathion and dimethoate, are generally used against *S. pyri* in Turkey (Anonymous, 2020). Organophosphates are among the most widely used insecticides today. Pesticides in this group have many undesirable effects on humans and the environment, and can remain in the soil for long periods (Srivastava & Kesavachandran, 2019; Rakhimol et al., 2020). In recent years, IPM has been focusing on minimizing the damage caused by pesticides (Peshin & Pimentel, 2014). In IPM, using bacterial insecticides (e.g., spinosad), botanical insecticides (e.g., azadirachtin) and particle film technology (e.g., kaolin) to control pests has become increasingly important (Rakhimol et al., 2020).

Azadirachtin, a plant-derived insecticide, consisting of compounds from different parts of the neem tree [*Azadirachta indica* A. Juss, 1830 (Sapindales: Meliaceae)]. Azadirachtin is used as a broad-spectrum insecticide as well as bactericide and fungicide. Azadirachtin is effective on more than 400 insect species including species in the family Tingidae. Azadirachtin, which is lethal to insects, negatively affects the metamorphosis, feeding, oviposition and reproduction of insects. Azadirachtin gives useful control of insects that are resistant to synthetic pesticides. Azadirachtin is also an insect growth regulator (Isman, 2006; Saha et al., 2011; Pener & Dhadialla, 2012; Pavela et al., 2013; Sánchez-Ramos et al., 2014; Vacante & Kreiter, 2018; Joseph, 2019; Pamela, 2019).

Spinosad, a bacterial pesticide, is a natural insecticide obtained from the soil-based bacteria *Saccharopolyspora spinos*a Mertz & Yao, 1990 (Actinomycetales: Pseudonocardiaceae) (Vacante & Kreiter, 2018). Spinosad is used against many harmful insect species, including some species in the family Tingidae, and has a range of effects on insects including as a repellant, antifeedant, and growth and mating inhibitor (Isman, 2006; Matthews, 2006; Joseph, 2020).

Particle film technology increasingly used against insect, particularly using kaolin (Akgül & Özgen, 2017). Damage caused by some fruit flies in citrus decreased and less eggs were deposited after kaolin application. Kaolin generally shows repellent effects on insects. After kaolin application, a thin layer is formed on the leaf surface, and this layer prevents the insects from piercing epidermis and depositing eggs. This layer also makes it difficult for insects to find the plant as it changes color perception (Glenn, 2012; Vacante & Kreiter, 2018; Rakhimol et al., 2020).

There have been no studies on the effects of azadirachtin, spinosad and kaolin on *S. pyri*. Access to safe and healthy food is always a primary concern for consumers, so it is important to study the effects of natural insecticides on harmful insects, as these present low risks to human health. This study was conducted to determine the effects of spinosad, azadirachtin and kaolin on the fourth instar nymphs and adults of *S. pyri*. Using the most effective product, identified by this study, when overwintering adults of *S. pyri* become active will contribute lowering the population throughout the growing season. In addition, the findings of this study will also be useful in keeping the population density of nymphs and adults in summer below the economic threshold.

## Materials and Methods

### Preparing almond sapling for stock culture of *Stephanitis pyri*

The study was conducted in Diyarbakır between August and October 2019. In order to have sufficient *S. pyri* during the study, first suitable conditions were prepared for a stock culture. For this purpose, a two-year-old certified almond sapling obtained from Dicle University Faculty of Agriculture almond plantation (37º53'20.4" N, 40º16'17.0" E) was planted in a pot measuring 50 x 60 cm (height x diameter). In addition to the maintenance of the sapling, nitrogen fertilizer supplements were applied to ensure vegetative growth. The almond sapling was placed in a cage measuring 80 x 80 x 150 cm (length x width x height) covered in insect-proof mesh (Figure 1). The cage was placed in a sun-exposed place at the Diyarbakır GAP International Agricultural Research and Training Center (37º56'41.9" N, 40º15'29.2" E).

Figure 1. Cage used for stock culture (80 x 80 x 150 cm).

### Rearing *Stephanitis pyri* under laboratory condition

An 8-year-old almond orchard of the Diyarbakır GAP International Agricultural Research and Training Center, to which pesticide was not applied, was selected for collection of *S. pyri* for the stock culture. At the beginning of August, one branch of the two randomly selected trees was shaken gently, so the insects fell onto white cloth measuring 50 x 50 cm (width x length) and adults were collected with a mouth aspirator. In total, 150 adults were released on the caged almond sapling. Nymphs, hatching from eggs, started to fed under the leaves and were used as the stock culture.

To provide suitable conditions for nymphs and adults, 10 plastic containers, measuring 18 x 10 x 5 cm (length x width x height) were prepared (Figure 2). Two small holes were made on the both ends of the lid with a pin for ventilation. In order to keep the leaves alive as long as possible, the MS growth medium (Murashige & Skoogl, 1962) was used. The growth medium was dispensed into tubes 6.5 x 1.2 cm (height x diameter). A wooden platform measuring 5 x 2 x 3 cm (length x width x height) was prepared so that the

tubes can stand horizontally in the container. The platform was fixed to bottom. Almond leaves were obtained from Dicle University Faculty of Agriculture almond plantation. Healthy leaves, with no apparent diseases and pests were selected. Leafy shoots collected from the plantation were kept in the refrigerator (4ºC) in a black plastic bag and replaced every 2 d. These leaves are also used for treatments. The petiole of the leaf was placed in the tube containing the growth medium. The cap of the tube was covered with white modeling clay (Figure 2).

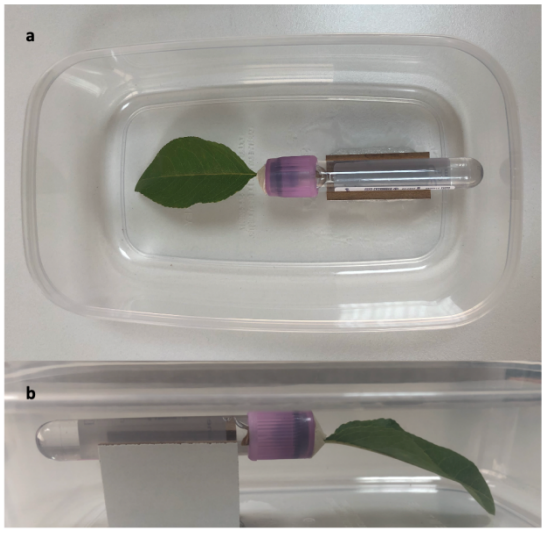


Figure 2. The container used to obtain nymphs and adults of *Stephanitis pyri*: a) top view, and b) side view.

A sufficient number of new generation adults were collected from the cage and brought to the laboratory in a plastic container. These adults were separated into 100 males and 100 females using the methods of Drake & Ruhoff (1965) and Péricart (1983). Five females and five males were placed in each container as indicated in Figure 2. The containers were placed in a growth chamber (Daihan Scientific, Wonju, Gangwon, South Korea) at 25 ± 1ºC, 65 ± 5% RH and 16:8 h L:D photoperiod. The containers were checked daily to see if females had laid eggs. After observing that the females laid enough eggs, the adults were removed from the containers. The containers with growth medium were renewed for the hatching of the eggs. The hatched nymphs were transferred to a container with the help of a soft tip brush. The growth chamber was checked daily and the nymphs were transferred to other containers according to their stages.

**Bioassays with fourth instar nymphs and adults**

Almond leaves used in the experiment were prepared as described above. In order to keep the leaves alive during the experiment (6 d), moistened cotton was wrapped around the stems (Figure 3). Petri dishes were secured with rubber bands to prevent insects from escaping. The study was conducted in the growth chamber (Daihan Scientific) located in the Laboratory of GAP International Agricultural Research and Training Center at 25 ± 1ºC, 65 ± 5% RH, and 16:8 h L:D photoperiod.

Aysal & Kıvan (2008) determined the life parameters of *S. pyri* on apple [*Malus domestica* Borkhausen (Rosales: Rosaceae)] under the laboratory conditions at 26 ± 1ºC, 60-70% RH and 16:8 h L:D photoperiod, and they found that the lowest nymph mortality (0%) was observed in the third, fourth and fifth instar stages. Based on these data, the fourth instar was used in this study. Fourth instar nymphs younger than 24 h old were selected for four product replicates and four control replicates for each product. Six fourth instar nymphs were placed in each Petri dish by means of a soft tip brush. In this way, six fourth instar nymphs were placed in each of eight Petri dishes with a total of 48 fourth instar nymphs used for each product. A total of 144 fourth instar nymphs were used for the three products in the study.

Figure 3. Petri dish used in the study.

For adult's selection, nymphs were monitored regularly and adults younger than 24 h old were selected for the study. Six adults, three females and three males, were placed in each Petri dish by using a soft tip brush. Six adults were placed in each of the eight Petri dishes, four product replicates and four control replicates for each product with a total of 48 adults were used for each product. A total of 144 adults, 72 females and 72 males, were used in the study for three products.

**Insecticide exposure test**

The products used and the doses applied are given in Table 1. A 500-ml capacity hand spray bottle was used to apply the products to the leaves. After the products were prepared, they were sprayed from the same distance until the whole leaf was wet, and the leaves were then allowed to dry. Dried leaves were then transferred to Petri dishes. Pure water was used for the leaves in the control group.

Table 1. Natural insecticides used against *Stephanitis pyri* in the study

| Trade name | Active ingredient | Concentration | Recommended maximum field concentration | Supplier | Concentration applied |
|---|---|---|---|---|---|
| Nimbecidine | azadirachtin | 0,3 g $L^{-1}$ (EC) | 5 mL $L^{-1}$ | Agrobest Grup Tarım İlaçları Toh. İml. İth. İhr. San. ve Tic. A.Ş.- İzmir/Türkiye | 1.5 mg $L^{-1}$ |
| Laser | spinosad | 480 g $L^{-1}$ (SC) | 0.2 mL $L^{-1}$ | Dow AgroSciences A.Ş.- Ataşehir/İstanbul | 96 mg $L^{-1}$ |
| Utelka Kaolin Kili | kaolin | 950 gr $kg^{-1}$ (WP) | 50 mg $L^{-1}$ | Utelka Maden San. Tic. Ltd. Şti.- Sındırgı/Balıkesir | 47.5 mg $L^{-1}$ |

**Mortality ratio**

Nymphs and adults placed in the growth chamber were observed for 6 d. The Petri dishes were checked on day 3 and 6 and the mortality ratio calculated. For the determination of dead insects, the Petri dishes were taken out of the growth chamber, the lid of the Petri dish was opened and the individuals were observed. Individuals that fell to the bottom of the Petri dish were presumed dead and transferred to another Petri dish. These dead individuals were observed for 30 min and included in the calculations after making sure that they died. After calculating mortality ratios using the method specified in the statistical analysis section of this manuscript, corrected mortalities were found.

**Feeding activity**

After 6 d, the leaves in the Petri dishes were examined to determine the feeding activity and damage rate of the pest. Tingidae family species leave behind black sticky excrements under the leaf after feeding. In some cases, as a result of intensive feeding, the entire lower surface of the leaf is covered with excrement, which prevents the leaf from photosynthesis and makes it susceptible to disease and pest attacks. The amount of excrement left by *S. pyri* was included in the calculations because it is an important indicator of pest density and damage rate. To measure the feeding activity of *S. pyri*, the lower surfaces of the leaves were examined and the deposits of excrement they left after feeding was counted (Figure 4).

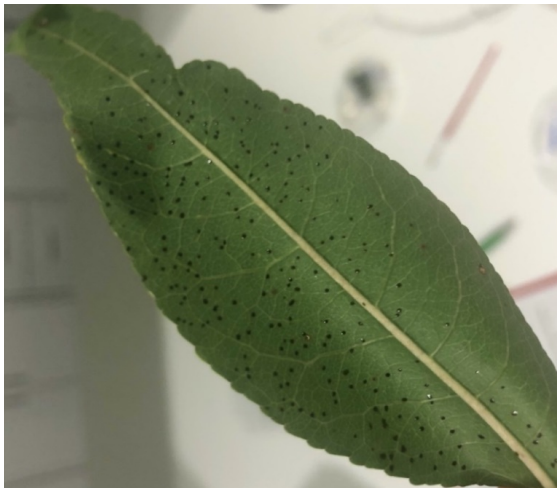

Figure 4. Deposits of excrement on the lower surface of leaf left by fourth instar nymphs and adults of *Stephanitis pyri* after feeding.

**Damage rate**

Tingidae family species live on the undersides of the leaves and feed on by sucking plant sap by piercing the parenchyma tissue with their stylet. Whitish spots appear at the feeding sites. The density of these whitish spots on the leaf surface is an important indicator of pest density and damage rate. The damage index was based on this parameter using the method developed by Sánchez-Ramos et al. (2014) for the analyzing of feeding damage (Figure 5) on the upper surface of the leaf after feeding fourth instar nymphs and adults: 0, no damage on the leaf surface; 1, a third of the leaf surface is damaged; 2, between one and two thirds of the leaf surface is damaged; and 3, more than two thirds of the leaf surface is damaged.

Figure 5. Feeding damage on the upper surface of the leaf after feeding fourth instar nymphs and adults of *Stephanitis pyri*.

### Statistical analysis

Total mortality data of fourth instar nymphs and adults were transformationed [√(x/100)]. Corrected mortalities were found by using the method suggested by Abbott (1925).

$$\text{Corrected mortality} = \frac{\text{(\% observed mortality - \% control mortality)}}{\text{(100 - \% control mortality)}}$$

All the other parameters were transformed by √(x + 1). The level of significance was $P < 0.05$ in all cases. All parameters were analyzed by means of mixed-model factorial analysis of variance with four replicates. In cases where the examined features were found to be statistically significant ($P < 0.05$), the averages of the applications were grouped by the multiple comparison test, LSD ($P < 0.05$) test. Correlation analyses were made between the examined features. Variance analysis, LSD ($P < 0.05$) and correlation analyses were performed using JMP 7.0 (SAS Institute Inc., Cary, NC, USA) statistical package.

## Results

### Mortality

The mortality of nymphs with of spinosad, azadirachtin and kaolin on day 3 was 49, 44 and 11% and on day 6 was 54, 51 and 47%, respectively. Maximum mortality of nymphs on the third day was with spinosad and azadirachtin (Table 2).

Table 2. The mortality ratios of fourth instar nymphs of *Stephanitis pyri* on 3 and 6 day after kaolin, spinosad and azadirachtin application (n = 24)

| | Mortality (%) | | Corrected mortality (%) | |
|---|---|---|---|---|
| | Day 3 | Day 6 | Day 3 | Day 6 |
| Kaolin | 21 ± 7.3 bc | 59 ± 11.2 | 11 b | 47 |
| Spinosad | 55 ± 5.5 a | 64 ± 4.2 | 49 a | 54 |
| Azadirachtin | 50 ± 13.0 ab | 62 ± 16.1 | 44 a | 51 |
| Control | 11 ± 5.9 c | 22 ± 8.2 | | |
| F (df = 3,9) | 48.3 | 2.46 | 48.3 | 2.46 |
| P | 0.02 | 0.12 | 0.02 | 0.12 |
| Mean | 34 | 52 | 35 | 50 |
| CV (%) | 57 | 49 | 56 | 48 |
| LSD (%) | 31.3 | ns | 30.4 | ns |

The mortality of adults with spinosad, azadirachtin and kaolin on day 3 was 75, 11 and 14%, and on day 6 is 89, 18 and 12%, respectively. Maximum mortality of adults on day 3 and 6 was with spinosad (Table 3).

Table 3. The adult mortality ratios of *Stephanitis pyri* on 3 and 6 day after kaolin, spinosad and azadirachtin application (n = 24)

| | Mortality (%) | | Corrected mortality (%) | |
|---|---|---|---|---|
| | Day 3 | Day 6 | Day 3 | Day 6 |
| Kaolin | 14 ± 4.7 b | 18 ± 3.2 b | 14 b | 12 b |
| Spinosad | 75 ± 5.6 a | 90 ± 2.6 a | 75 a | 89 a |
| Azadirachtin | 12 ± 7.4 b | 23 ± 7.0 b | 11 b | 18 b |
| Control | 1 ± 4.3 b | 6 ± 5.0 b | | |
| F (df = 3,9) | 268 | 45.0 | 268 | 47.0 |
| P | <0.0001 | <0.001 | <0.001 | <0.001 |
| Mean | 25 | 34 | 33 | 34 |
| CV (%) | 51 | 32 | 51 | 32 |
| LSD (%) | 20.8 | 17.6 | 20.3 | 17.6 |

### Damage index

The damage index of spinosad, azadirachtin and kaolin for nymphs is 1.25, 1.50 and 1.50, respectively and the only significant difference was between the treatments and the control (Table 4).

The damage index of spinosad, azadirachtin and kaolin for adults was 1.00, 2.00 and 2.00, respectively, and, there was a significant difference between spinosad and the control (1.00 versus 2.53).

Table 4. Damage index* for *Stephanitis pyri* with kaolin, spinosad and azadirachtin application (n = 24)

| | Fourth instar nymphs | Adults |
|---|---|---|
| Kaolin | 1.50 ± 0.38 a | 2.00 ± 0.37 a |
| Spinosad | 1.25 ± 0.16 a | 1.00 ± 0.10 b |
| Azadirachtin | 1.50 ± 0.47 a | 2.00 ± 0.36 a |
| Control | 1.88 ± 0.20 a | 2.53 ± 0.10 a |
| F | 0.476 | 4.21 |
| Df | 0.70 | 0.04 |
| P | 1.53 | 1.88 |
| Mean | 50 | 33 |
| CV (%) | ns | 0.99 |
| LSD (%) | ns | 0,99 |

* Damage index: 0, no damage on the leaf surface; 1, a third of the leaf surface is damaged; 2, between one and two thirds of the leaf surface is damaged; and 3, more than two thirds of the leaf surface is damaged.

### Feeding activity

After application of spinosad, azadirachtin and kaolin, the deposits of excrement left by nymphs were 55, 98 and 75, respectively, with kaolin (75 versus 167) and spinosad (55 versus 167) being significantly different from the control (Table 5). After the application of spinosad, azadirachtin and kaolin, the deposits of excrements left by adults were 15, 98 and 93, respectively, with spinosad significantly different (15 versus 121) from the control (Table 5).

Table 5. The deposits of excrement per leaf left by fourth instar nymphs and adults of *Stephanitis pyri* after kaolin, spinosad and azadirachtin application (n = 24)

| | Fourth instar nymphs | Adults |
|---|---|---|
| Kaolin | 75 ± 19.6 b | 93 ± 9.2 a |
| Spinosad | 55 ± 8.4 b | 15 ± 5.5 b |
| Azadirachtin | 98 ± 28.6 ab | 98 ± 19.4 a |
| Control | 167 ± 14.1 a | 121 ± 6.2 a |
| F (df = 3, 9) | 4.82 | 12.1 |
| P | 0.029 | 0.002 |
| Mean | 99 | 82 |
| CV (%) | 45 | 32 |
| LSD (%) | 70.8 | 42.4 |
| LSD (%) | 70,78 | 42,410 |

### Correlation between variables

Negative correlations were found between the deposits of excrement left after feeding and the total mortality and between the damage index and the total mortality. Positive correlations were found between the damage index and the deposits of excrement left after feeding (Table 6).

Table 6. Correlation analysis of the three response variables measured in this study for fourth instar nymphs and adults of *Stephanitis pyri* after kaolin, spinosad and azadirachtin application

| Comparison | Fourth instar nymphs | | Adults | |
|---|---|---|---|---|
| | R | P | R | P |
| Excrement vs total mortality (%) | -0.930 | <0.001 | -0.871 | <0.001 |
| Damage index vs total mortality (%) | -0.810 | <0.001 | -0.685 | 0.0034 |
| Damage index vs excrement | 0.810 | <0.001 | 0.822 | <0.001 |

## Discussion

In the present study, spinosad was determined as the most effective product in all parameters except the nymph damage index. Spinosad suppressed the feeding activity of *S. pyri* and as a result, the lowest damage index values were obtained. No studies on the effects of spinosad on *S. pyri* have previously been published. However, in a study conducted under the laboratory conditions on the repellency of some insecticides, including spinosad, on *Stephanitis pyrioides* (Scott, 1874) (Hemiptera: Tingidae), spinosad was found to be a repel *S. pyrioides* (Joseph, 2020). In the present study, the repellency of spinosad was not studied. It is considered that, however, the significant suppressive effect of *S. pyri* on feeding activity is a potential indictor of repellency. In another study, spinosad with added 1% petroleum caused a low mortality rate of 10% in adults of avocado lace bug, *Pseudacysta perseae* (Heidmann, 1908) (Hemiptera: Tingidae), which was probably due to the feeding habits of this pest (Humeres et al., 2009). The results of that study differ from the present study probably as a consequence of different doses and different target pest.

In the present study, it was determined that azadirachtin had useful effects on mortality rates of fourth instar nymphs. In terms of total mortality of fourth instar nymphs on day 3, azadirachtin showed a significant difference from spinosad as compared to the control. No studies on the effects of azadirachtin on *S. pyri* have previously been published. Sánchez-Ramos et al. (2014) found that azadirachtin caused a high mortality (97%) in the fourth instar nymphs, and 32% in adults of *Monosteira unicostata* (Mulsant & Rey, 1852) (Hemiptera: Tingidae), one of the most important pests of almonds in Spain, at similar application rates to the present study. The researchers reported that azadirachtin being a growth regulatory was contributing to nymphal mortality. According to Joseph (2019), azadirachtin caused transovarial activity and as a result, there was a decrease in the number of nymphs hatching from eggs of adults with azadirachtin applied as a topical spray compared to the control. In another study injecting azadirachtin in the trunks of *Platanus* sp. under natural conditions, it was found that the density of nymphs and adults of *Corythucha ciliata* (Say, 1832) (Hemiptera, Tingidae) decreased significantly over 2 years compared to the control (Pavela et al., 2013). Similarly, azadirachtin was found to cause a high mortality of fourth instar nymphs in the present study.

In the present study, it was determined that kaolin had less impact on both fourth instar nymph and adult mortality compared to spinosad and azadirachtin. Although, kaolin significantly reduced on the feeding of fourth instar nymphs compared to the control, It did not significantly reduce the damage index for fourth instar nymphs and adults, and the feeding of adults. No studies on the effect of kaolin on *S. pyri* have previously been published. In a study of the effects of kaolin on nymphs and adults of *M. unicostata*, the mortality of fourth instar nymphs and adults on day 6 was 43 and 44%, respectively (Sánchez- Ramos et al., 2014) and kaolin significantly suppresses the reproduction and feeding of *M. unicostata*. In the present study, it was similarly determined that after application of kaolin, feeding of nymphs decreased, and kaolin increased the mortality fourth instar nymph and adult. In a field study with kaolin in 2009 and 2010, Marcotegui et al. (2015), determined that the density of *M. unicostata* halved in 2009 and damage decreased by 26%, and in 2010, the density decreased by a third and the damage decreased by 11%. Since we did not perform any application under the field conditions in the present study, it was not possible

to compare our data with that study. However, it is considered that the pest density may have decreased due to the fact that kaolin suppresses nymphs similarly in the present study.

The present study, spinosad was determined to be the most effective product in all parameters except for the fourth instar nymphs damage index. Spinosad gave higher mortality of adults than nymphs. Therefore, it is considered that spinosad can be used to control overwintering adults as the reemerge in spring and also to decrease the population density in the summer when the adult density is high and the generation time is short. Kaolin, which had significant effects on the feeding of the fourth instar nymphs, and azadirachtin, which caused high mortality in the fourth instar nymphs, are considered to be able to decrease the population density of *S. pyri* if applied on first generation nymphs. To confirm this suggestion, it is recommended that the effects of spinosad, kaolin, and azadirachtin on *S. pyri* and its natural enemies be studied under field conditions. In addition, the effects of these products on nymphs and adults of *S. pyri* should be studied in detail by contact application under both laboratory and field conditions, because only the leaf exposure method was used in the present study.

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*Original article (Orijinal araştırma)*

# *Cercopis sanguinolenta* (Scopoli, 1763) (Hemiptera: Auchenorrhyncha: Cercopidae) dilemma and redescription of rare *Cercopis* Fabricius, 1775 species from Turkey[1]

*Cercopis sanguinolenta* (Scopoli, 1763) (Hemiptera: Auchenorrhyncha: Cercopidae) ikilemi ve Türkiye'den nadir *Cercopis* Fabricius, 1775 türlerinin yeniden tanımlanması

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## Abstracts

This study was conducted to determine the Cercopidae Leach, 1815 (Hemiptera: Auchenorrhyncha) fauna of the Central and Southern Kuseyr Plateau (Hatay/Turkey). For this purpose, samples were collected with a net, hand and tweezers from the different habitats of the region in April-June 2014-2015. However, in the process of evaluating the samples, it was found that *Cercopis sanguinolenta* (Scopoli, 1763) and *Cercopis intermedia* Kirschbaum, 1868 species were frequently confused with each other. This is because two different approaches were adopted that have resulted in a dilemma over the type of *C. sanguinolenta* before the "Principle of Priority" was established. With this study, not only the current controversy is eliminated, but also redescriptions of *Cercopis distincta* (Melichar, 1896), *C. intermedia* and *Cercopis septemmaculata* (Melichar, 1903) is made with informative images and morphometric data. Among these species, complete locality record has been provided for an uncertain Turkey records of *C. septemmaculata* and the first local record of *C. distincta*, which is defined as an endemic species in Turkey but whose terra typica is unknown, was also revealed with this study. New distribution maps of these species are presented with local and worldwide distributional data, and also an identification key is given, including other *Cercopis* Fabricius, 1775 species that occur in Turkey.

**Keywords:** *Cercopis distincta*, *Cercopis intermedia*, *Cercopis septemmaculata*, fauna

## Öz

Bu çalışma, Orta ve Güney Kuseyr Platosu'nun (Hatay/Türkiye) Cercopidae Leach, 1815 (Hemiptera: Auchenorrhyncha) faunasını belirlemek için yapılmıştır. Bu amaçla 2014-2015 yıllarının Nisan-Haziran aylarında, bölgenin farklı habitatlarından; ağ, el ve pensle örnekler toplanmıştır. Ancak örneklerin değerlendirilmesi sürecinde *Cercopis sanguinolenta* (Scopoli, 1763) ve *Cercopis intermedia* Kirschbaum, 1868 türlerinin sıklıkla birbirleriyle karıştırıldığı görülmüştür. Bunun nedeni, "Öncelik İlkesi" tesis edilmeden önce *C. sanguinolenta*'nın tipi üzerinde bir ikilemle sonuçlanan iki farklı yaklaşımın benimsenmiş olmasıdır. Bu çalışma ile sadece mevcut tartışma ortadan kaldırılmakla kalınmaz, aynı zamanda *Cercopis distincta* (Melichar, 1896), *C. intermedia* ve *Cercopis septemmaculata* (Melichar, 1903) türlerinin, aydınlatıcı görseller ve morfometrik verilerle birlikte yeniden tanımlanmaları yapılmaktadır. Bu türlerden *C. septemmaculata*'nın kesin olmayan Türkiye kayıtları için tam lokalite kaydı sağlanmış ve ülkemize endemik bir tür olarak tanımlanan ancak terra tipi dahi bilinmeyen *C. distincta*'nın ilk yerel kaydı da yine bu çalışma ile ortaya konulmuştur. Yerel ve dünya yayılış verileri ile bu türlerin yeni dağılım haritaları sunulmakta ve ayrıca Türkiye'de görülen diğer *Cercopis* Fabricius, 1775 türlerini de kapsayan bir teşhis anahtarı verilmektedir.

**Anahtar sözcükler:** *Cercopis distincta*, *Cercopis intermedia*, *Cercopis septemmaculata*, fauna

[1] This study was a part of a master's degree thesis of the second author's that completed at the Graduate School of Natural and Applied Sciences of Hatay Mustafa Kemal University in 2019. The preliminary results of the data obtained from this thesis have been shared as an oral presentation at the "8th European Hemiptera Congress" in Katowice-Zawiercie, Poland, on 24-29 June in 2018.

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## Introduction

Cercopoidea Leach, 1815 (Hemiptera: Cicadomorpha), one of the superfamilies of Cicadomorpha Evans, 1946 (Hemiptera: Auchenorrhyncha), is represented in the world by 366 genera and 2,612 (2,500-3,000) species (Bartlett et al., 2018; Soulier-Perkins, 2020). However, only 42 genera and 158 species are recorded in the Palearctic (Nast, 1972, 1987; Bartlett et al., 2018) (Table 1). Of the species in Palearctic, 51 belong to the Aphrophoridae Amyot & Serville, 1843, 93 belong to the Cercopidae and 14 belong to the Machaerotidae Stål, 1866 (Brambila & Hodges, 2008; Soulier-Perkins, 2020). Despite some doubtful records, the previous studies have shown that this superfamily is represented by 22 species in Turkey, 15 of which are Aphrophoridae and seven of which are Cercopidae (Nast, 1972; Lodos & Kalkandelen, 1981, 1988; Önder et al., 2011).

Table 1. The distribution of Cercopoidea (Hemiptera: Auchenorrhyncha) genera and species by family and zoogeographical areas (Bartlett et al., 2018)

| Regions / Taxa | NEA[a] | | NEO | | PAL | | IND | | AFR | | AUS | | OCE | | World | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Ge[b] | Sp | Ge | Sp | Ge | Sp | Ge | Sp | Ge | Sp | Ge | Sp | Ge | Sp | Ge | Sp |
| Cercopoidea | 12 | 48 | 71 | 482 | 42 | 158 | 91 | 726 | 46 | 239 | 24 | 61 | 44 | 233 | 366 | 2 612 |
| Aphrophoridae | 5 | 8 | 4 | 4 | 17 | 51 | 11 | 24 | 9 | 35 | 11 | 28 | 16 | 58 | 157 | 925 |
| Cercopidae | 6 | 8 | 63 | 418 | 20 | 93 | 56 | 620 | 32 | 196 | 8 | 22 | 24 | 165 | 173 | 1 480 |
| Clastopteridae | 1 | 32 | 1 | 56 | 0 | 0 | 2 | 3 | 0 | 0 | 0 | 0 | 0 | 0 | 3 | 85 |
| Epipygidae | 0 | 0 | 3 | 4 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 3 | 4 |
| Machaerotidae | 0 | 0 | 0 | 0 | 5 | 14 | 22 | 79 | 5 | 8 | 5 | 11 | 4 | 10 | 30 | 118 |

[a] Zoogeographic Regions: NEA, Nearctic Region; NEO, Neotrophic Region; PAL, Palearctic Region; IND, Indomalayan Region; AFR, Afrotropical Region; AUS, Australasia Region; OCE, Oceania Region.
[b] Hierarchical Categories: Ge, Genera; Sp, Species.

Cercopidae, the largest family of the Cercopoidea, are characterized by bright colors and patterns, and because of their production of large amounts of protective foam they are called spittlebugs (Carvalho & Webb, 2005). Numerous Cercopidae species have patterns with a combination of red, orange and yellow with black (Bartlett et al., 2018). Most of the Cercopids are aposematically colorful and many exhibits reflex bleeding (Peck, 2000).

It is possible to classify the studies that have conducted on Cercopidae that included one of the two families in the Cercopoidea in Turkey from the Palearctic Region, as follows: nomenclatural changes (Puton, 1881; Cavanna, 1882; Royer, 1906; Kirkaldy, 1907), checklists (Fabricius, 1775; Le Peletier de Saint-Fargeau & Audinet-Serville, 1825; Walker, 1851; Fieber, 1872; Oshanin, 1910; Lallemand, 1912; Metcalf, 1961; Nast, 1972, 1987), descriptions of new subspecific forms (Péneau, 1912; Haupt, 1919, 1922; Nast, 1933; Lallemand, 1949), an encyclopedic article (Olivier, 1797) and new taxon description studies. When these studies were evaluated as a whole, it was found that there is longstanding confusion regarding the diagnosis of *Cercopis* Fabricius, 1775 genus-group species. Given that many *Cercopis* spp. sensu lato, which are now considered as species, either have been incorrectly synonymized or were defined as subspecific forms. Indeed, it can be seen from the erroneous diagnoses in some studies conducted recently that this dilemma still continues. One of the reasons for this is that almost all of the work was reported only textually and contained little or insufficient images. However, the main reasons are, it is difficult to readily diagnose Cercopidae (Carvalho & Webb, 2005), the diagnostic keys alone are insufficient due to the convergences seen in color patterns between taxa and the distinctive characters between male genital structures are limited, especially as reported in the literature (Cryan, 2005).

It is clearly understood from the frequent synonym errors made that the most difficult species in the diagnosis of this group are *Cercopis sanguinolenta* (Scopoli, 1763) and *Cercopis intermedia* Kirschbaum,

1868, which are often confused with each other. The main reason for this is, two different type descriptions were adopted by different researchers for *C. sanguinolenta* before the “Principle of Priority” was established. While one group performed their studies using the Scopoli's description, which is currently accepted, other studies were based on Linnaeus’s description. In many cases, the interpretations were made assuming these two descriptions to be the same, and this has allowed the problem to persist.

Cercopids are represented by three genera and seven species in Turkey, of these five species belong to the genus *Cercopis*. However, the existence of *Cercopis septemmaculata* (Melichar, 1903) has been always seen as a doubtful record. In this study, the status of three *Cercopis* spp. has been occurring the Central and Southern parts of Kuseyr Plateau (Hatay, Turkey) has been reassessed with the addition of new data. Accordingly, *C. septemmaculata*, *Cercopis distincta* (Melichar, 1896) and *C. intermedia* have been redescribed using morphometric data for the important taxonomic characters and supported by detailed images that have not provided by the previous researchers. Local and Palearctic distribution maps of these species are updated, "Turkish *Cercopis* Fabricius, 1775 Identification Key" is included with the addition of the two other *Cercopis* spp. that occur in Turkey along with some zoogeographical comments on these species.

## Materials and Methods

The samples were collected from 12 habitats and altitudes of the Central and Southern Kuseyr Plateau (Hatay) by hand, tweezers and sweep net in April to June 2014-2015.

The collected samples were killed in jars containing 70% ethanol and then stored as dried specimens. After morphological examination, in order to dissecting the genital structures of males, the dry pinned samples were moisturized and their genital capsules extracted under Boeco BSZ-405 model stereo microscope and treated with 10% KOH for 15 min by the bain-marie method.

Local, Turkey and Palearctic distribution maps of species were prepared with the ArcView v3.3 software using the data obtained from a Garmin Monterra GPS (Global Positioning System) and by compiling details of previous studies (Nast, 1972, 1987; Lodos & Kalkandelen, 1981, 1988; Holzinger et al., 2003; Önder et al., 2011). The photos of the dorsal, lateral and ventral habitus of the diagnosed species were with a Nikon D750 camera with Nikon AF-S VR Micro-NIKKOR 105mm f/2.8G IF-ED lens, and the photos of the male genitals have been taken with Leica S9D model trinocular stereo microscope attached to a Nikon D750 camera, and then these photos were edited using GIMP (GNU Image Manipulation Program) software. For the terminology of morphology and genital parts, Ossiannilsson et al. (1970) and Lallemand (1949) were followed. The diagnosed samples are preserved in the Zoology Research Laboratory of the Department of Biology at the Faculty of Arts and Science in Hatay Mustafa Kemal University.

## Results

Through evaluating 42 samples, 22♂♂ and 20♀♀ collected from the field, three *Cercopis* spp. were determined as occurring in the study area. The diagnosed species with relevant details are listed alphabetically below.

***Cercopis distincta* (Melichar, 1896)**

*Triecphora distincta* Melichar, 1896

**Redescription/Diagnosis**

The male length is 12 mm with wings and 9.2 mm without wings (Figure 1a-c) (Table 2). The vertex, frontal plate and the longitudinal slits between the ocelli and compound eyes have almost identical characteristics as reported in previous studies. However, these are approximately at the same level in

lateral view and the two pits behind the frontal plate are divided the back edge of the frontal plate into approximately three equal parts. The tempe is divided into two equal parts with the bases of antennae. The postclypeus is domed or tulip-like, because of its longitudinal carina that is only prominent in the middle. The pronounced transverse grooves are parallel to each other and to the ground in lateral view but their back tips are appeared to be bent downwards in ventral view. Head is completely with long yellow setulae, except the area between the compound eyes and the postclypeus.

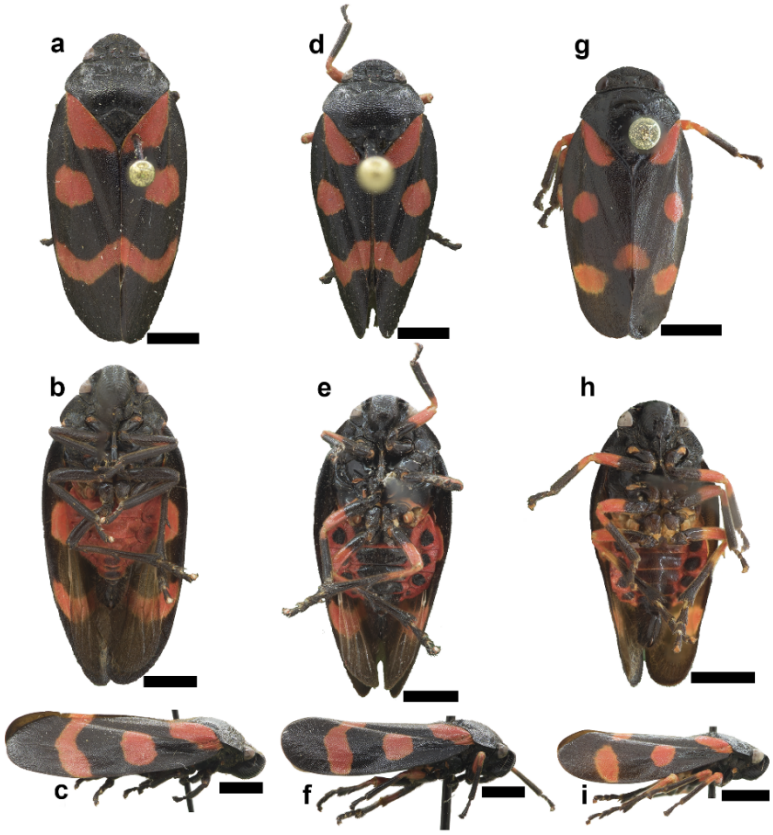


Figure 1. Dorsal, ventral and lateral views of *Cercopis* spp. occurring in the Central and Southern parts of Kuseyr Plateau (Hatay, Turkey). *Cercopis distincta*: a) dorsal habitus, b) ventral habitus, c) lateral habitus; *Cercopis intermedia*: d) dorsal habitus, e) ventral habitus, f) lateral habitus; *Cercopis septemmaculata*: g) dorsal habitus, h) ventral habitus, i) lateral habitus (scale bar = 2 mm).

The dorsal appearance of the pronotum is hexagonal. The front side edges are slightly raised to upward laterally. The front edge of this hexagon is almost straight and its back edge is perceived as having two spherical lobes. There are shallow but large symmetrical depressions on both sides of the pronotum with a vaguely longitudinal carina up to the front half of its midline. Due to the structure of the postclypeus, the head is long and the pronotum is distinctly in the form of hump in lateral view.

The wings have patterns of red on black and these are composed of four red spots and a red stripe. Two of the red spots extending towards the corium are at the anterior end of the clavus, and the back edges perpendicular to the body are recessed and aligned with the back end of the scutellum. The other two spots are located in the middle of corium and they seem to be rectangular. The outer edges of these spots do not reach the costa; the inner edges contact the claval suture but do not cross the clavus. The tips of the red stripe at the posterior of the wings are more or less touching the costa and they resembles the end of a scimitar. The stripe width is consistent over the rest of the area (Figure 1a, c).

The legs are entirely black, except that the outside of the hind tibia is yellowish-brown. There are two thorns with the same color on the outer part of this tibia (Figure 1b).

Table 2. Important morphometric data of *Cercopis* spp. determined for specimens collected in the study area

| Character | *C. distincta* | *C. intermedia* | *C. septemmaculata* |
|---|---|---|---|
| Vertex width/length | 2.0 | 2.2 | 2.4 |
| Frontal plate width/length | 1.6 | 1.4 | 1.6 |
| Diameter of pits (µm) | 41.0 | 66.0 | 37.0 |
| Distance between pits (µm) | 238.0 | 195.0 | 168.0 |
| Distance between pits and lateral edges of frontal plate (µm) | 273.0 | 149.0 | 209.0 |
| Compound eye length/width (Dorsal) | 1.8 | 1.7 | 1.7 |
| Compound eye length/width (Ventral) | 1.4 | 1.5 | 1.2 |
| Distance between compound eyes (µm) | 1717.0 | 1615.0 | 1509.0 |
| Distance between compound eyes and frontal plate (µm) | 488.0 | 522.0 | 448.0 |
| Distance between compound eyes and posterior edge of vertex (µm) | 136.0 | 117.0 | 74.0 |
| Diameter of ocelli (µm) | 124.0 | 93.0 | 60.0 |
| Distance between ocelli (µm) | 216.0 | 221.0 | 172.0 |
| Distance between ocelli and compound eyes (µm) | 617.0 | 583.0 | 588.0 |
| Distance between ocelli and frontal plate (µm) | 232.0 | 201.0 | 161.0 |
| Distance between ocelli and posterior edge of vertex (µm) | 184.0 | 145.0 | 113.0 |
| Width of tempe (µm) | 577.0 | 497.0 | 538.0 |
| Postclypeus length/width | 1.3 | 1.2 | 1.0 |
| Postclypeus dorsal edge/ventral edge | 2.1 | 1.9 | 1.3 |
| Length of anteclypeus (µm) | 906.0 | 737.0 | 667.0 |
| Width of anteclypeus dorsal edge (µm) | 626.0 | 577.0 | 698.0 |
| Width of anteclypeus ventral edge (µm) | 197.0 | 221.0 | 187.0 |
| Width of anteclypeus in middle (µm) | 398.0 | 501.0 | 356.0 |
| Length of lorum/width | 2.0 | 2.2 | 2.1 |
| Length of rostrum (µm) | 1497.0 | 1399.0 | 1378.0 |
| First segment length of rostrum (µm) | 537.0 | 515.0 | 515.0 |
| Last segment length of rostrum (µm) | 571.0 | 501.0 | 501.0 |
| Pronotum width/length (in middle) | 2.1 | 2.0 | 2.0 |
| Pronotum width/length (on lateral) | 1.9 | 1.8 | 1.9 |
| Pronotum anterior edge/posterior edge | 1.3 | 1.5 | 1.4 |
| Pronotum anterior side edge/posterior side edge | 1.2 | 1.3 | 1.3 |
| Front wings length/width | 3.7 | 3.9 | 3.3 |
| Claval suture length/width | 5.4 | 6.3 | 4.8 |
| Length of hind tibia with apical platellae (mm) | 3.4 | 3.7 | 2.5 |
| Length of hind tibia without apical platellae (mm) | 3.3 | 3.4 | 2.3 |
| Distance between hind tibia small thorn and knee (µm) | 653.0 | 720.0 | 549.0 |
| Distance between hind tibia large thorn and the knee (µm) | 1578.0 | 1796.0 | 1248.0 |
| Length of second thorn/first thorn | 2.0 | 1.5 | 1.7 |
| Genital plate length/width | 2.1 | 2.2 | 2.2 |
| Paramere length/width | 1.8 | 1.9 | 2.0 |
| Aedeagus length/height | 2.5 | 2.0 | 2.2 |
| Base thickness of aedeagus (µm) | 232.0 | 242.0 | 203.0 |
| Thickness of aedeagus in middle (µm) | 145.0 | 159.0 | 131.0 |
| Narrowest point of aedeagus (µm) | 77.0 | 106.0 | 114.0 |
| Distance from phallobase to appendages/appendages to tip | 2.2 | 3.6 | 3.5 |
| Long appendages length/width | 12.0 | 28.2 | 29.8 |
| Short appendages length/width | 10.5 | 15.2 | 17.7 |
| Long appendages length/short appendages | 1.5 | 1.7 | 1.7 |
| Long appendages width/short appendages | 1.3 | 0.9 | 1.0 |

The abdomen is red, except for the weak blackish coloration in the middle of the last few of the abdominal sterna and the distinct blackness in the genital capsule (Figure 1b).

The dorsal part of the genital plate tip is distinctively ramuscule towards to the posterior. There is a short and straight edge located perpendicular to the ground just below at this structure. The ventral edge of the plate is slightly but completely convex. The surface of the plate is covered with setulae except only along the narrow strip-shaped area of the ventral edge.

The middle of the posterior edge of the paramere is ended conical and the tip of this conic structure seems like a "head of match". A weak chitinous setulae cluster is located on the dorsal corner of this structure. The shoulder-shaped structure just placed below this one is reduced and it nearly looks like a part of the ventral edge of paramere. There are few weak chitinous long setulae both on the first 1/3 part of the ventral edge and the shoulder. The elevation on the right in the middle of the dorsal edge is sphere-like and has a few long and non-chitinous setae. The ventral edge of paramere is completely convex and naked, with the exception of 1/3 of the posterior tip.

There are two pairs of appendages on the ventral posterior of the gonopore of the aedeagus: one is short and located ahead, the other one is long and located just behind it. These long appendages from base to the terminal end up forming a pointed tip, by bending first inward, then outward and finally inward. On the other hand, the short appendages are narrow at the base, thickens in the middle, and finalizes by forming a pointed tip narrowed both two sides equally at the terminal. The part after the appendages reminds the crocodile head in the lateral view.

The length of anal tube from the dorsal is 763 µm and the widest part is 345 µm.

Material examined. 6♂♂, 2♀♀ Hatay, Defne (Antakya), Döver, Piknik Alanı, 36°12’39" N, 36°14’4" E, 279 m, 17.IV.2015; ♂, 3♀♀, Hatay, Defne, Balıklıdere, Sinanlı, 36°10’12" N, 36°9’6" E, 35 m, 17.IV.2015; ♂, Hatay, Defne, Sinanlı, Dağdüzü, 36°7’52" N, 36°8’36" E, 346 m, 17.IV.2014; 2♀♀, Hatay, Defne, Döver, Harbiye Yolu, 36°7’47" N, 36°8’30" E, 236 m, 15.V.2015; 2♂♂, Hatay, Defne, Sinanlı-Dağdüzü Yolu, Dağdüzü Girişi, 36°4’30" N, 36°5’3" E, 336 m, 15.V.2015; ♀, Hatay, Yayladağı, Dağdüzü-Karacurun Arası, 36°2'32"N, 36°5’37" E, 686 m, 15.V.2015; ♂, Hatay, Yayladağı, Sürütme-Sungur Yolu, 36°3'52"N, 36°5’44" E, 788 m, 15.V.2015; 3♀♀, Hatay, Defne-Döver Yolu, 36°7’16" N, 36°8’46" E, 459 m, 05.VI.2015 (Figure 2c).

Distribution in Palearctic Region. Turkey (Anatolia) (Melichar, 1896; Nast, 1933; Lallemand, 1949; Nast, 1972) (Figure 2a).

Distribution in Turkey. Exact terra typica location of this species is not specified in the original description (Taurus). This is the first report of this species from Turkey with a defined locality record, even after a 124-year delay (Figure 2b).

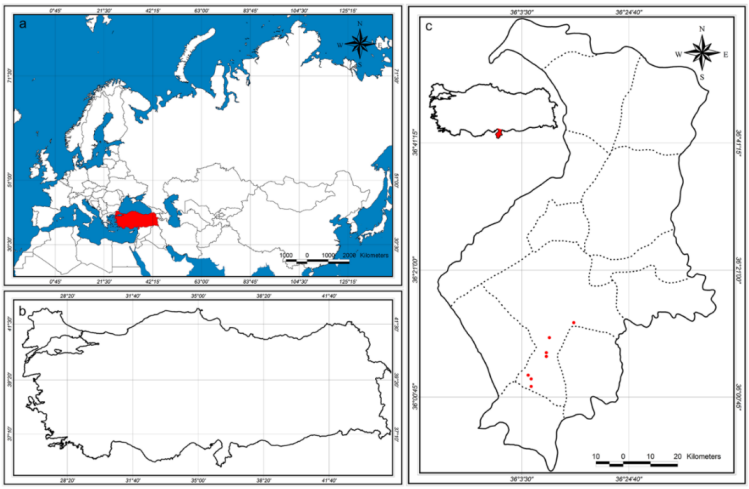


Figure 2. Distribution of *Cercopis distincta* (Melichar, 1896): a) Palearctic; b) Turkey; c) local.

***Cercopis intermedia* Kirschbaum, 1868**

*Cercopis obliterata* Kirschbaum, 1868

*Triecphora intermedia nigra* Royer, 1906

*Triecphora intermedia simulans* Peneau, 1912

*Cercopis sanguinolenta turkestanica* Lindberg, 1923

*Cercopis sanguinolenta intermedia bipunctata* Ribaut, 1946

*Cercopis sanguinolenta intermedia quadrimaculata* Ribaut, 1946

*Cercopis sanguinolenta intermedia septempunctata* Ribaut, 1946

*Cercopis sanguinolenta intermedia sexmaculata* Ribaut, 1946

**Redescription/Diagnosis**

The male length is 11 mm with wings, 8.8 mm without wings (Figure 1d-f) (Table 2). The vertex more or less semi-elliptic. The frontal plate with rare setulae and indistinct carina is a broad and shallow pentagonal view; the pits, longitudinal slits and the tempe are positioned just like in the *C. distincta*. The ocelli and compound eyes are approximately at the same level in lateral, however, the frontal plate is under the level of the ocelli in the middle. The postclypeus is in the form of a three-faced prism due to its three carinae. The lateral longitudinal carinae are parallel to each other at the upper side of the frons and they close up together towards to the anteclypeus but terminated much before without contact. The parallel transverse grooves are distinct and uninterrupted in lateral surfaces but become indistinct towards the weak mid-carina. In contrast with *C. distincta*, the side surfaces of the postclypeus, the lorum, anteclypeus, and the lower back part of the compound eye are covered with shorter dark colored setulae. The other parts of the head is naked or covered rarely with setulae so, all these areas are metallic black.

The front side edges of the pronotum are nearly straight in laterally. Its front edge is straight, the back edge is shallower wavy than the *C. distincta* and the back ends of its lobes are almost flat. There is no depression or a longitudinal carina in the front half of the pronotum, however, there are indistinct elevations on both sides. The upper part of the pronotum is metallic black and covered with sparsely black setulae. Due to the structure of the postclypeus, the head is short and the pronotum is weakly in the form of hump in lateral view.

The posterior edges of the red spots on the anterior of the clavus are convex and these edges are reaching only up to the middle half of the scutellum. The red spots in the corium are smaller and rounded, also they do not cross the clavus. And last but not least, the red band at the posterior of the wing whose tips touch more or less to the costae, is formed by combination of two obround red spots on the corium and the trapezoid spot on the clavus (Figure 1d, f).

The half parts of the first and second pairs of the femora and the tip parts of the tibia are red but half of the outer part of the both two segments are red on the third pairs, the inner parts are yellowish-brown. The large black thorn is nearly in the middle of hind tibia but the small yellow-reddish one is near the femur. All the coxae are more or less metallic black (Figure 1e).

The middle of the abdomen sterna, the centers of the connexiva and the genital capsule are black but the other areas are red (Figure 1e).

The genital plate is divided into two equal parts because of the indentation in the middle of the posterior edge. The part remaining in the dorsal shows a wavy edge structure and turns to the anterior then

ends with a prominently pointed protrusion distally, but the ventral part ended with a rounded corner. The surface and the ventral edge of the plate is showed similarity to the *C. distincta*.

The posterior edge of the paramere finalizes like *C. distincta* but unlike from it, the shoulder-shaped structure very prominent and has a small number of weakly chitinous setulae. There is an overturned trapezium-shaped elevation in the middle of the dorsal rim of the paramere, which has weak, long but numerous chitinous setae. Also, a secondary, very small and nodule-shaped elevation is located on the posterior of this structure. The ventral edge of the paramere is completely bare and with a concave curvature at the first 1/3 of the posterior part of it.

The appendages of aedeagus are almost straight-edged, and while the tips of the long appendages are tapered in both directions, bent slightly towards anteriorly in short ones. There is a formation that looks like an Adam's apple at the anterior of the appendages and also the tip part of gonopore resembles a Fowler's head.

The length of anal tube from the dorsal is 720 µm and the widest part is 372 µm.

Material examined. 2♂♂, Hatay, Yayladağı, Kulaç Yolu, 35°55'7" N, 36°7'31" E, 792 m, 17.IV.2015; ♀, Hatay, Yayladağı, Ziyaret Dağı, Hırbi Yayla, Türbinler Mevkii, 36°03'08" N, 36°07'48" E, 825 m, 15.V.2015 (Figure 3c).

Distribution in Palearctic Region. Albania, Algeria, Armenia, Bulgaria, France, Georgia, Germany, Greece (Holotype), Iran, Israel, Italy, Lebanon, Morocco, Portugal, Russia (Dagistan), Spain, Switzerland, Syria, Turkey (Anatolia), Turkmenistan, Ukraine, Uzbekistan (Nast, 1972, 1987; Holzinger et al., 2003) (Figure 3a).

Distribution in Turkey. Adıyaman, Aksaray, Amasya, Ankara, Antalya, Artvin, Balıkesir, Çanakkale, Çorum, Diyarbakır, Elâzığ, Eskişehir, Gaziantep, Giresun, Gümüşhane, Hakkâri, Hatay, Isparta, İzmir, Kahramanmaraş, Kayseri, Kırıkkale, Kırklareli, Konya, Kütahya, Mardin, Rize, Samsun, Siirt, Şanlıurfa, Tokat, Uşak (Dlabola, 1971; Lodos & Kalkandelen, 1981; Kartal et al., 1994; Demir, 2006a, b, 2008, 2019; Önder et al., 2011) (Figure 3b).

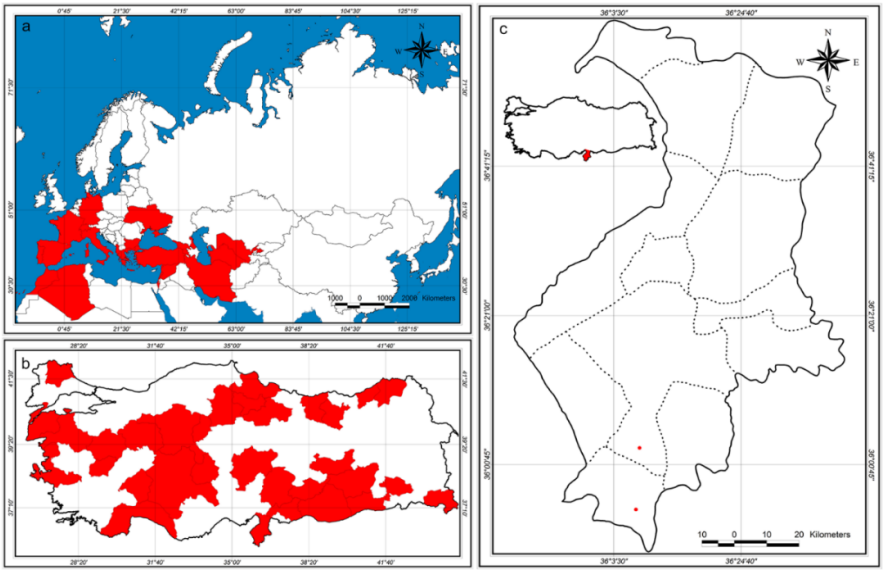


Figure 3. Distribution of *Cercopis intermedia* Kirschbaum, 1868: a) Palearctic; b) Turkey; c) local.

***Cercopis septemmaculata* (Melichar, 1903)**

*Triecphora septemmaculata* Melichar, 1903

**Redescription/Diagnosis**

The male length is 9 mm with wings, 7.8 mm without wings (Figure 1g-i) (Table 2). The vertex is slightly curved banana-shaped. The frontal plate is almost semicircular, with vaguely carinated and setulae. The two pits distance located behind the frontal plate equal to each other on the side parts. Unlike the other two species, the ocelli are higher than the other structures and the longitudinal depressions are not clear. The tempe is divided into two equal parts with the bottom of the antenna as in *C. distincta* and *C. intermedia.* Longitudinal carina in the middle of postclypeus is vague and complete but, the lateral carinae are incomplete. However, the face still gives the impression of with three parts. The transverse grooves are not apparent and do not touch to each other in the middle. The lorum, gena, back parts of the compound eye and anteclypeus are covered with sparsely and weak yellowish setulae.

The front side edges of the pronotum are short and straight in the lateral view. The front edge of the pronotum nearly straight but the back edge shallowly undulated form. Consequently, the lobes are not fluffy and prominent. There is no carina on the front half of the metallic black pronotum but there is an undulation, which is very lightly polished from the center towards sides and resembles a dickey bow without a knot. This area is nearly naked but the remaining areas are covered with sparse setulae and small pits. The pronotum does not swell like a hump towards to the anterior in lateral view.

The front wings have a pattern on black, consisting of seven red spots. The two spots on located anterior of clavus with convex back edges and front tips have a black edge formation on the area of facing the pronotum and scutellum. The two red spots on the corium are almost circular and neither contact the clavus nor the costa. The strip on the posterior of the wing is replaced by three red spots (Figure 1g, i).

The inner half parts of the third pairs of tibiae are yellowish-red and the rest are blackish brown. The large thorn is dark brown-blackish and in the middle of the hind tibia but the small and yellow-reddish one is close to the femur. All the coxae are more or less metallic brown-black (Figure 1h).

Nearly all the abdomen sterna, the centers of connexiva and the genital capsule are black, the remaining areas are red (Figure 1h).

The genital plate is divided into two equal parts because of the shallow indentation in the middle of the posterior edge. The part remaining in the dorsal shows a straight edge structure and turns towards the anterior, then ends with an indistinct blunt protrusion at the distal but the ventral part ended without creating any corners at the distal. Almost the entire surface of the plate is covered with setulae, except only a narrow area of the posterior half of the ventral edge.

The shoulder-shaped structure on the posterior edge of the paramere and its ventral corner has a small number of weakly but long chitinous setulae and the neck structure above this is thicker. There is a parallelogram-shaped elevation in the middle of the dorsal edge with a small number of long weak chitinous setae. Also, there is a very slightly single-curled at the posterior of this structure. A concave curvature is ranged in the middle of the ventral edge of the paramere.

The longer appendages of aedeagus first curling outward, second inward and finally outward again then ends up with a pointed tip. The short ones are located in the form of a mirror image of the long ones from the middle to the distal. However, the first fold is outward, just like the long ones. The part after the gonopore resembles the mouth of an antique pitcher.

The length of anal tube from the dorsal is 659 µm and the widest part is 270 µm.

Material examined. 2♂♂, 3♀♀, Hatay, Defne, Balıklıdere, Sinanlı, 36°10'12" N, 36°9'6" E, 35 m, 17.IV.2015; 4♂♂, ♀, Hatay, Yayladağı, Kösrelik Köyü Çıkışı, Leylekli Barajı, 35°56'36" N, 36°3'44" E, 510 m, 17.IV.2015; ♀, Hatay, Defne, Sinanlı, Dağdüzü, 36°7'52" N, 36°8'36" E, 346 m, 17.IV.2014; 3♂♂, ♀, Hatay, Yayladağı, Kulaç Yolu, 35°55'7" N, 36°7'31" E, 792 m, 17.IV.2015; ♀, Hatay, Defne, Döver, Bahçeköy (Aşağı Döver), 36°7'36" N, 36°6'55" E, 73 m, 15.V.2015; ♀, Hatay, Defne-Döver Yolu, 36°7'16" N, 36°8'46" E, 459 m, 05.VI.2015 (Figure 4c).

Distribution in Palearctic Region. Israel, Jordan, Lebanon, Palestine, Syria (Dlabola, 1965; Nast, 1972) (Figure 4a).

Distribution in Turkey. The distribution record of this species has been given by Lodos and Kalkandelen as Adana, İzmir, Kastamonu, Mardin and Siirt Provinces but missing the other important information (Lodos & Kalkandelen, 1988). This is the first regional and complete locality record for this species (Figure 4b).

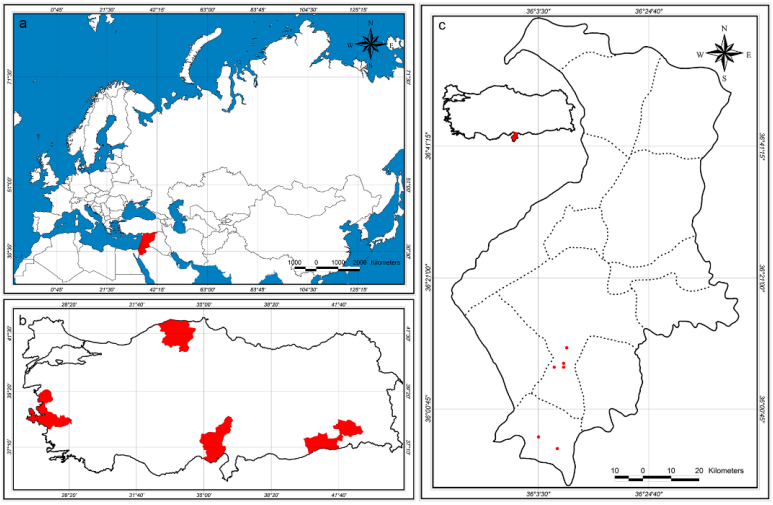


Figure 4. Distribution of *Cercopis septemmaculata* (Melichar, 1903): a) Palearctic; b) Turkey; c) local.

## Discussion

Through the evaluation of the samples from the study area it was determined that three *Cercopis* spp., *C. distincta*, *C. intermedia* and *C. septemmaculata*, occur in the region. *Cercopis distincta* is the most common species with 22 samples collected from five locations, followed by *C. septemmaculata* with 17 samples from four locations. *Cercopis intermedia* was the least commonly collected species.

When the quantitative data (Table 2) and general appearances of these morphological structures are evaluated together, the features of the vertex, the diameter of the ocelli, the pronotum and the ratios of the long to short appendages of the aedeagus are the most distinctive. The vertex is triangular in *C. distincta*, semi-ellipsoid in *C. intermedia* and banana-shape in *C. septemmaculata* (Figure 5 a, d, g). Also, the vertex width/length ratio differs from species to species (Table 2). Ocelli diameters appear to be markedly different between these species. While the smallest eye diameter occurs in *C. septemmaculata*, *C. intermedia* is 1.5-times and *C. distincta* is two-times larger (Table 2). Given the shape of the undulation on the posterior edge of the hexagonal pronotum results in variable proportions between the species (Table 2). However, the anterior edge to posterior edge ratio is smallest in the relatively larger *C. distincta* (Table 2). The ratios of the long and short appendages of the aedeagus are one of the important characteristics used in the diagnosis of *Cercopis* Fabricius, 1775 species. However, the ratio of the long appendages length/width or the short appendages length/width revealed more useful differences for the identification of species.

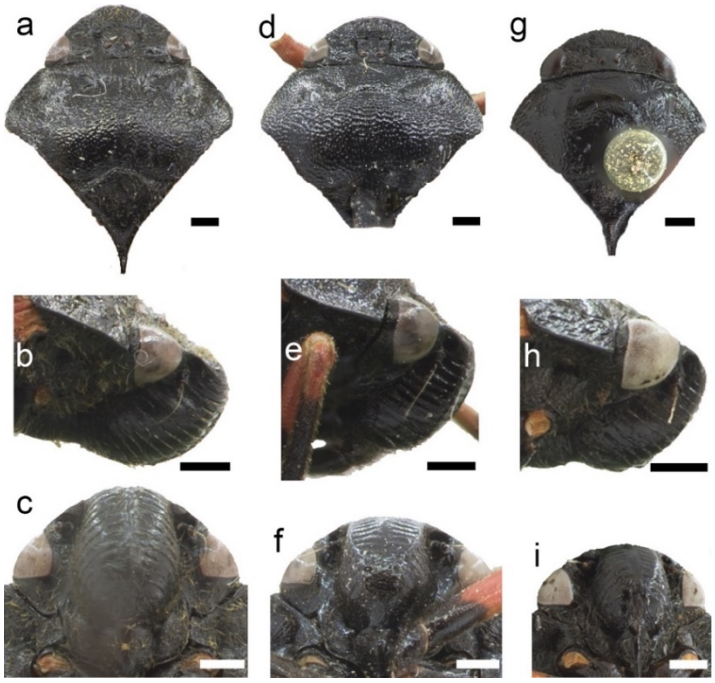


Figure 5. Head of *Cercopis* spp.: *Cercopis distincta*: a) male adult, dorsal view, b) male adult, lateral view, c) male adult, ventral view; *Cercopis intermedia*: d) male adult, dorsal view, e) male adult, lateral view, f) male adult, ventral view; *Cercopis septemmaculata*: g) male adult, dorsal view, h) male adult, lateral view, i) male adult, ventral view (scale bar = 500 µm).

The doubtful Syrian record for *C. distincta* (Lallemand, 1912; Nast, 1933) was corrected in subsequent studies (Metcalf, 1961; Nast, 1972; Lodos & Kalkandelen, 1981). It was confirmed as an endemic species for Turkey at least for now by these studies. Despite this the terra typica of this species is unknown and no local record has been found in any publication before this study.

The color pattern descriptions of *C. distincta* show similarities with the previous reports (Melichar, 1896; Nast, 1933). Although, it was stated by Nast that the postclypeus extends beyond the edge of the vertex in dorsal view (Nast, 1933), it is considered that this was due to the incorrect perspective (Figure 5a). In the same study, although the frontal plate was declared to be carinated or traced, this is not the case in our specimens, nor is it mentioned in the original definition (Figure 5a). The entire body of the adult is covered with yellow-brown setulae (Figure 5a-c).

Despite slight differences, the definitions of the wing band and the legs are similar to these publications, however, the red spot in the corium does not appear to touch the claval suture in the illustrations of wings (Nast, 1933). On the contrary, in our specimens, the spot is touching the suture as broadly as in the original definition (Melichar, 1896) (Figure 1a, c). The detailed characteristics of the spots were not provided in either publication.

Again, the male genital structures are not described in detail in these reports. Although parts other than the anal tube are given in Nast's paper, the drawings for the genital plate and the paramere are especially far from sufficient (Figure 7a-e).

*Cercopis intermedia* was originally described quite simply, then given as a subspecies of *C. sanguinolenta* by Nast (1933) and last revaluated as a valid species by Dlabola (1965). Neither of these reports provided images for this species, except the lateral view of its aedeagus (Dlabola, 1965). Even if we disregard other ambiguous statements in the original description; the inconsistent matter is: *C. intermedia* species with red knees, has been likened to *Cercopis distinguenda* Kirschbaum, 1868 which is now known to be a synonym of the species *C. sanguinolenta*. Given that the legs of *C. distinguenda* were

described as completely black by the author in the same publication without leaving room for doubt (Kirschbaum, 1868) (Figure 6). The more ironic thing is this apparent characteristic was almost given as the only difference between the nominative subspecies from *Cercopis sanguinolenta intermedia* Kirschbaum, 1868 by Nast (Nast, 1933). Nevertheless, this publication does not mention what the genital differences of these two subspecies are (Figure 7f-j). For many other morphological characters preferred in the definitions, ambiguous or inconsistent expressions were often used. All these problems make the identification key dysfunctional and ineffectual. In the study of Giustina, while there are insufficient images of adults from the dorsally and genital structures only from the lateral of the paramere and the aedeagus, illustrations from other perspectives, and genital structures that will facilitate the diagnosis of the species were not included (Giustina, 1983).

Figure 6. Mating adults of *Cercopis sanguinolenta* (Scopoli, 1763) (Holzinger, 2008).

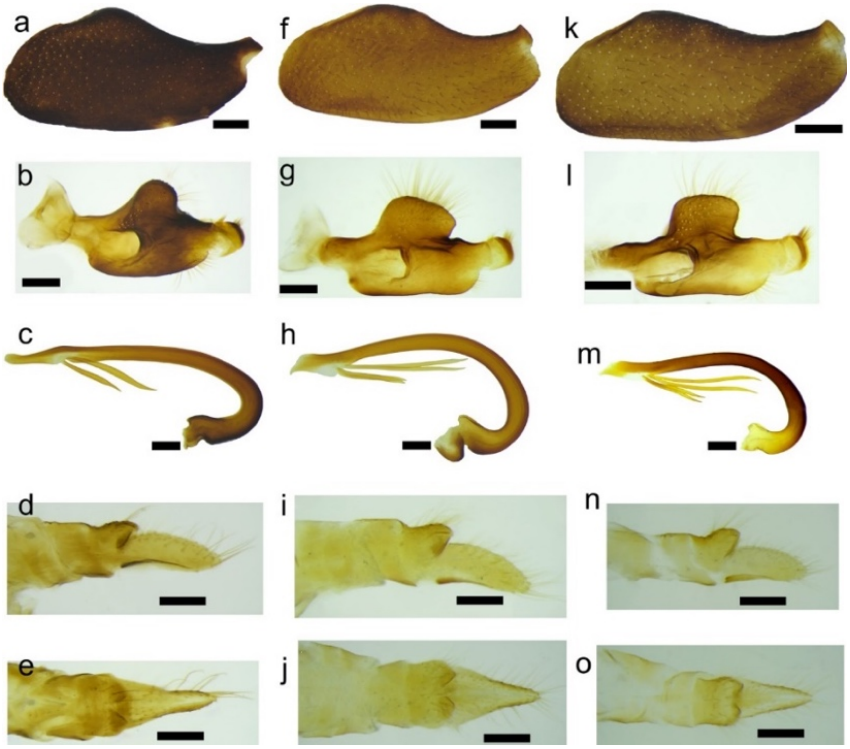


Figure 7. Genitalia parts of *Cercopis* spp.: *Cercopis distincta*: a) ventrolateral view of the left genital plate, b) exterior view of the left paramere, c) lateral view of the aedeagus, d) lateral view of the anal tube, e) dorsal view of the anal tube; *Cercopis intermedia*: f) ventrolateral view of the left genital plate, g) exterior view of the left paramere, h) lateral view of the aedeagus, i) lateral view of the anal tube, j) dorsal view of the anal tube; *Cercopis septemmaculata*: k) ventrolateral view of the left genital plate, l) exterior view of the left paramere, m) lateral view of the aedeagus, n) lateral view of the anal tube, o) dorsal view of the anal tube (scale bar = 250 µm).

Considering the current distributions of *C. sanguinolenta* and *C. intermedia* species, which are often confused due to color, pattern and size similarities; While *C. sanguinolenta* mostly occurs in the countries in the middle and north of the Western Palearctic Region, it is seen that the *C. intermedia* species is stuck in the middle and south border of the same region. The color pattern convergence of both species cause the existing uncertainty especially where their occurrence overlap and it is considered that this problem remains in many regions. Therefore, locality records given in the past for both species from the north and northwest of Turkey should be reviewed using freshly collected specimens from these regions.

The incorrect local records provided for *C. intermedia* in previous studies (Lodos & Kalkandelen, 1981; Kartal et al., 1994) have been identified or corrected. Accordingly, Bitlis, İstanbul, Niğde and Trabzon Provinces were removed from the distribution of this species, and Tokat, Aksaray and Gümüşhane Provinces added.

Austria, Czech Republic, Hungary, Poland, Romania and Slovakia given by Önder et al. as the Palearctic distribution of *C. intermedia* are not included in this publication because the source details were not specified (Önder et al., 2011).

The adults of *C. intermedia* can be collected from annual plants like *Astragalus* L., *Onopordum* L., *Verbascum* L., *Medicago sativa* L. and trees such as *Pistacia vera* L., *Prunus domestica* L., *Acacia* spp., *Salix* spp. and *Alnus* spp. from the start of May until the beginning of August (Lodos & Kalkandelen, 1981).

With the exception of the simple description made by Melichar for three samples collected from Palestine and material provided by Dlabola (Dlabola, 1965), there is no available data on *C. septemmaculata*. In Melichar's description, this species is illegitimately associated with *Triecphora sanguinolenta* Linnaeus, 1767 which has no validity today. Also, in his study, Dlabola provided a drawing of only the lateral view of the aedeagus and made a description with no useful distinction from the original but only shortened (Dlabola, 1965). Although Nast considered this species to be one of the three melanotic aberrations of *C. intermedia* (Nast, 1933), when the morphological and male genital structures of adults were examined (Figure 7k-o), it is concluded that is completely different.

Although this species is fundamentally given as a new record for Turkey (Lodos & Kalkandelen, 1988) (Figures 4 & 8), it has always been considered doubtful because of data deficiencies (date, collector, number of specimens etc.). Consequently, this record is the first regional and complete locality record for this species. Addition to this, if all these deficiencies was ignored and the distribution information given is processed on the map, it is clear that occurrence in the provinces of İzmir and Kastamonu would be inconsistent with the current distribution of the species and its known habitats. Therefore, it is considered appropriate to exclude these provinces from the distribution information of this species until proven otherwise. It is clear from the original work that Leopold MELICHAR did not select either of his specimens as a holotype for *C. septemmaculata*, so their syntype status is preserve until a lectotype is designated.

Family: Cercopidae Leach, 1815.

** Cercopis septemmaculata (Melichar, 1903).

Distribution: Adana (Ceyhan), İzmir (Bozdağ), Kastamonu (İnebolu), Mardin (Ömerli), Siirt (Aydınlar).

** Poophilus nebulosus (Lethierry, 1876).

Distribution: Mardin (Cizre, Hasankeyf).

Figure 8. An image of the record given by Lodos & Kalkandelen (1988).

The Turkish *Cercopis* Fabricius, 1775 identification key below was created by joining the two other species occurring in Turkey [*Cercopis vulnerata* Rossi,1807 and *C. sanguinolenta*] with the redescribed species in this study.

Turkish *Cercopis* Fabricius, 1775 Identification Key

1. All leg segments black (Figure 1b)..…… ..... 2

— Femora and tibiae half red (Figure 1e, h)…. ..... 4

2. Ratio of black and red nearly equal, transverse red stripe at the back tip of the wing thick forming horseshoe shape on wing corium… ..... ***C. vulnerata***

— Black predominant over red with thin, more or less curled, transverse red stripe on the rear tip of the wing…..... 3

3. Connexiva of the ventral plates red with central black spot.. ..... ***C. sanguinolenta***

— Connexiva of the ventral plates completely red (Figure 1b)…..... ***C. distincta***

4. Postclypeus without lateral keel (Figure 5h, i) ..... ***C. septemmaculata***

— Postclypeus with two lateral keels (Figure 5e, f).…….. ..... ***C. intermedia***

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*Original article (Orijinal araştırma)*

# Non-target effects of insecticides commonly used against lepidopteran pests on the predator, *Nesidiocoris tenuis* (Reuter, 1895) (Hemiptera: Miridae), under greenhouse conditions[1]

Lepidopter zararlılara karşı kullanılan bazı insektisitlerin sera koşullarında *Nesidiocoris tenuis* (Reuter, 1895) (Hemiptera: Miridae)'e yan etkileri

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## Abstract

*Nesidiocoris tenuis* (Reuter, 1895) (Hemiptera: Miridae) is the most widely used biological control agent of tomato pests, particularly tomato leafminer. Five treatments, spinetoram, chlorantraniliprole + abamectin, chlorantraniliprole + thiamethoxam, emamectin benzoate and dimethoate were tested on *N. tenuis* under greenhouse conditions in summer and autumn of 2018 in Malatya Province, Turkey. After insecticide application, *N. tenuis* were counted on days 1, 4, 7, 14, 21 and 28. The non-target effects of insecticides are classified according to IOBC toxicity categories. Spinetoram caused 24 and 52% mortality in summer and autumn experiments, respectively and is compatible with *N. tenuis* considering mortality in both seasons. Therefore, it is recommended for IPM. Chlorantraniliprole + abamectin was classified as slightly harmful in the summer experiment as it resulted in 45% mortality, however, in autumn conditions, it was resulted in 79% mortality and classified as harmful. This effect seen under cooler conditions should be consider in planning IPM. Chlorantraniliprole + thiamethoxam caused 62 and 63% mortality which was increasing up to the final day of autumn assessment, whereas emamectin benzoate caused high mortality of 86 and 87% in summer and autumn, respectively. Thus, it is concluded that these latter two insecticides are not compatible with *N. tenuis*.

**Keywords:** Biological control, inoculative releasing, insecticides, protected cultivation, side effects, tomato

## Öz

*Nesidiocoris tenuis* (Reuter, 1895) (Hemiptera: Miridae), başta Domates güvesi olmak üzere domates zararlılarına karşı en yaygın kullanılan biyolojik mücadele etmenidir. Bu çalışmada, 2018 yılı yaz ve sonbahar dönemlerinde Malatya İli sera koşullarında spinetoram, chlorantraniliprole + abamectin, chlorantraniliprole + thiamethoxam, emamectin benzoate ve dimethoate etken maddeli 5 farklı pestisitin *N. tenuis*'e yan etkilerinin belirlenmesi amaçlanmıştır. İnsektisit uygulamaları yapıldıktan sonra 1, 4, 7, 14, 21 ve 28. günlerde *N. tenuis* sayımları yapılmıştır. İnsektisitlerin yan etkileri IOBC toksisite kategorisine göre sınıflandırılmıştır. İki sezonun ortalaması dikkate alındığında, spinetoram, sırasıyla yaz ve sonbahar denemelerinde %24 ve 52 ölüme neden olmuş ve aynı zamanda *N. tenuis* ile de uyumlu olduğu saptanmıştır. Böylece IPM programlarında önerilebilir. Chlorantraniliprole + abamectin yaz denemesinde %45 ölüm oranı ile zararsız veya az zararlı sınıfında yer alırken, sonbahar denemesinde ise %79 ölüme neden olmuş ve zararlı olarak sınıflandırılmıştır. Serin şartlarda görülen bu etki IPM programları hazırlanırken dikkate alınmalıdır. Chlorantraniliprole + thiamethoxam yaz ve sonbahar denemelerinde, özellikle sonbahar denemesinde son sayım gününe kadar artmaya devam eden %62 ve 63 ölüme neden olmuşken, emamectin benzoate ise %86 ve 87 oranında yüksek ölüme neden olmuştur. Bu yüzden, bu iki insektisitin de *N. tenuis* ile uyumlu olmadığı düşünülmektedir.

**Anahtar sözcükler:** Biyolojik mücadele, aşılama salımı, insektisitler, örtüaltı yetiştiriciliği, yan etki, domates

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## Introduction

Turkey, with over 12 Mt of tomato production, ranks as the fourth highest producer after China, India and the USA (FAO, 2018). There are biotic factors that limit tomato production in Turkey including the pest invertebrates, tomato leafminer [*Tuta absoluta* (Meyrick, 1917) (Lepidoptera: Gelechiidae)], whitefly [*Bemisia tabaci* (Gennadius, 1889) (Hemiptera: Aleyrodidae)], vegetable leafminer [*Liriomyza trifolii* (Burgess in Comstock, 1880) (Diptera: Agromyzidae)], western flower thrips [*Frankliniella occidentalis* (Pergande, 1895), onion thrips [*Thrips tabaci* Lindeman, 1889 (Thysanoptera: Thripidae)] and two-spotted spidermite [*Tetranychus urticae* Koch, 1836 (Acarina: Tetranychidae)] (Ulubilir & Yabaş, 1996; Yasarakıncı & Hıncal, 1999; Bulut & Göçmen, 2000; Keçeci et al., 2007; Kılıç, 2010).

The common use of chemical pest control leads to negative consequences including destruction of natural pest predators and the development of insecticide resistance in pests (Devonshire & Field, 1991). Integrated pest management (IPM) has become important in pest control and alternative methods have been developed (Desneux et al., 2007; van Lenteren, 2009; Bueno & van Lenteren, 2010; Yücel et al., 2013). Although chemical pest control is mostly preferred by producers, there is also an increase in the use of biological control of greenhouse vegetable pests. Before *T. absoluta* was introduced to European tomato crops, the primary pests were whiteflies with biological control achieved by a combination of *Macrolophus pygmaeus* (Rambur, 1839) (Hemiptera: Miridae), *Encarsia formosa* Gahan, 1924 (Hymenoptera: Aphelinidae) and *Eretmocerus mundus* Mercet, 1931 (Hymenoptera: Aphelinidae). However, the arrival of *T. absoluta*, which in now found in almost all tomato production areas in the Mediterranean basin and Europe (Biondi et al., 2018), especially in greenhouse tomato cultivation between 2006 and 2010, led to changes in biological control approaches. The current biological control is now based on *Nesidiocoris tenuis* (Reuter, 1895) (Hemiptera: Miridae), which feeds on preadult stages of whiteflies as well as the eggs and larvae of *T. absoluta* (Yucel et al., 2013; Pérez-Hedo & Urbaneja, 2016; Topakcı & Keçeci, 2017).

The following insecticides, used in tomato production, could potentially impact on *N. tenuis* and its effectiveness as a biocontrol agent. Chlorantraniliprole is a ryanodine receptor modulator. Insect ryanodine receptors activated by chlorantraniliprole stimulate the release of calcium from muscles, causing impaired muscle regulation, paralysis and ultimately death. Abamectin is a glutamate-gated chloride channel allosteric modulator and activates this channel stimulating the release of γ-aminobutyric acid from presynaptic inhibitory membranes and resulting in an increased flow of chloride ions into the cell blocking nerve signals. Another insecticide applied with chlorantraniliprole is thiamethoxam which belongs to neonicotinoids group (MoA group 4A). This group of insecticides bind to the acetylcholine sites on nicotinic acetylcholine receptors causing some symptoms such as hyperexcitation, lethargy and paralysis. Emamectin benzoate is an activator of the chloride channel (MoA group 6) causing neuronal and muscular system malfunctions. Spinetoram is nicotinic acetylcholine receptor allosteric activator (MoA group 5) causing hyperexcitation of neurons in the central nervous system (IRAC, 2020).

Non-target effects pesticides are mostly assessed under laboratory and semi field conditions given that field studies can be time-consuming and expensive. However, field tests should provide more reliable results (Thomson & Hoffmann, 2006; Pozzebon et al., 2015). The non-target effects of insecticides on *N. tenuis* have mostly been assessed under laboratory conditions. Thus, it is important to also conduct assessments under standard tomato production conditions. Only a single published study has been conduction on the effects of chlorantraniliprole on *N. tenuis* under greenhouse conditions (Dáder et al., 2020). However, there are no studies investigating the impact of newer products containing abamectin or thiamethoxam. Similarly, although there are a number of studies on spinosad, a spinosyns group agent, there are no studies on the new active ingredient spinetoram.

For successful IPM, the compatibility of pesticides with biological control agents is vital when applied in combination with biological control. This study aimed to determine the potential non-target effects of the

some commonly-used insecticides on the predator insect, *N. tenuis*, under greenhouse conditions. The insecticides assessed, dimethoate and the 4 larvicides, spinetoram, chlorantraniliprole + abamectin, chlorantraniliprole + thiamethoxam and emamectin benzoate are commonly used to control cotton bollworm and tomato leafminer in tomato production.

## Materials and Methods

### *Nesidiocoris tenuis* rearing

A population of *N. tenuis* was obtained from Biobest Corporation, Antalya, Turkey and was subsequently reared on tomato seedlings in cages covered with fine netting. The cages were placed in a controlled environment room at 25 ± 1ºC, 60 ± 10% RH and 16:8 h L:D photoperiod. *Ephestia kuehniella* Zeller, 1879 (Lepidoptera: Pyralidae) eggs were used as the food source for these cultures (Sanchez et al., 2009; De Puysseleyr et al., 2013; Keçeci & Öztop, 2017).

### Insecticides and their applications

The effect of the insecticides (Table 1) spinetoram, chlorantraniliprole + abamectin, chlorantraniliprole + thiamethoxam and emamectin benzoate were compared to the highly-toxic insecticide, dimethoate, under greenhouse conditions. These insecticides are known to be effective on larval stages, and are widely used for cotton bollworm, *Helicoverpa armigera* (Hübner, 1808) (Lepidoptera: Noctuidae) and tomato leafminer, *T. absoluta* (IRAC, 2017; Anonymous, 2020; Kandil et al., 2020).

Table 1. Active ingredients, trade names, chemical groups, target pests and application rate of the tested insecticides

| Treatment (active ingredient and formulation*) | Trade name (company) | Chemical group | Target pest | Recommended field rates of products in Turkey |
|---|---|---|---|---|
| Spinetoram (120 g/L, SC) | Radiant (Dow AgroSciences) | Spinosyns | tomato leafminer | 500 mL/kL water |
| Chlorantraniliprole and thiamethoxam (100 and 200 g/L, SC) | Durivo (Sygenta) | Diamides and neonicotinoids | tomato leafminer | 800 mL/ha |
| Chlorantraniliprole and abamectin (45 and 18 g/L, SC) | Voliam Targo (Sygenta) | Diamides and Avermectins | cotton bollworm | 900 mL/kL water |
| Emamectin benzoate (5%, SG) | Surrender (Agrobest) | Avermectins | cotton bollworm | 300 g/kL water |
| Dimethoate (400 g/L, EC) | Poligor (Hektaş) | Organophosphates | various** | 1 L/kL water |

* EC, emulsion concentrate; SC, suspension concentrate; SG, soluble granules;
** Dimethoate is broad-spectrum, highly-toxic insecticide included as a positive control.

The experiments were conducted in the summer and autumn of 2018 under greenhouse conditions in the Department of Crop Protection, Faculty of Agriculture, Malatya Turgut Özal University. Net (50 mesh) cages (2 x 2.5 x 2 m, width x length x height) were used with 10 tomato cv. Bigmek F1 plants per cage planted as seedlings. The experiments were laid out in a randomized complete block design with three replicate cages per treatment, and included three untreated controls.

In the summer, tomato seedlings were planted on 16 April. *N. tenuis* were released (2 adults/$m^2$) (Calvo & Urbaneja, 2004) in all cages on 21 June. After predator release, 0.14 g *E. kuehniella* eggs were placed six times on each of the 10 tomato plants at 7-9-d intervals as a food source and to enable establishment of the *N. tenuis*. On 5 August, the plants were sprayed with the insecticide treatments at the maximum recommended concentration for field use (Table 1). Spinetoram, chlorantraniliprole + abamectin, emamectin benzoate and dimethoate were applied as a foliar application in 1.15 L of water/cage with a backpack sprayer. Chlorantraniliprole + thiamethoxam was applied to the tomato plant roots with 0.5 L of water/plant. Control plants was only sprayed with water. Supplementary food was provided only once, 3 d after application of the treatments. No other organisms that could potentially be a food source for the predators were observed on plants during the experiment.

In the autumn, tomato seedlings were planted on 13 September, and *N. tenuis* released on 28 September with four *E. kuehniella* egg applications as in the summer experiment. Insecticides were applied on 30 October.

**Sampling**

Adults and nymphs of *N. tenuis* on all parts of three plant in each cage were counted using a magnifying glass 1 d before the insecticide application and 1, 4, 7, 14, 21 and 28 d after insecticide application.

**Data analyses**

Counts adults plus nymphs of *N. tenuis* per plant were converted to percentage mortality for each assessment time using Henderson-Tilton formula (Henderson & Tilton, 1955) based on the numbers in the untreated control for each experimental block. The data was examined using exploratory statistics (Tukey, 1997) and assumptions tests for least squares hypothesis testing, and found to contain significant non-normality (Shapiro-Wilk test, $p < 0.001$ and by examining Q-Q plots) and lack of homogeneity of variance (Levene's test $p < 0.001$). So, the assumption required for a least square repeated measures ANOVA were not met. Therefore, the data was initially analyzed by fitting linear mixed-effects models using restricted maximum likelihood with R function "lmer" in the "lme4" package (Bates et al., 2015). The model formula used include blocks and assessment times as random effects to account for the nesting and repeated measures in the experimental design. Main effects (treatment and time) were significant for both seasons and response variates (counts and mortalities), but no interactions were found to be significant. Therefore, nonlinear least squares asymptotic fits were made with the R function "nls" and model, Response ~ SSasymp (Cycle, Asym, R0, lrc), where SSasymp function is a self-start model to evaluate the asymptotic regression and its gradient in the R package "lme4" (Bates et al., 2015). For the mortality data, where the parameter Asym was statistically significant the estimate obtains represented the equilibrium mortality as there was no evidence of population increase in the untreated controls over the 28-d assessment period. The logarithmic rate constant (lrc) indicates the rate of response to the treatments but these rate changes are evident in the plotted regressions, so only Asym values are presented here. These mortality estimates are also used to classify insecticide treatments based on International Organization for Biological and Integrated Control toxicity categories for semi field and field conditions: N, harmless or slightly harmful for 0-50% mortality; M, moderately harmful for 51-75% mortality; and T, harmful for >75% mortality (Boller et al., 2006).

## Results

In the summer experiment, on the day before insecticide application, the mean predator population ranged between 15 and 24 individuals/plant. In the autumn experiment, predator population in all plot were 7 to 10 individuals/plant before treatment. During the summer and autumn experiments, the mean daily temperatures (and range) the duration of the experiments were 25.2ºC (22.7-27.0ºC) and 16.7ºC (10.7-18.9ºC), respectively. Due to the low temperatures during the autumn experiment, the predator population did not reach levels as high as those observed in the summer period. Therefore, each experiment was analyzed separately.

**Summer experiment**

The results for the summer experiment are presented in Figure 1. During the summer experiment, there was a slight decrease in the number of *N. tenuis* in the control plots. However, except for spinetoram, predator population declined rapidly with insecticide treatment. Spinetoram reduced the number of *N. tenuis* more slowly from 1 to 7 d after treatment, then the population stabilized. Chlorantraniliprole + abamectin effect quickly reduced the number of *N. tenuis* rapidly 1 to 4 d after treatment. In contrast, number of *N.*

*tenuis* with chlorantraniliprole + thiamethoxam treatment, applied as a soil drench, did not decrease up to 4 d after treatment but rapidly decreased by 7 d after treatment before stabilizing. Given this disjuncture in the rate of decline, the asymptotic fit for this treatment was not as tight as the other treatments, but the equilibrium morality appeared to be reliably estimated (Figure 1d, i & Table 2). With emamectin benzoate the population decrease was complete by 7 d after treatment. The control insecticide, dimethoate, caused almost complete mortality a 1 d and 100% by day 4.

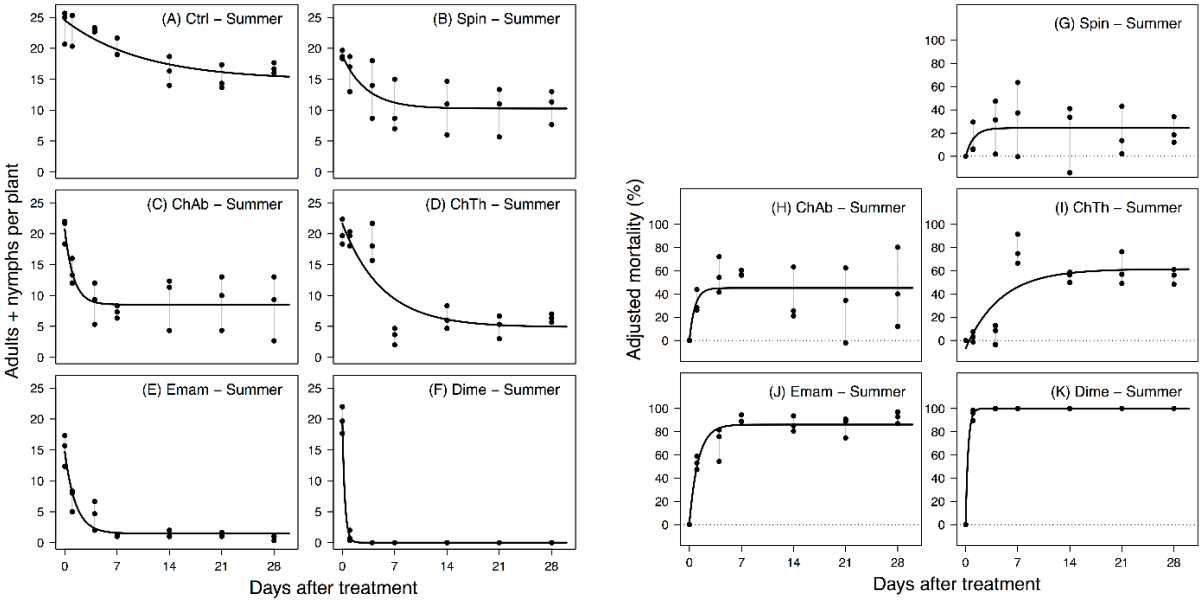


Figure 1. A-F, Numbers of *Nesidiocoris tenuis* for an untreated control and five insecticide treatments applied in summer 2018; and G-K, adjusted mortalities (%) for the insecticide treatments. Mortalities were adjusted using the Henderson-Tilton formula (Henderson & Tilton, 1955). Points represent the values for each of the replicates and the regression lines are for a nonlinear least squares fits of the model, Response ~ SSasymp(Day, Asym, R0, lrc), where SSasymp is a self-start model to evaluate the asymptotic regression and its gradient in the R package "lme4" (Bates et al., 2015). See Table 2 for the values and significance of the Asym parameters. Treatment codes: Ctrl, control; ChAb, chlorantraniliprole + abamectin; ChTh, chlorantraniliprole + thiamethoxam; Dime, dimethoate; Emam, Emamectin benzoate; and Spin, spinetoram. Treatments are displayed from lowest to highest equilibrium mortality.

**Autumn experiment**

The results for the summer experiment are presented in Figure 2. In the autumn experiment, the initial *N. tenuis* population was lower than that in the summer. The population only decreased slightly in control cages, probably because of cooler conditions. In contrast to summer experiment there was a significant decrease in predator numbers with spinetoram application and nearly half of the population was affected. With chlorantraniliprole + abamectin, a rapid decline was evident on day 1 and had stabilized by day 4. Emamectin benzoate had caused a significant reduction in population 1 d after treatment. The asymptotic fits were relatively tight for the population changes in all treatments, however, changes the less uniform decline in the control population compared to the summer experiment (Figures 1a vs 2a) feed some variability in the mortalities adjusted by the Henderson-Tilton formula. Nevertheless, the equilibrium toxicities were reliably estimated (Table 2) excepted with chlorantraniliprole + thiamethoxam treatment. For that treatment no statistically, significant asymptote was estimated because the mortality progress over 28 d of the experiment. So, for classification of that treatment, the 28 d toxicity (62%, Table 2) was used.

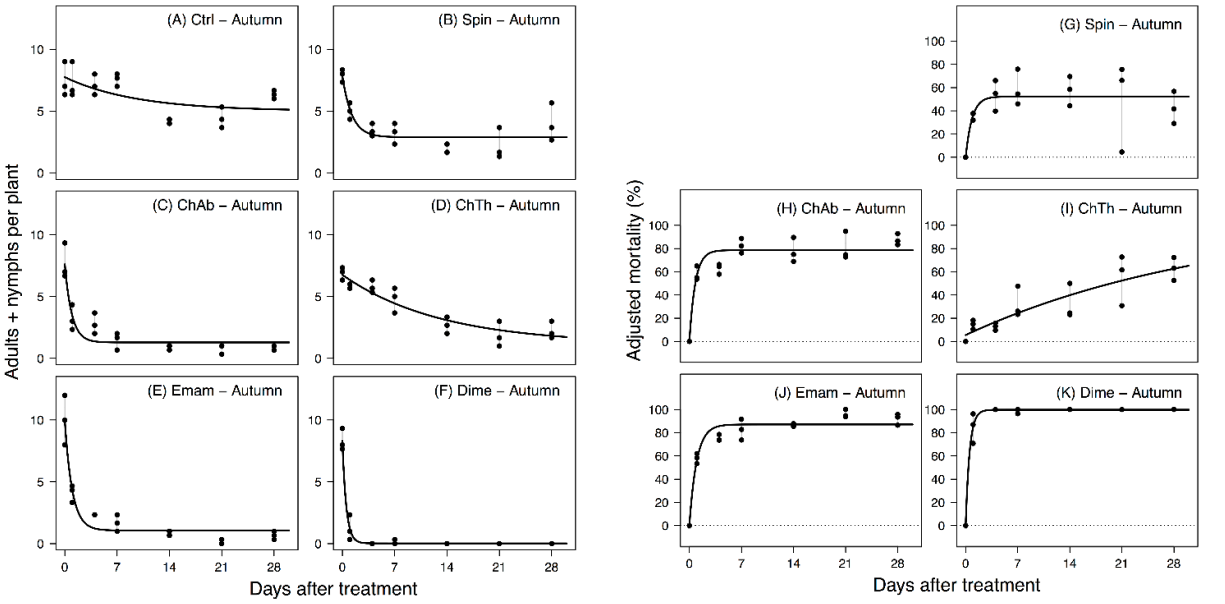


Figure 2. A-F, Numbers of *Nesidiocoris tenuis* for an untreated control and five insecticide treatments applied in autumn 2018; and G-K, adjusted mortalities (%) for the insecticide treatments. Mortalities were adjusted using the Henderson-Tilton formula (Henderson & Tilton, 1955). Points represent the values for each of the replicates and the regression lines are for a nonlinear least square fits of the model, Response ~ SSasymp (Day, Asym, R0, lrc), where SSasymp is a self-start model to evaluate the asymptotic regression and its gradient in the R package "lme4" (Bates et al., 2015). See Table 2 for the values and significance of the Asym parameters. Treatment codes: Ctrl, control; ChAb, chlorantraniliprole + abamectin; ChTh, chlorantraniliprole + thiamethoxam; Dime, dimethoate; Emam, Emamectin benzoate; and Spin, spinetoram. Treatments are displayed from lowest to highest equilibrium mortality for the summer data presented in Figure 1.

Table 2. Values and significance of the Asym parameters (estimated equilibrium mortality) for regression lines for nonlinear least squares fits of the model, Response ~ SSasymp(Day, Asym, R0, lrc), where SSasymp is a self-start model to evaluate the asymptotic regression and its gradient in the R package "lme4" (Bates et al., 2015) shown in Figures 1 and 2. Mortalities were adjusted using the Henderson-Tilton formula (Henderson & Tilton, 1955) and classified according to Organization for Biological and Integrated Control (IOBC) toxicity categories

| Treatment | Season | Estimated equilibrium mortality (%) | Standard error | T value | P value | IOBC toxicity category* |
|---|---|---|---|---|---|---|
| Spinetoram | summer | 24.3 | 5.25 | 4.6 | <0.001 | N |
| | autumn | 52.3 | 4.42 | 11.8 | <0.001 | M |
| Chlorantraniliprole +abamectin | summer | 45.1 | 5.44 | 8.3 | <0.001 | N |
| | autumn | 78.6 | 2.60 | 30.2 | <0.001 | T |
| Chlorantraniliprole + thiamethoxam | summer | 61.5 | 7.53 | 8.2 | <0.001 | M |
| | autumn | 108.0** | 88.60 | 1.2 | ns | M |
| Emamectin benzoate | summer | 86.1 | 2.50 | 34.5 | <0.001 | T |
| | autumn | 87.1 | 1.98 | 43.9 | <0.001 | T |
| Dimethoate | summer | 100.0 | 0.39 | 253.5 | <0.001 | T |
| | autumn | 99.8 | 1.13 | 88.3 | <0.001 | T |

* N: Harmless or slightly harmful; M: Moderately harmful; and T: Harmful;

** An estimated of the equilibrium mortality for the chlorantraniliprole + thiamethoxam treatment in autumn could not be reliably obtained by this nonlinear regression method, so it means final adjusted morality (62.7%) was used for determining its IOBC toxicity category.

The estimated equilibrium mortality and insecticide toxicity categories for *N. tenuis*, determined in the summer and autumn experiments, are shown in Table 2. Spinetoram caused only 24% mortality in the summer experiment and is classified harmless or only slightly harmful. Chlorantraniliprole + abamectin caused mortality of nearly half of the predator population, but is also categorized as harmless or slightly harmful. However, spinetoram and chlorantraniliprole + abamectin caused significant reduction in *N. tenuis* population and is classified as moderately harmful and harmful, respectively, under cooler conditions. Chlorantraniliprole + thiamethoxam is classified as moderately harmful in both experiments, while emamectin benzoate and dimethoate were classed as harmful (Table 2).

## Discussion

Biological control has increased in importance as an alternative pest control method in IPM. However, the effectiveness of a biological control agent can be compromised, particularly if pesticides are used when against unexpected secondary pest outbreaks. Therefore, it is essential to determine the non-target effects of pesticides to ensure successful and sustainable IPM. Consequently, in the present study, the non-target effects of 5 insecticides, namely spinetoram (a spinosyn), chlorantraniliprole + thiamethoxam (diamide and neonicotinoid), chlorantraniliprole + abamectin (diamide and avermectin), emamectin benzoate (avermectin) and dimethoate (organophosphate), on the predator insect, *N. tenuis,* were determined.

Spinetoram caused 24 and 52% mortality of *N. tenuis* in summer and autumn, respectively. Despite in high mortality under cooler conditions, based mean mortality of two season, spinetoram can be classified as harmless or less harmful (category N). Similarly, it was reported that spinetoram did not cause mortality of the adults and nymphs of a mirin bug, *Macrolphus basicornis* (Stal, 1860) (Soares et al., 2019). Martinou et al. (2014) reported that the active ingredient of spinosad another insecticide classified in the same group as spinetoram (spinosyns) was harmless or slightly harmful (category N) to *M. pygmaeus*. This is consistent with the findings of Arnó & Gabarra (2011), who found less than 13% mortality on predatory insects *M. pygmaeus* and *N. tenuis* in response to spinosad application. However, they also reported that spinosad exposure of females of *M. pygmaeus* and *N. tenuis* caused a significant decrease in number of progenies. Thus, they concluded that the sublethal effects of spinosad on predator reproduction should not be overlooked. In contrast, in other research, spinosad was reported as moderately harmful (category M) or harmful (category T) to *N. tenuis* (Sukhoruchenko et al., 2015; Portakaldalı & Satar, 2015a). Although, those studies reported high mortality from spinosad, this might have been due to the different insecticide formulation or conditions.

Chlorantraniliprole + thiamethoxam and chlorantraniliprole + abamectin gave differing results indicating the secondary insecticide was influential. Chlorantraniliprole + thiamethoxam was categorized as moderately harmful (category M) in both experiments. However, in the autumn experiment, its mortality effect did not stabilize, but it is unlikely that further unestimated decline in the population would push it into the next category. The low initial mortality of this insecticide was due to it being applied as a drench taking time for it to reach the upper canopy of the plant where the predator mainly resided.

Chlorantraniliprole + abamectin was classified as harmless or less harmful (category N) under warmer summer conditions, but was classed as harmful (category T) in autumn. This could be due to the slow breakdown of the insecticide under cooler conditions (Op de Beeck et al., 2017). In an experiment conducted under greenhouse conditions, Dáder et al. (2020) reported that chlorantraniliprole was harmless to *N. tenuis*. Another study also reported that chlorantraniliprole was harmless or less harmful with a mortality of less than 25% to the mirid, *M. pygmaeus* (Martinou et al., 2014). Therefore, it is suspected that the addition of abamectin may have a synergistic effect on the chlorantraniliprole against *N. tenuis* under greenhouse conditions.

Emamectin benzoate was classified as harmful (category T) in both experiments. These findings are consistent with a study conducted under laboratory conditions by Portakaldalı & Satar (2015b) who reported 74-79% mortality to *N. tenuis*. These findings are also consistent with those of some previous studies on *Pilophorus typicus* (Distant, 1909) (Heteroptera: Miridae) (Nakahira et al., 2010) and *Orius laevigatus* (Fieber) (Hemiptera: Anthocoridae) (Biondi et al., 2012). In contrast, Martinou et al. (2014) found that emamectin benzoate was harmless to another mirid, *M. pygmaeus* adults, under laboratory experiments. In addition, Amor et al. (2012) reported similar findings under semifield conditions for *M. pygmaeus*. Dáder et al. (2020) also determined that emamectin benzoate was only slightly harmful in a study conducted under greenhouse conditions with natural prey, including *T. absoluta* and *B. tabaci*. It is probable that different insecticide application rates, more than double used in the present study, and the insufficiency of the artificial diet provided led to increased movement of the predators on the plant and higher exposure to the insecticide, and thereby higher mortality. Also, emamectin benzoate is a systemic insecticide with a translaminar property; it is possible that omnivorous insects, such as *N. tenuis*, could ingest more toxic substances due to feeding directly the plant rather than just insecticide-contaminated prey.

One of the most important goals of IPM is the inclusion of insecticides that not harmful to the natural enemies of target pests. In the present study, it was revealed that spinetoram could be employed safely in IPM programs. Chlorantraniliprole + abamectin could potentially be used taking into consideration a possible effect on the predator population, which could be reduced by around 50%. Chlorantraniliprole + thiamethoxam has long lasting negative effects on the predator, so cannot be recommended. Caution should also be exercised in the use of emamectin benzoate, particularly at the application rates used in the present study, due to its harmful effects on *N. tenuis*.

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# Reduction of some insecticide residues from grapes with washing treatments

Üzümdeki bazı insektisit kalıntılarının yıkama işlemleriyle azaltılması

**Burak POLAT**[1*]

## Abstract

Insecticide application is the most common method of insect control in agriculture. Efficiency of washing treatments in reduction of insecticide (chlorpyrifos-methyl and lambda-cyhalothrin) residues from grapes were investigated in this study. The trial was established in a Sultana seedless vineyard in Sarıgöl District, Manisa Province, Turkey in 2020. Method verification was performed with the recovery, limit of quantification and precision. Pesticide-free grapes were spiked with 0.5, 1 and 5 times of MRL for pesticides. The recovery of chlorpyrifos-methyl and lambda-cyhalothrin were 102 and 101% respectively. QuEChERS method yielded an overall-recovery of 101%. These figures were within the SANTE recovery limits (60-140%) and the detection limits of the insecticides were below the MRLs. Grapes in a vineyard were sprayed with insecticides four times and harvested 0, 2, 4 and 7 d after the last spray. Washing (tap water, citric and acetic acid) and ultrasonic cleaning treatments were applied to harvested grapes. Washing treatments decreased residue levels and reductions increased with prolonged washing durations. Reductions also decreased with prolonged harvest durations from the last spray. The citric and acetic acid washing, and ultrasonic-cleaning methods provided more efficient reduction than washing with tap water.

**Keywords:** Chlorpyrifos-methyl, grape washing process, lambda-cyhalothrin, Manisa, pesticide residue reduction

## Öz

İnsektisit kullanımı tarımda zararlı kontrolü için en yaygın metottur. Bu çalışmada yıkama işlemlerinin üzümler üzerindeki insektisit kalıntılarının (chlorpyrifos-methyl ve lambda-cyhalothrin) azaltılmasına etkisi araştırılmıştır. Deneme Türkiye'de Manisa İli-Sarıgöl ilçesinde 2020 yılında Sultana çekirdeksiz üzüm bağında kurulmuştur. Metot doğrulama, geri kazanım, ölçüm limiti, tekrarlanabilirlik ve kesinlik ile gerçekleştirilmiştir. İnsektisit içermeyen üzüm numuneleri her pestisit için 0.5, 1 ve 5 kat MRL seviyelerinde sabitlenmiştir. Chlorpyrifos-methyl ve lambda-cyhalothrin geri alımları, sırasıyla %102 ve %101 olarak bulunmuştur. Tüm QuEChERS yönteminin geri kazanımı %101 olarak bulunmuştur. Bu rakamlar SANTE geri kazanım limitleri (%60-140) arasındadır. İnsektisitlerin tespit limitleri, MRL'nin altında bulunmuştur. Bağda üzümlere dört defa insektisit uygulanmıştır. Son insektisit uygulamasının 0., 2., 4. ve 7. günlerinde üzümler hasat edilmiş ve çeşme suyu, sitrik ve asetik asit ve ultrasonik yıkama işlemlerine tabi tutulmuştur. Yıkama işlemi kalıntıları azaltmış ve artan yıkama süresiyle kalıntının azalma oranları artmıştır. İlerleyen hasat zamanları ile kalıntının giderilmesi azalmıştır. Sitrik ve asetik asit yıkama ve ultrasonik yıkama, musluk suyu ile yıkamadan daha etkili bulunmuştur.

**Anahtar sözcükler:** Chlorpyrifos-methyl, üzüm yıkama işlemi, lambda-cyhalothrin, Manisa**,** pestisit kalıntı azaltılması

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## Introduction

Grapes and their processed products are extensively consumed all around the world. The world grape production is about 79 Mt in 2018. With an annual production of 4 Mt, Turkey has the sixth largest producer. In Turkey, 38% (1.5 Mt) of this production is in Manisa Province (TÜİK, 2019). About 83% of grapes exported from Turkey are sent to EU countries. Grapes are either consumed fresh or processed into different products such as wine, vinegar, raisin and molasses. Turkey's exports of raisins were about 243 kt in 2019, about 30% of globally traded raisins (TÜİK, 2019). Grapes are rich in carbohydrates, thus highly prone to pest damage. Pests including *Lobesia botrana* (Denis & Schiffermüller, 1775) (Lepidoptera: Tortricidae), *Daktulosphaira vitifoliae* (Fitch, 1855) (Phylloxeridae: Hemiptera), *Otiorhynchus* spp. Germar, 1822 (Coleoptera: Curculionidae), *Tetranychus urticae* Koch, 1836 (Acari: Tetranychidae) create significant problems in viticulture and result in serious economic losses each year. *Lobesia botrana* causes 45-92% product loss every year (Önçağ, 1975). Insecticides are commonly used in vineyards against these pests. Chlorpyrifos-methyl is used against *L. botrana*, lambda-cyhalothrin is applied against *Otiorhynchus* spp. and *L. botrana*.

In 2017, about 54 t of pesticides were used in Turkey with nearly 5 t used in Manisa Province. Manisa Province ranks the second highest user of insecticides at 1 t/year after Antalya province (1.2 t/year) in Turkey (TÜİK, 2018). Despite the advantages of pesticide use in agricultural fields and vineyards in terms of pests and disease control, residues pose serious risks for human health. Insecticides used in vineyards may leave a problematic level of residue on grape berries. Since chlorpyrifos-methyl is neurotoxic and cholinesterase inhibitor and lambda-cyhalothrin is endocrine disruptor, eye irritant and skin irritant (PPDB, 2020), such residues may constitute a major concern for consumers. Residues also generate significant problems for international trade, they are even a criterion for import bans.

There are many studies on pesticide residues in grapes. These are generally monitoring studies conducted by sampling from markets and vineyards. There is limited information on the residues left after pesticide treatment. Some researchers have investigated pesticide residues in grapes.

Zengin & Karaca (2017) determined pesticide residues on grape berries directly sampled from a vineyard. These researchers applied GAP (good agricultural practice) in a vineyard located in Uşak Province, Turkey. About 45% of the samples had no residues, 55% had pesticide residues but none exceeded the maximum residue levels (MRL), with 13 pesticides detected. These researchers reported that lambda-cyhalothrin was the most commonly detected pesticide. They also emphasized the importance of GAP, especially in terms of pesticide application. Turgut et al. (2011) conducted a study in vineyards of Denizli, İzmir and Manisa Provinces and reported the most common pesticide residues were lambda-cyhalothrin (22 samples) and chlorpyrifos-methyl (15 samples). Gölge & Kabak (2018) investigated residues of pesticides in table grapes collected from local markets in four provinces of Turkey and reported residue of one or more pesticide in 60% of the samples. The residues were greater than MRL in 20% of the samples. Chlorpyrifos, azoxystrobin, boscalid and cyprodinil were identified as the most common residues encountered on grape samples. In another study conducted on fresh Sultana grapes, İçli & Tahmas Kahyaoğlu (2020) encountered lambda-cyhalothrin and iprodione residues in majority of the samples.

Researchers have mostly focused on efficient means of pesticide residue reduction from fruits and vegetables. Majority of them employed washing treatments with tap water and reported some positive outcomes with washing (Zhou et al., 2019; Corrias et al., 2020). Heshmati et al. (2020) worked on residues of some pesticides in unwashed and washed grapes and reported decreased residues with washing treatments. Tap water, acetic acid and sodium bicarbonate ($NaHCO_3$) were used in washing treatments and $NaHCO_3$ was reported as the most efficient means of pesticide residue reduction. Researchers reported that $NaHCO_3$ treatments yielded about 95% reduction for diazinon, 95% reduction for penconazole, 94% for hexaconazole, 72% for ethion and 63% for phosalone. However, Zhou et al. (2019) reported that

ultrasonic cleaning was the most efficient means of residue reduction in grape samples with reduction of 72-100%. These different degrees of reduction can be attributed to surface structure of the matrix.

Efficiency of washing treatments with different solutions in reduction of pesticide residues depends on the mode of action mode, solubility and physicochemical characteristics of the relevant pesticides (Hassan et al., 2019). Mode of action designates the behavior of the pesticides in the treated plant. Thus, mode of action (systemic or contact) is considered as an important factor for reduction of pesticide residues with washing treatments. It is harder to reduce systemic residues within fruits and vegetables than contact pesticides with remain on the fruit surface, whereas systemic pesticides absorbed by the leaves, fruits, shoots or stems, and penetrate deep into the plants and are transported to different locations within the plants (Lozowicka et al., 2013; Acoglu et al., 2018). In previous studies, it was found that it was easier to remove highly-soluble pesticides with small partition coefficients by washing (Kong et al., 2012; Lozowicka et al., 2016; Heshmati et al., 2020).

The PHI (preharvest interval), the time between the last spray and the harvest, is another factor to be taken into consideration in reduction of pesticide residues by washing. Özel & Tiryaki (2019) reported decreasing reduction with decreasing PHI. Solutions used for washing also significantly influence efficiency of the process. While some researchers used tap water, the others used acetic acid and ultrasonic cleaning processes for reduction of residues from fruits and vegetables (Khadre et al., 2001; Lozowicka et al., 2013; Polat & Tiryaki, 2020; Çatak et al., 2020). Previous research also focused on reduction of residue levels with different food processing techniques (Randhawa et al., 2104a; Lozowicka et al., 2016; Zhou et al., 2019; Heshmati et al., 2020).

Osman et al. (2014) reported that washing with acetic acid and citric acid (1-2%) was more efficient for reduction of insecticide (chlorpyrifos) residues than the other procedures, such as tap water washing and $KMnO_4$ and $H_2O_2$. Randhawa et al. (2014a) studied the effects of various acid concentrations on residue reduction in capsicum and cucumber samples, and reported that greater acid concentration (9%) had higher reduction than the other concentrations (1.5, 3 and 6%). In another study, ultrasonic cleaning was found to be more efficient in reduction of pesticide residues from strawberries than the other methods (Lozowicka et al., 2016).

Efficiency of washing (tap water, citric acid and acetic acid) and ultrasonic cleaning in reduction of insecticide (chlorpyrifos-methyl and lambda-cyhalothrin) residues from Sultana grapes were investigated in this study with different PHI (days 0, 2, 4 and 7) and different washing durations (2 and 5 min). The QuEChERS-AOAC 2007.01 method coupled with UPLC-MS/MS detection was used for the pesticide residue analyses. Method reliability was verified based on international guidelines (EURACHEM, 2014; SANTE, 2019; TURKAK, 2019).

## Materials and Methods

### Field experiments and sampling

Field experiments were conducted in a vineyard located in Sarıgöl District, Manisa Province in 2020. Relevant cultural practices were conducted as needed throughout the growing season. Trials, sprays and sampling were conducted according to published standard trial methods for residue experiments with plant protection products (Anonymous, 2011). Lambda-cyhalothrin (Karate Zeon CS, 50 g/L, Syngenta) and chlorpyrifos-methyl (Reldan 22 EC, 227 g/L, Dow AgroSciences) insecticides were sprayed with at 25 and 200 mL/100 L water. Four sprays (15-d intervals) were applied throughout the growing season. Sultana seedless grapes were harvested 4 h (day 0) and 2, 4 and 7 days after the last spray. At each harvest, 5 kg grapes were collected (60 kg in total). Harvested samples were brought to laboratory in an icebox. Disposable polyethylene gloves were used to prevent contamination during the collection of samples.

**Instruments**

For washing treatments, about 1 kg of harvested grapes (EC, 2002) were submerged in tap water (5L, 20ºC), acetic acid and citric acid solutions (5L, 9%, 20ºC) for 2 and 5 min (Randhawa et al., 2014a). Samples were then dried for 30 min at room temperature before analysis. Analyses were conducted in four replicates. An ultrasonic cleaner (Medisson 12UT, Turkey) was used in ultrasonic cleaning treatments applied by immersing air-dried samples in 5 L tap water for 2 and 5 min (Figure 1). Unwashed samples were also analyzed for insecticide residues and resultant values were used to assess the efficacy of washing treatments.

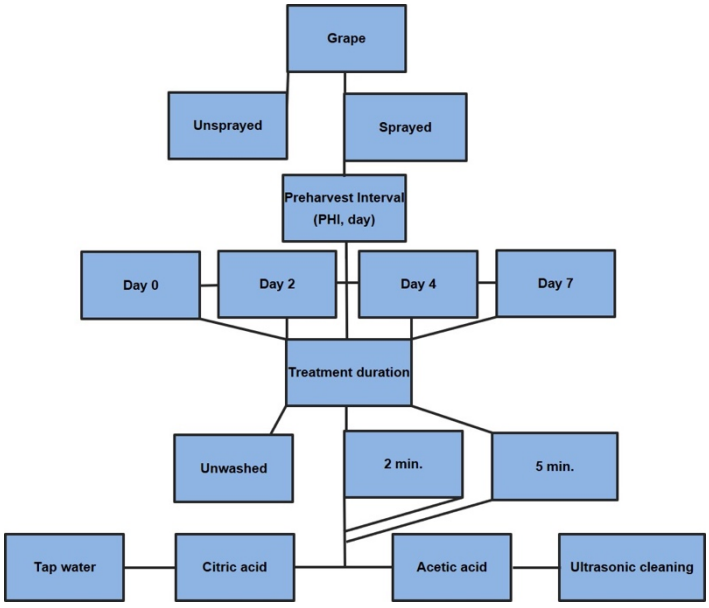


Figure 1. Steps of washing processes and sampling details of various washing treatments.

The processing factor (PF) for the washing treatments (mg/kg) (OECD, 2008) was calculated as the ratio of residue concentration in processed over unprocessed product. PF values of <1 indicate decreased concentrations with washing treatment (Dong et al., 2012). For assessing of pesticide residues in processed product, PFs is published by Turkish Ministry of Agriculture and Forest General Directorate of Food and Control (Anonymous, 2020).

**Chemicals, reagents and solutions**

Insecticide (chlorpyrifos-methyl and lambda-cyhalothrin) standards were purchased from a Dr. Ehrenstorfer Lab., GmbH (Wesel, Germany) at purity of 99.69 and 98.73%, respectively; and anhydrous $MgSO_4$, sodium acetate (NaAC), methanol (MeOH) and acetonitrile (MeCN) from Merck Co. (Darmstadt, Germany) at purities of 99.5, 99.0, 99.9 and 99.9%, respectively. PSA (primary-secondary amine 40 µm, 100 g) was sourced from Agilent (Santa Clara, CA, USA). Toxicological and physicochemical properties of insecticides are given in Table 1.

Stock solutions (400 µg/mL) were prepared adding 10 mg pesticide into 25 mL flasks and completing the final volume to 25 mL with acetonitrile. Working solutions (1.0 µg/mL) were prepared through dilution of the stock solutions. Calibration solutions were prepared over ranges of 20-4000 pg/µL and 10-2000 pg/µL. Spiking solutions equal to 0.5, 1 and 5 times of MRL were prepared. The standards and solutions were stored at 4ºC in the dark. Representative apple matrix was used for matrix-matched calibrations and quantification, as indicated in Codex Alimentarius Commission Guidelines (CAC, 2003) and SANTE Guidelines (SANTE, 2019). Spiking with five times MRL was diluted to fit the calibration range.

Table 1. Physicochemical and toxicological properties and mode of action of insecticides (WHO, 2009; EU, 2020; IRAC, 2020; PPDB, 2020)

| | Parameter | Chlorpyrifos-methyl | Lambda-cyhalothrin |
|---|---|---|---|
| | Chemical formula | $C_7H_7Cl_3NO_3PS$ | $C_{23}H_{19}ClF_3NO_3$ |
| | Group | Organophosphates (1B) | Pyrethroids (3A) |
| | Mode of action | Non-systemic acetylcholinesterase (AChE) inhibitors nerve action | Non-systemic sodium channel modulators nerve action |
| Physicochemical property | Log P (Octanol-water partition coefficient) | 4.7 | 5.5 |
| | Solubility in water at 20°C (mg/L) | 2.74 | 0.005 |
| | Degradation point (ºC) | 175 | 275 |
| | Molecular mass (g/mol) | 322.53 | 449.85 |
| Toxicological property | EU MRL (µg/kg) | 1000 | 80 |
| | ADI (mg/kg/bw/day) | 0.010 | 0.0025 |
| | ARfD (mg/kg bw /day) | 0.10 | 0.005 |
| | Mammals -Acute $LD_{50}$ (mg/kg) | 5000 | 56 |
| | Mammals-Inhalation $LC_{50}$ (mg/L) | >0.67 | 0.06 |
| | WHO classification * | III | II |
| | Health issues | Reproductive / Growth effects Neurotoxic, Cholinesterase inhibitor | Endocrine disruptor Eye irritant, Skin irritant |

* II, moderately hazardous; III, less hazardous.

**Sample preparation and analyses**

The QuEChERS method was published in 2003 and the method has been widely used in residue analyses since. The QuEChERS-AOAC Official Method 2007.01 version has also been employed in residue analyses in agricultural commodities (Lehotay, 2007; Omeroglu et al., 2012; Polat & Tiryaki, 2019; Heshmati et al., 2020).

QuEChERS method should be verified under local conditions. Recovery assessment is the first step of method validation (SANTE, 2019). Therefore, fortification trials were performed. For this aim, 1 kg of blank (pesticide-free sample, no pesticide applied sample) grape sample was homogenized with a blender (Waring Commercial Blender). Then, 15 g homogenized sample (analytical portion) were placed into tubes supplemented then with 100 µL lambda-cyhalothrin and chlorpyrifos-methyl corresponding to 0.5, 1 and 5 x MRL spiking levels in four replicates (analytical portion) (Table 2). Resultant mixture was vortexed for 30 s and left for 15 min. After the salting step ($MgSO_4$ and NaAC), the extract was centrifuged (Hettich EBA 280) for 5 min at 5000 rpm. For further analyses, the steps in Figure 2 were followed. Analyses (extraction and cleanup) of all spiked and processed grapes were performed with the QuEChERS AOAC Method 2007.01 and LC-MS/MS (Lehotay et al., 2005). Both spiked and processed samples were analyzed in four replicates (analytical portion). About 1 mL of extract was sampled once for processed samples and three times for spiked samples into GC vials. The recovery was calculated as the percentage of measured over spiked concentration (Çatak & Tiryaki, 2020).

Table 2. Spiking pattern of grapes with chlorpyrifos-methyl and lambda-cyhalothrin

| Spike | Code | Fortification (µg/kg) | |
|---|---|---|---|
| | | Chlorpyrifos-methyl | Lambda-cyhalothrin |
| 0.5 x MRL* | F1/1-4 | 500 | 40 |
| 1.0 x MRL | F2/1-4 | 1000 | 80 |
| 5.0 x MRL | F3/1-4 | 5000 | 400 |
| Control | F0/1-3 | none | none |

* EU maximum residue limit, µg/kg.

Method precision and recovery rates were tested in accordance with SANTE European Guidelines (SANTE, 2019). Method linearity was checked for the ranges of 20-4000 pg/µL for chlorpyrifos-methyl and 10-2000 pg/µL for lambda-cyhalothrin.

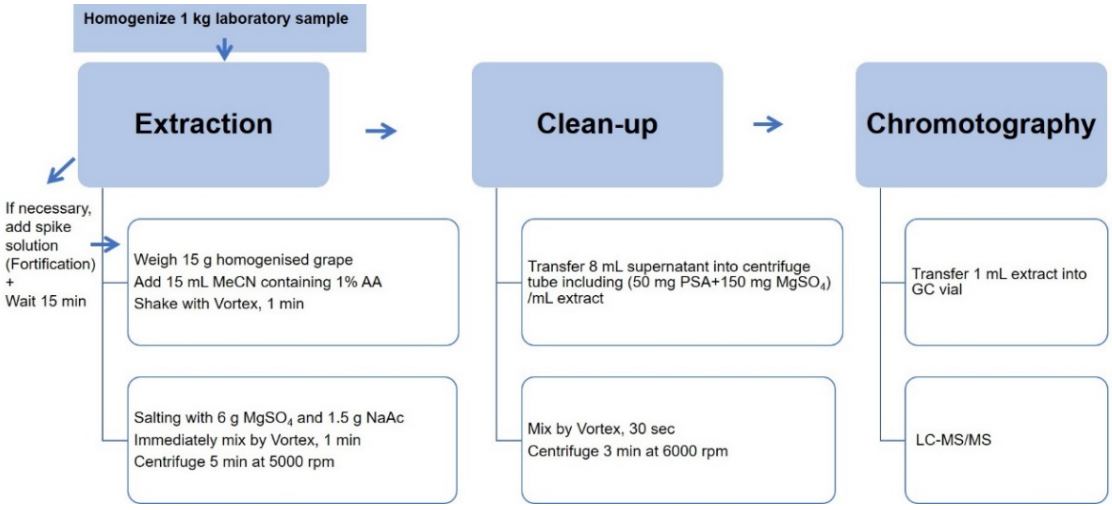


Figure 2. Analysis of chlorpyrifos-methyl and lambda-cyhalothrin in sultana grapes with the QuEChERS method.

### UPLC-MS/MS analysis

Chromatographic analyses were conducted with an LC-MS/MS (Waters I Class Plus UPLC + Xevo TQ-S micro MS Detector; ESI + mode) equipped with Acquity UPLC BEH $C_{18}$ column (1.7 µm, 2.1 x 100 mm). Total run time was 15 min. Injection volume was 1 µL (in MeCN) and flow rate was 0.35 mL/min. A gradient program including 10 mM $NH_4CH_4CO_2$, 95% MeOH (B) and 10 mM $NH_4CH_4CO_2$ pH 5, water (A) were used (Table 3). Selected ion groups, retention time and other parameters such as calibration ranges and limit of quantification of insecticides in LC-MS/MS analysis are shown in Table 4.

Table 3. Gradient solvent flow program (flow rate 0.35 mL/min)

| | Time (min) | A% | B% |
|---|---|---|---|
| 1 | | 98.0 | 2.0 |
| 2 | 0.25 | 98.0 | 2.0 |
| 3 | 11.75 | 1.0 | 99.0 |
| 4 | 13.25 | 1.0 | 99.0 |
| 5 | 13.35 | 98.0 | 2.0 |
| 6 | 15.00 | 98.0 | 3.0 |

Table 4. Selected ion groups, retention time and other parameters insecticides in LC-MS/MS analysis

| Parameter | Chlorpyrifos-methyl | Lambda-cyhalothrin |
|---|---|---|
| Quantification ion | 321.80 > 124.93 | 467.22 > 225.04 |
| Confirmation ion | 323.99 > 124.93 | 467.22 > 141.06 |
| Retention time (tR. min) | 10.13 | 11.25 |
| Calibration range, ppb, pg/µL | 20-4000 | 10-2000 |
| LOQ, ppb (µg/kg) | 20 | 10 |

### Method verification

Analytical method was verified in accordance with the SANTE guidelines. Recovery (including relative standard deviation, RSD), linearity, precision, limit of quantification and accuracy assessments were performed for method verification (SANTE 2019).

### Data analysis

SAS statistical software was used for statistical evaluation (SAS, 1999). Data were subjected to analysis of variance. Tukey's test was used to compare significant mean values.

## Results and Discussion

### Method performance verification

The calibration curves for standard solutions of chlorpyrifos-methyl and lambda-cyhalothrin were y = 314.041x + 398.601 and y = 639.498x + 2277.72, respectively. The curves were linear with the 20-4000 pg/µL range for chlorpyrifos-methyl and 10-2000 pg/µL range for lambda-cyhalothrin with $r^2 \geq 0.999$. Analytical functions were used as regression equations in matrix-matched calibrations and they were used for analyte (pesticide) quantification. The retention times for lambda-cyhalothrin and chlorpyrifos-methyl in matrix-matched solutions are presented in Table 4. Limit of quantification of chlorpyrifos-methyl and lambda-cyhalothrin for running method were 20 and 10 µg/kg, respectively (Table 4). These values were substantially lower than the MRLs specified by EC. MRLs of chlorpyrifos-methyl and lambda-cyhalothrin for grape were 1000 and 80 µg/kg, respectively (Table 2).

The recovery ratios varied between 84-127% (n = 72) (Table 5). Recovered chlorpyrifos-methyl from the grape matrix was 102% with the 36 samples ranging from 89-116%. For method precision, RSD was identified as 7.9% (n = 36). Recovery of lambda-cyhalothrin and RSD value were 100% and 10.5, respectively. Overall recovery was 101% with a standard deviation of 9.4% and RSD of 9.3% (Table 5). The recovery ratios and RSDs were within the SANTE recovery limits (60% ≤ Q ≤ 140%; RSD ≤ 20%) (SANTE, 2019; UGRL, 2020). Based on the accuracy values (Table 5), the QuEChERS-LC-MS/MS method yielded sufficient recovery ratios for lambda-cyhalothrin and chlorpyrifos-methyl insecticides in Sultana grapes.

Table 5. Method verification results (3 spiking level x 4 analytical portion x 3 times 1 mL for LC-MS/MS x 2 pesticide, n = 72)

| Analyte | Concentration, µg/kg | | Recovery % | RSD % |
|---|---|---|---|---|
| | Spiked | Measured | (As a tool for trueness) | (As a tool for precision) |
| Chlorpyrifos-methyl | 500 | 465 | 93 | 4.6 |
| | 1000 | 1087 | 109 | 4.4 |
| | 5000 | 5208 | 104 | 4.7 |
| | Mean recovery, n=36, Recovery range= 89-116% | | 102 | 7.9 |
| Lambda-cyhalothrin | 40 | 38 | 95 | 11.3 |
| | 80 | 78 | 98 | 4.1 |
| | 400 | 431 | 108 | 10.4 |
| | Mean recovery, n=36, Recovery range =84-127% | | 100 | 10.5 |
| | Overall recovery of the QuEChERS (method accuracy):101% (n=72, SD= 9.4, RSD=9.31) Recovery range: 84-127% | | | |

### Analysis of unwashed grapes

Unwashed were also analyzed to compare the performance of washing methods through PF values. In day 0 samples, chlorpyrifos-methyl residues (1156 µg/kg) slightly exceeded MRL at 1000 µg/kg. However, lambda-cyhalothrin residues (325 µg/kg) were about four times the MRL (80 µg/kg). The residues of chlorpyrifos-methyl in day 2, 4 and 7 samples were 742, 124 and 42 µg/kg, respectively. For lambda-cyhalothrin, these values were 276, 75 and 62 µg/kg, respectively (Table 6). Chlorpyrifos-methyl and lambda-cyhalothrin residues decreased with increasing time after the last spray. This again highlights the importance of PHI.

### Effects of washing treatments

The effects of different washing treatments on residue reduction are shown in Table 6. Increasing residue reduction were seen for both insecticides with longer washing. Processing factors of washing treatments are shown in Table 6. Since PF were all less than 1, the treatments were efficient in reduction of insecticide residues from Sultana grapes. Results of statistical analyses for insecticide residue levels are shown in Table 7.

Table 6. Pesticide residues of unwashed samples, reduction rates and their PFs achieved with the present applications (n = 4)

| Application | PHI (d) | Time (min) | Chlorpyrifos-methyl | | | Lambda-cyhalothrin | | |
|---|---|---|---|---|---|---|---|---|
| | | | Mean residue (µg/kg) (±SD) | PF | Reduction (%) | Mean residue (µg/kg) (±SD) | PF | Reduction (%) |
| Unwashed | 0 | - | 1156 ± 38.8 | | | 325 ± 33.7 | | |
| | 2 | - | 742 ± 56.5 | | | 276 ± 11.6 | | |
| | 4 | - | 124 ± 37.0 | | | 75 ± 4.5 | | |
| | 7 | | 42 ± 8.2 | | | 62 ± 3.6 | | |
| Tap water | 0 | 2 | 549 ± 26.6 | 0.47 | 52.6 | 176 ± 11.9 | 0.54 | 45.9 |
| | | 5 | 430 ± 35.0 | 0.37 | 62.8 | 142 ± 16.6 | 0.44 | 56.1 |
| | 2 | 2 | 380 ± 27.8 | 0.51 | 48.8 | 170 ± 6.7 | 0.62 | 38.3 |
| | | 5 | 307 ± 20.4 | 0.41 | 58.7 | 164 ± 10.5 | 0.59 | 40.5 |
| | 4 | 2 | 94 ± 15.8 | 0.76 | 24.1 | 55 ± 7.3 | 0.73 | 26.7 |
| | | 5 | 88 ± 8.0 | 0.72 | 28.4 | 50 ± 16.3 | 0.67 | 33.0 |
| | 7 | 2 | 35 ± 7.9 | 0.86 | 13.9 | 52 ± 7 | 0.85 | 15.3 |
| | | 5 | 33 ± 3.5 | 0.79 | 20.7 | 44 ± 2.8 | 0.71 | 29.2 |
| Citric acid | 0 | 2 | 485 ± 41.2 | 0.42 | 58.1 | 124 ± 9.5 | 0.38 | 61.7 |
| | | 5 | 397 ± 25.2 | 0.34 | 65.7 | 109 ± 3.4 | 0.34 | 66.4 |
| | 2 | 2 | 339 ± 21.5 | 0.46 | 54.4 | 132 ± 3.1 | 0.48 | 52.3 |
| | | 5 | 270 ± 15.9 | 0.36 | 63.6 | 127 ± 6 | 0.46 | 54.1 |
| | 4 | 2 | 83 ± 10.3 | 0.68 | 32.5 | 50 ± 4.2 | 0.67 | 33.5 |
| | | 5 | 66 ± 7.0 | 0.53 | 46.8 | 38 ± 1.4 | 0.51 | 48.8 |
| | 7 | 2 | 34 ± 4.6 | 0.81 | 19.5 | 41 ± 8.5 | 0.67 | 33.2 |
| | | 5 | 29 ± 4.0 | 0.69 | 31.2 | 40 ± 3.6 | 0.64 | 35.8 |
| Acetic acid | 0 | 2 | 516 ± 13.8 | 0.45 | 55.3 | 143 ± 7.4 | 0.44 | 56.1 |
| | | 5 | 386 ± 17.9 | 0.33 | 66.7 | 119 ± 6.8 | 0.37 | 63.4 |
| | 2 | 2 | 351 ± 23.3 | 0.47 | 52.8 | 154 ± 15.7 | 0.56 | 44.3 |
| | | 5 | 298 ± 14.1 | 0.40 | 59.8 | 141 ± 2 | 0.51 | 49.0 |
| | 4 | 2 | 82 ± 12.2 | 0.67 | 33.5 | 49 ± 5.5 | 0.65 | 34.8 |
| | | 5 | 75 ± 8.7 | 0.61 | 39.1 | 42 ± 2.9 | 0.56 | 43.9 |
| | 7 | 2 | 30 ± 2.2 | 0.72 | 28.0 | 40 ± 4.5 | 0.66 | 34.4 |
| | | 5 | 27 ± 3.6 | 0.66 | 34.5 | 31 ± 2.3 | 0.51 | 49.0 |
| Ultrasonic cleaning | 0 | 2 | 451 ± 39.1 | 0.39 | 61.0 | 140 ± 13.2 | 0.43 | 56.9 |
| | | 5 | 334 ± 55.5 | 0.29 | 71.1 | 104 ± 15.0 | 0.32 | 68.0 |
| | 2 | 2 | 311 ± 10.2 | 0.42 | 58.1 | 151 ± 5.0 | 0.55 | 45.4 |
| | | 5 | 274 ± 16.6 | 0.37 | 63.1 | 132 ± 4.3 | 0.48 | 52.2 |
| | 4 | 2 | 82 ± 7.4 | 0.66 | 33.9 | 52 ± 6.9 | 0.70 | 30.3 |
| | | 5 | 69 ± 8.2 | 0.55 | 44.5 | 48 ± 4.4 | 0.64 | 35.5 |
| | 7 | 2 | 36 ± 2.8 | 0.86 | 13.9 | 44 ± 5.7 | 0.72 | 28.4 |
| | | 5 | 30 ± 3.7 | 0.71 | 28.8 | 41 ± 3.8 | 0.67 | 32.8 |

Table 7. Significant differences in insecticide residue level of the washing applications

| Active ingredient | Application* | Residues at harvest (µg/kg) | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | Day 0 | | | Day 2 | | | Day 4 | | | Day 7 | | |
| Chlorpyrifos-methyl | Raw | 1156 | A** | a*** | 742 | A | b | 124 | A | c | 42 | A | d |
| | Tap water (2) | 549 | B | a | 380 | B | b | 94 | B | c | 36 | AB | d |
| | Tap water (5) | 430 | EF | a | 307 | DEF | b | 88 | BC | c | 33 | BC | d |
| | Citric acid (2) | 485 | CD | a | 339 | CD | b | 83 | BC | c | 34 | BC | d |
| | Citric acid (5) | 397 | F | a | 270 | F | b | 66 | C | c | 29 | BC | d |
| | Acetic acid (2) | 516 | BC | a | 351 | BC | b | 82 | BC | c | 30 | BC | d |
| | Acetic acid (5) | 386 | F | a | 298 | EF | b | 75 | BC | b | 27 | C | d |
| | Ultrasonic C (2) | 451 | DE | a | 311 | DE | b | 82 | BC | c | 36 | AB | d |
| | Ultrasonic C (5) | 334 | G | a | 274 | EF | b | 69 | C | c | 30 | BC | c |
| | Mean | 523 | | a | 364 | | b | 85 | | c | 33 | | d |
| Lambda-cyhalothrin | Raw | 325 | A | a | 276 | A | b | 75 | A | c | 62 | A | c |
| | Tap water (2) | 176 | B | a | 170 | B | a | 55 | B | b | 52 | B | b |
| | Tap water (5) | 142 | C | b | 164 | BC | a | 50 | BC | c | 44 | C | c |
| | Citric acid (2) | 124 | CDE | a | 132 | FG | a | 50 | BC | b | 41 | C | b |
| | Citric acid (5) | 109 | E | b | 127 | G | a | 38 | D | c | 40 | C | c |
| | Acetic acid (2) | 143 | C | a | 154 | CD | a | 49 | BC | b | 40 | C | b |
| | Acetic acid (5) | 119 | DE | a | 141 | EF | a | 42 | CD | c | 31 | D | d |
| | Ultrasonic C (2) | 140 | CD | a | 151 | DE | a | 52 | B | b | 44 | C | b |
| | Ultrasonic C (5) | 104 | E | b | 132 | FG | a | 48 | BC | c | 41 | C | c |
| | Mean | 154 | | a | 161 | | b | 51 | | c | 44 | | d |

* Bracketed values are the time of application (min);
** Means followed by the same uppercase letters within the same column are not significantly different at $p \leq 0.01$;
*** Means followed by the same lowercase letters within the same row are not significantly different at $p \leq 0.01$.

**Washing with tap water**

Effects of tap water washing treatments on residue reduction are given in Table 6. Significant differences were observed in residue levels in unwashed grapes of chlorpyrifos-methyl in day 0, 2, 4 and 7 samples (Table 7). For lambda-cyhalothrin, significant differences were only observed in unwashed samples between the day 0 and 2 samples. With washing of 2 and 5 min, significant changes were only observed for chlorpyrifos-methyl between day 0 and 2 samples. However, this was valid for only in day 0 samples for lambda-cyhalothrin.

With 5-min washing, day 0, 2, 4 and 7 samples with tap water washing, respectively, gave 63, 59, 28 and 21% reductions for chlorpyrifos-methyl, respectively, with PF = 0.37, PF = 0.41, PF = 0.72 and PF = 0.79. The values for lambda-cyhalothrin were 56, 41, 33 and 29%, respectively, with PF = 0.44, PF = 0.59, PF = 0.67 and PF = 0.71.

Washing tap water significantly decreased insecticide residues on the grape samples. Increasing reductions were found with increasing washing duration corresponding to decreasing PF values. Similarly, decreasing reduction ratios were found with increasing PHI values corresponding to increasing PF values. Similarly, Lozowicka et al. (2016) also reported decreasing PF values for increasing washing duration.

Zhou et al. (2019) reported that reductions for five pesticides (difenoconazole, azoxystrobin, abamectin, thiamethoxam, tebuconazole) from grape samples were higher than 60%, except for abamectin, with tap water washing. Heshmati et al. (2020) reported the greatest efficiency in reductions of pesticide residues with tap water washing and the greatest for $NaHCO_3$ washing. Çelik et al. (1995) investigated insecticide (diazinon) reduction with tap water washing and reported 18% reduction in grapes, 10% in apples and 9% in tomatoes.

Holland et al. (1994) noted that water solubility and octanol-water partition coefficient (log P) are important for pesticide residue reduction. Previous researchers emphasized that highly-soluble pesticides with lower Log P could be removed more efficiently with the use of washing treatments (Kong et al., 2012; Randhawa et al., 2014b; Zhao et al., 2014; Lozowicka et al., 2016). Polat & Tiryaki (2020) investigated the efficiency of washing applications in reduction of pesticide residues. In day 0, 2 and 3 samples, they reported 64, 18 and 40% reduction for acetamiprid, respectively, with PF values of 0.36, 0.82 and 0.6, with tap water washing. Reductions were 38, 32% and 33% for chlorpyrifos and 76, 70 and 69% for formetanate hydrochloride, respectively.

In present study, chlorpyrifos-methyl gave greater reduction (63%) and lower PF (0.37) with lower log P (4.74) and high solubility value (2.74 mg/L) (Table 1). However, lambda-cyhalothrin (log P = 5.5, Sw = 0.005 mg/L) had lower reduction (56%) and higher PF (0.44) (Table 6). Such results indicated that log P and solubility were a significant factor in reduction of pesticides residues. Besides water solubility, mode of action of pesticide is also important in reduction of pesticide residues. In present study, both insecticides were non-systemic.

**Washing with acid solutions**

In day 0 samples, significant differences were found between residues in 5-min acid washed and unwashed grapes for both pesticides (Table 7). The significant differences were found between 2- and 5-min citric acid washing for chlorpyrifos-methyl in day 0 and 2 samples. The differences between citric acid treatment duration for lambda-cyhalothrin in day 2 and 4 samples were significant and between acetic acid treatment durations of chlorpyrifos-methyl and lambda-cyhalothrin in day 0 and 2 samples (also day 7 samples for lambda-cyhalothrin).

The 5-min citric acid wash reduced chlorpyrifos-methyl by 66% (PF = 0.34) and lambda-cyhalothrin 66% (PF = 0.34) in day 0 samples. Acetic acid wash reduced chlorpyrifos-methyl by 67% (PF = 0.33) and lambda-cyhalothrin by 63% (PF = 0.37) (Table 6).

Since both insecticides were non-systemic, the reductions were not significantly different. These findings reveal that acid washing was more efficient in reduction of insecticide residues than the tap water. Randhawa et al. (2014a) conducted a similar study with acid washing treatments and reported a reduction 72% with citric acid washing and 69% with acetic acid. Polat & Tiryaki (2020) conducted a study with acetic acid washing and reported a maximum reduction of 78% for chlorpyrifos, followed by reduction rate of rate of 72% for formetanate hydrochloride and 34% for acetamiprid. Researchers reported the reduction rates of citric acid washing respectively as 78, 76 and 36%.

**Washing with ultrasonic cleaner**

Ultrasonic cleaning was effective in day 0 samples. The reductions with 5-min treatment were 71% for chlorpyrifos-methyl and 68% for lambda-cyhalothrin. In day 7 samples, the reductions were 29% and 33%, respectively (Table 6). Decreasing reduction rates were observed with increasing PHI. In day 4 samples, 5-min ultrasonic cleaning reduced chlorpyrifos-methyl by 45% (PF = 0.55) and lambda-cyhalothrin by 36% (PF = 0.64). Ultrasonic cleaning significantly reduced residues. In day 0 samples, the differences between unwashed grapes and 2 and 5-min treatments were significant (Table 7). Compared to tap water, ultrasonic cleaning gave greater reductions in residues of both insecticides. Polat & Tiryaki (2020) conducted a study with ultrasonic cleaning and reported a reduction of 30% for acetamiprid, 82% for chlorpyrifos and 74% for formetanate hydrochloride. In day 0 grape samples, such reduction rates were 77, 86 and 89%. Zhou et al. (2019) investigated the efficiency of different washing treatments in reduction of pesticide residues and found that ultrasonic washing more efficient than tap water treatment. Buakham et al. (2012) also reported greater reductions with ultrasonic washing.

Çatak et al. (2020) reported significant effects of different washing processes on reduction of pirimiphos-methyl residues. The order of reduction of pirimiphos-methyl residues (from highest to lowest) was ultrasonic cleaning > citric acid > acetic acid > tap water. The maximum reduction (87%) of pirimiphos-methyl was with ultrasonic cleaning. Lozowicka et al. (2016) reported reductions of 92% for ultrasonic cleaning, 20-68% for tap water and 36-75% for ozonated-water washing.

In the present study, citric acid washing and ultrasonic cleaning were more efficient in reduction of pesticide residues in grape samples than the acetic acid and tap water washing. The lowest residues (30 µg/kg for chlorpyrifos-methyl and 41 µg/kg for lambda-cyhalothrin) were achieved in day 7 samples with 5-min ultrasonic cleaning. In day 4 samples with 2-min washing, chlorpyrifos-methyl residues did not exceed the MRL (1000 µg/kg) but lambda-cyhalothrin residues (325 µg/kg) were about four times the MRL (80 µg/kg) in unwashed grapes. Residues of lambda-cyhalothrin were below the MRL with citric acid (50 µg/kg), acetic acid (49 µg/kg) and ultrasonic (52 µg/kg) washing.

## Conclusions

These findings reveal increased residue reduction with longer washing. However, decreased residue reduction was observed with increasing PHI values. Greater reductions were achieved for the highly-soluble insecticide, chlorpyrifos-methyl, with smaller log P. Chlorpyrifos-methyl and lambda-cyhalothrin residues decreased with PHI, highlighting the importance of PHI. The residues of chlorpyrifos-methyl were below the MRL in day 2 ,4 and 7 samples. With both 2- and 5-min washings, the residue of chlorpyrifos-methyl were reduced below MRL with all washing methods. For lambda-cyhalothrin, the residue levels were below the MRL in day 4 and 7 samples. The residues of lambda-cyhalothrin were above the MRL in day 0 and 2 samples with ultrasonic cleaning, citric and acetic acid and tap water washing treatments. It is concluded that as PHI increases the reduction of pesticide decreases with the various washing treatments and harvesting should be conducted according to the recommended PHI. Combined applications (for instance, ultrasonic cleaner plus acid solutions) are recommended for future studies.

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## Türkiye Entomoloji Dergisi Yayın İlkeleri

Derginin yayın ilkeleri aşağıda özet olarak sunulmuştur. Ayrıntılar için web adresine (www.entomoloji.org.tr) bakınız.

1. Dergi, entomoloji ve tarımsal zooloji bilim dallarıyla ilişkili konulara açıktır.
2. Dergide Türkçe veya İngilizce yazılmış orijinal araştırmalar yayımlanır.
3. Yayımlanması istenilen eserlerin kısmen veya tamamen herhangi bir yerde yayınlanmamış veya yayımlanmayacak olması zorunludur.
4. Daha önce Kongre/Sempozyum vs. de sözlü/poster bildiri olarak sunulmuş ancak sadece kısa özet olarak basılmış eserler, dipnotta belirtilmesi koşuşuyla kabul edilir.
5. Lisansüstü tezleri veya TÜBİTAK, DPT, BAP gibi çeşitli kurumlarca desteklenen proje bulgularından kısımlar içeren eserler ilgililerinden gerekli izinler alındıktan sonra hazırlanmalı, ilgi durum dipnotta mutlaka belirtilmelidir.
6. Türkiye veya herhangi bir bölge için, başta karantina listesinde bulunan türler olmak üzere, yeni tür kayıtlarını içeren eserler gönderilmeden önce mutlaka ilgili kurumlara bilgi verilmiş olmalıdır.
7. Dergide yayımlanması istenilen eserler, web sayfasında sunulan “eser başvurusu” bölümünde açıklandığı gibi hazırlanarak, üst yazı, imzalı telif hakları formu ve başvuru ücreti dekontu ile dergi e-posta adresine gönderilmelidir.
8. Yayımlanması istenilen eserler web sayfasında sunulan “örnek makale taslağı” kullanılarak, gereksiz tekrar, şekil ve cetvellerden kaçınılarak, özden uzaklaşmayacak şekilde hazırlanmalı ve 16 sayfadan fazla olmamalıdır.
9. Yayın ilkelerine uygun olmayan eserler istenilen şekle göre yeniden düzenlenmek üzere yazara geri gönderilir. Detaylar için web sayfasında sunulan “eser değerlendirme süreci” ne bakınız.
10. Bir eser yayıma kabul edildiğinde, telif hakları formu tüm yazarlar tarafından imzalanıp dergimize gönderilmeden yayımlanmaz. Sorumlu yazara eserin pdf formatında hazırlanmış hali e-posta ile gönderilir, ayrıca telif ücreti ödenmez. Yayımlanan eserlere ait şekil dışı sorumluluklar yazarlarına aittir.


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