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*Original article (Orijinal araştırma)*

# A faunistic study on the Tachinidae (Diptera) family in Mersin (Türkiye) province with new records for Türkiye[1]

## Türkiye için yeni kayıtlar ile Mersin (Türkiye) ilinde Tachinidae (Diptera) familyası üzerine faunistik bir çalışma

**Hasan Alper SEDENLER**[2] **Turgut ATAY**[3*]

## Abstract

This study was conducted between 2020 and 2021 to reveal the Tachinidae (Diptera) fauna of Mersin province. For this purpose, Tachinidae specimens from agriculture, forest and other areas (grassland, pasture, etc.) of 8 districts (Anamur, Çamlıyayla, Erdemli, Gülnar, Mezitli, Silifke, Toroslar, Yenişehir) selected to represent the province were collected together with the plants they visited. Additionally, insect species from different orders were reared in a laboratory to determine their status as hosts for Tachinidae species. In total, 32 species were determined and identified during the study. These species were categorized into subfamilies and genera: Exoristinae subfamily: 6 genera and 7 species; Tachininae subfamily: 5 genera and 7 species; Dexiinae subfamily: 6 genera and 8 species; Phasiinae subfamily: 4 genera and 10 species. Among them *Prosopea nigricans* (Egger, 1861), *Estheria hertingi* Cerretti & Tschorsnig, 2012 and *Stomina calvescens* Herting, 1977 were recorded for the first time in Türkiye. *P. nigricans* is the first record of the genus *Prosopea* Rondani, 1861 from Türkiye. The distribution in Türkiye, visited plants and hosts known from Türkiye of the determined species were also given. In addition, *Leucostoma crassa* (Kugler, 1966) was reared from *Spilostethus pandurus* (Scopoli, 1763) (Hemiptera: Lygaeidae), and it was determined that this host-parasitoid-couple is a new record for Türkiye. This is the first comprehensive research of the Tachinidae family in Mersin province.

**Keywords:** Fauna, Mersin, new records, Tachinidae, Türkiye

## Öz

Bu çalışma Mersin ilinin Tachinidae (Diptera) faunasını ortaya koymak amacıyla 2020 ve 2021 yıllarında gerçekleştirilmiştir. Bu hedef doğrultusunda ili temsil edecek şekilde seçilen 8 ilçenin (Anamur, Çamlıyayla, Erdemli, Gülnar, Mezitli, Silifke, Toroslar, Yenişehir) tarım, orman ve diğer (çayır, mera vb.) alanlarından Tachinidae örnekleri, ziyaret ettikleri bitkiler ile birlikte toplanmıştır. Ayrıca, Tachinidae türlerinin konukçularını tespit etmek için farklı böcek takımlarına ait türler laboratuvar koşullarında yetiştirilmiştir. Çalışma sonucunda, Exoristinae altfamilyasından 6 cinse ait 7 tür, Tachininae altfamilyasından 5 cinse ait 7 tür, Dexiinae altfamilyasından 6 cinse ait 8 tür ve Phasiinae altfamilyasından 4 cinse ait 10 tür olmak üzere 32 tür belirlenmiştir. Bunlardan *Prosopea nigricans* (Egger, 1861), *Estheria hertingi* Cerretti & Tschorsnig, 2012 ve *Stomina calvescens* Herting, 1977 Türkiye için yeni kayıt niteliğindedir. Yine *Prosopea* Rondani, 1861 cinsi Türkiye'de ilk defa *P. nigricans* ile temsil edilmiştir. Belirlenen türlerin Türkiye'deki yayılışlar, ziyaret ettikleri bitkiler ve Türkiye'den bilinen konukçuları ile ilgili bilgiler sunulmuştur. Ayrıca *Spilostethus pandurus* (Scopoli, 1763) (Hemiptera: Lygaeidae)'dan *Leucostoma crassa* (Kugler, 1966) elde edilmiş ve bu konukçu-parazitoit çiftinin Türkiye için yeni kayıt niteliğinde olduğu belirlenmiştir. Bu çalışma Mersin ilinde Tachinidae familyasına yönelik ilk detaylı çalışma niteliğindedir.

**Anahtar sözcükler:** Fauna, Mersin, yeni kayıtlar, Tachinidae, Türkiye

[1] This study was a partial summary of partial summary of the Master thesis of the first author.

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## Introduction

The Tachinidae (Diptera) is a family which has an important biodiversity in the Diptera, with approximately 8592 species around the world, and 2112 species in the Palearctic region (O'Hara et al., 2021). In Türkiye, the number of known species belonging to the family is 341 (Kara et al., 2020). When this number is compared with the number of Tachinidae species in some neighboring countries such as Greece (Cerretti & Ziegler, 2004-334 species), Bulgaria (Hubenov, 2008-409 species) and Serbia (Hubenov, 2008-188 species) and considering the area of Türkiye, it can be seen that the tachinid fauna of Türkiye is still insufficiently investigated. All Tachinidae species are parasitoids of other arthropods, mainly insects. They attack mainly Lepidoptera larvae but also other insects such as Coleoptera (larvae and adults), Heteroptera (nymphs and adults) and Hymenoptera Symphyta (larvae). They serve a crucial role in naturally controlling the populations of major insect pests (Grenier, 1988; Stireman et al., 2009; Tschorsnig, 2017). Therefore, studies on determining species diversity and revealing host-parasitoid interactions can provide useful information for utilizing tachinids as biocontrol agents. In addition, the presence of suitable and sufficient number of plants for adult parasitoids to feed on has a positive effect on the ability of females to find hosts and parasitize, the number of eggs laid, and sex ratio (Berndt & Wratten, 2005). For this reason, studies to determine the plants visited by tachinids and to maintain the presence of the determined plants in the environment are of great importance in terms of supporting the populations of these beneficials and increasing their effectiveness.

Kara & Tachorsnig (2003) compiled all known hosts of tachinids in Türkiye and mentioned hosts of 95 tachinids. In addition, Kara et al. (2008) prepared a catalogue containing a total of 27 tachinids which are parasitoids of forest pests in Türkiye. Although there are some detailed studies conducted to reveal the species richness of the Tachinidae family in Türkiye, it is seen that the number of these studies is very low when the country is considered in general (Doğanlar, 1975; Kara, 1998; Aksu, 2005; Korkmaz, 2007; Atay & Kara, 2014; Balkan et al., 2015; Lekin et al., 2016; Atay, 2017; Uysal & Atay, 2021; Soykan & Atay, 2022). Finally, the work by Lutovinovas et al. (2018) is a significant contribution to the knowledge of Tachinidae species in southern Türkiye. Publishing a list of 139 tachinid species from this region, with 52 of them being new records for Türkiye, represents an important update to the tachinid diversity in the country.

Mersin, located in southern Türkiye, exhibits a notable variation in climate across its regions. The coastal areas of Mersin are characterized by Mediterranean climate. Inland and the more distant areas from the coast tend to have a continental climate. The variation in climate across different regions within Mersin province can contribute to an increase in insect biodiversity. The study on the Tachinidae fauna in Mersin province is of great importance, especially considering that only a limited number of tachinid species have been previously documented in the region (Yabaş & Zeren, 1987; Şimşek et al., 1994; Bystrowski, 2011; Aytar et al., 2021). This study focuses on the Tachinidae fauna of Mersin province.

## Materials and Methods

Tachinid specimens were collected from various types of environments, including agricultural fields, weeds, forest trees, and ornamental plants, across multiple locations in the Mersin province (Anamur, Çamlıyayla, Erdemli, Gülnar, Mezitli, Silifke, Toroslar, Yenişehir) during 2020-2021. The random collection approach helps ensure a representative sample of the local tachinid fauna. Specimens collected with an insect net and aspirator were killed with ethyl acetate. The latitude and altitude of the site where the tachinids were collected were recorded using GPS. In addition, the plants on which the adult flies were found were photographed and herbariums were made. For host detection studies, insects belonging to different orders were collected from agricultural and forest areas. After collection, insects were taken to the laboratory and reared with the plants they fed on in separate rearing boxes. Culture boxes were maintained at 25±2°C and 60 70% and monitored periodically. For the identification of some specimens, male genital preparations were prepared. For this purpose, the last part of the abdomen was removed from the insect body with forceps, boiled in 10% KOH solution and cleaned by separating the genitalia from the other parts in pure water (Tschorsnig, 1985). Genitalia were preserved in glycerin after being used for identification.

Tachinids were identified using Mesnil (1944-1965), Herting (1977), Herting (1983), Zimin et al. (1988), Tschorsnig & Herting (1994), Tschorsnig & Richter (1998), Cerretti (2005), Cerretti & Shima (2011), Cerretti & Tschorsnig (2012) and Glisian et al. (2013). Taxonomic status of tachinids is updated based on Herting & Dely-Draskovits (1993). The current names of the species are mostly taken from Herting & Dely-Draskovits (1993). Others are from O'Hara et al. (2021). Species showing intraspecific variation were photographed. A Leica MC170 digital camera mounted on a Leica M205 C stereomicroscope was used for photographing the tachinid specimens. Leica Application Suite Software v4.13.0, including the multifocus program was used for photography. The tachinid specimens are kept in the Plant Protection Museum in Tokat Gaziosmanpaşa University, Agricultural Faculty, Tokat, Türkiye. An asterisk (*) is used to indicate species newly recorded for Türkiye. The host belonging to the suborder Heteroptera was identified by Dr. Gülten YAZICI (Plant Protection Central Research Institute, Department of Entomology, Ankara, Türkiye) and the plants visited by adult tachinids were identified by Dr. Ünal ASAV (Department of Plant Protection, Faculty of Agriculture, Tokat Gaziosmanpaşa University, Tokat, Türkiye).

## Results and Discussion

A total of 32 tachinid fly species have been identified in the Mersin province of Türkiye. Among these, three species are reported as new records for the Turkish fauna: *Prosopea nigricans* (Egger, 1861), *Estheria hertingi* Cerretti & Tschorsnig, 2012 and *Stomina calvescens* Herting, 1977 (Diptera: Tachinidae).

**Subfamily: Exoristinae**

**Tribe: Exoristini**

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

Material examined. Silifke, N 36°26'10", E 34°5'43", 22.06.2021, 6m, ♂.

Distribution in Türkiye. İstanbul (Schimitschek, 1944), Trakya (Gürses, 1975), Erzurum (Doğanlar, 1975; Doğanlar, 1982a; 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., 2007), Lakes District (Avcı, 2009), Nevşehir (Bartsch & Tschorsnig, 2010), Mersin (Akdağcık, 2010; Aytar et al., 2021), Muğla (Lutovinovas et al., 2018).

Host in Türkiye. *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, 1982a; Atay & Kara, 2014), *Hyles centralasiae* (Staudinger, 1887) (Lepidoptera: Sphingidae) (Bartsch & Tschorsnig, 2010), *Lymantria dispar* (L., 1758) (Kara & Tschorsnig, 2003; Avcı, 2009; Aytar et al., 2021), *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* Doganlar & Avci, 2001 (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) (Mücksteinet al., 2007), *Pieris brassicae* (L., 1758) (Lepidoptera: Pieridae) (Akdağcık, 2010), *Hyles siehei* Püngeler, 1903 (Lepidoptera: Sphingidae) (Bartsch & Tschorsnig, 2010), *Utetheisa pulchella* (L., 1758) (Lepidoptera: Erebidae) (Aytar et al., 2021).

**Tribe: Wintheminii**

***Nemorilla floralis*** (Fallén, 1820)

Material examined. Erdemli, N 36°43'37", E 34°17'54", 29.09.2021, 678m, 2♂♂, 2♀♀.

Distribution in Türkiye. Burdur (Zeki et al., 1999; Lutovinovas et al., 2018), Tokat (Kara, 1998; Kara & Alaoğlu, 2002), Edirne (Tek & Okyar, 2018).

Host in Türkiye. *Pleuroptya ruralis* Scopoli, 1763 (Lepidoptera: Sphingidae) (Kara, 1998; Kara & Alaoğlu, 2002), *Depressaria daucivorella* Ragonot, 1889 (Lepidoptera: Elachistidae) (Zeki et al., 1999), *Acleris undulana* (Walsingham, 1900) (Lepidoptera: Tortricidae) (Kara & Tschorsnig, 2003), *Archips rosana* L., 1758 (Lepidoptera: Tortricidae) (Tek & Okyar, 2018).

**Tribe: Goniini**

***Pales pavida*** (Meigen, 1824)

Material examined. Toroslar, N 36°50'17", E 34°33'50", 16.04.2021, 85m, 2♂♂, collected from *Euphorbia helioscopia* L. (Euphorbiaceae); 19.09.2021, 62m, 4♂♂, 2♀♀.

Distribution in Türkiye. Ankara (Kara & Özdemir, 2000), Bolu (Robertson & Shaw, 2012), Erzurum (Doğanlar, 1975; Özbek & Çoruh, 2012), Kars (Doğanlar, 1982a; Özbek & Çalmaşur, 2010), Muğla (Lutovinovas et al., 2018), Isparta (Avcı, 2009), Sakarya (Balkan, 2014; Balkan et al., 2015), Samsun (Tuncer & Ecevit, 1996), Sivas (Robertson & Shaw, 2012), Tokat (Herting, 1983; Tschorsnig, 2005; Kara, 1998; Atay, 2011; Atay & Kara, 2014; Lekin, 2014; Lekin et al., 2016), Locality information is not provided (Cerretti, 2005), Amasya (Kara, 2001b), Muğla (Acatay, 1959).

Host in Türkiye. *Lymantria dispar* (L., 1758) (Lepidoptera: Lymantriidae) (Acatay, 1959; Avcı, 2009), *Malacosoma franconica* Esp. (Lepidoptera: Lasiocampidae) and *M. castrensis kırghisica* Stgr. (Lepidoptera: Lasiocampidae) (Doğanlar 1975, 1982a), *Hypantria cunea* (Drury, 1773) (Lepidoptera: Erebidae) (Tuncer & Ecevit, 1996; Kara & Tschorsnig, 2003), *Aglais urticae* (L., 1758) (Lepidoptera: Nymphalidae) and *Leucoma salicis* (L.) (Lepidoptera: Erebidae) (Kara, 1998), *Yponomeuta* sp. *(*Lepidoptera: Yponomeutidae) (Kara & Özdemir, 2000), *M. neustria* L. (Lepidoptera: Lasiocampidae) (Kara and Tschorsnig, 2003), *Abraxas pantaria* (L., 1767) (Lepidoptera: Geometridae) (Özbek & Çalmaşur, 2010), *Simyra dentinosa* Frr. (Lepidoptera: Noctuidae) and *Malacosoma neustria* (L.) (Lepidoptera: Lasiocampidae) (Atay, 2011; Atay & Kara, 2014).

***Dolichocolon paradoxum*** (Brauer et Bergenstamm, 1889)

Material examined. Yenişehir, N 36°50'42", E 34°33'21", 12.04.2021, 154m, ♂.

Distribution in Türkiye. Muğla (Lutovinovas et al., 2018).

****Prosopea nigricans*** (Egger, 1861)

Material examined. Erdemli, N 36°46'31", E 34°0'1", 07.10.2021, 1395m, ♀.

Distribution in Türkiye. Recorded for the first time from Türkiye.

Remark. Tschorsnig & Herting (1994), reported that the palps completely black, the middle tibia with 3 anterodorsal setae and the r-m vein is noticeably inclined towards the m vein. In the examined materials lower half of the palps blackish brown and the upper half lighter, the middle tibia with 5 anterodorsal setae (3 big and 2 small) and the r-m vein is not very noticeably slant to the m vein (Figure 1).

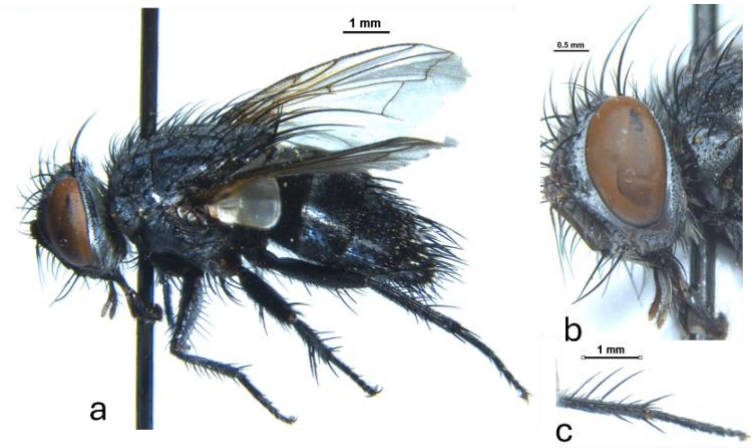


Figure 1. *Prosopea nigricans* ♀: a) General view, b) head, c) middle tibia.

***Spallanzania hebes*** (Fallén, 1820)

Material examined. Toroslar, N 36°51'28", E 34°33'23", 04.06.2021, 154m, ♂, collected from *Teucrium* sp. (Lamiaceae).

Distribution in Türkiye. Erzurum (Doğanlar, 1982a), Sakarya (Balkan, 2014; Balkan et al., 2015), Burdur (Lutovinovas et al., 2018), Manisa (Soykan, 2021; Soykan & Atay, 2022).

Host in Türkiye. *Agrotis* sp. (Lepidoptera: Noctuidae) (Tschorsnig, 2017).

***Spallanzania multisetosa*** (Rondani, 1859)

Material examined. Silifke, N 36°26'11", E 34°5'43", 07.04.2021, 16m, ♂.

Distribution in Türkiye. Eskişehir (Aksu, 2005).

**Subfamily: Tachininae**

**Tribe: Tachinini**

***Peleteria rubescens*** (Robineau-Desvoidy, 1830)

Material examined. Toroslar, N 37°1'51", E 34°35'22", 04.06.2021, 953m, 2♀♀, collected from *Melissa officinalis* L. (Lamiaceae).

Distribution in Türkiye. Erzurum (Doğanlar, 1975), Tokat (Kara, 1999a; Lekin et al., 2016), Ankara (Khan & Özer, 1984; Kansu et al., 1986; Kara & Özdemir, 2000), Zonguldak (Korkmaz, 2007), Sakarya (Balkan, 2014; Balkan et al., 2015), Çorum (Uysal, 2018; Uysal & Atay, 2021), Manisa (Soykan, 2021; Soykan & Atay, 2022).

Host in Türkiye. *Malacosoma castrensis* (L., 1758) (Lepidoptera: Lasiocampidae) (Doğanlar, 1975), *Agrotis* sp. (Lepidoptera: Noctuidae) (Khan & Özer, 1984; Kansu et al., 1986; Kara & Özdemir, 2000).

**Tribe: Ernestiini**

***Linnaemya comta*** (Fallén, 1810)

Material examined. Erdemli, N 36°46'31", E 34°0'1", 07.10.2021, 1395m, 4♀♀.

Distribution in Türkiye. Denizli (Kavut et al., 1974), Diyarbakır, Hakkari (Doğanlar, 1982b), Tokat (Kara, 1999a), Sakarya (Balkan, 2014; Balkan et al., 2015), Kastamonu (Atay, 2017).

Host in Türkiye. *Agrotis ipsilon* Hufnagel (Lepidoptera: Noctuidae) (Kavut et al., 1974).

**Tribe: Macquartiini**

***Macquartia praefica*** (Meigen, 1824)

Material examined. Gülnar, N 36°23'26", E 33°27'7", 19.05.2020, 1207m, ♀; Toroslar, N 36°50'3", E 34°35'1", 30.03.2021, 86m, ♀, collected from *Glebionis coronaria* (L.) Cass. ex Spach (Asteraceae); Yenişehir, N 36°50'42", E 34°33'21", 12.04.2021, 154m, ♀.

Distribution in Türkiye. Tokat (Kara, 1999a).

***Macquartia tenebricosa*** (Meigen, 1824)

Material examined. Toroslar, N 36°50'17", E 34°33'50", 16.04.2021, 85m, ♂, collected from *Euphorbia helioscopia* L. (Euphorbiaceae); 19.09.2021, ♂; N 36°50'24", E 34°33'46", 28.04.2021, 102m, ♂, ♀.

Distribution in Türkiye. Tokat (Kara, 1999a; Atay, 2018), Amasya (Kara, 2001b), Adana (Anay, 2000), Bartın (Korkmaz, 2007), Aydın and Muğla (Lutovinovas et al., 2018), Çorum (Uysal, 2018; Uysal & Atay, 2021).

Host in Türkiye. *Plebejus idas* (L., 1761) (Lepidoptera: Lycaenidae) (Anay, 2000), *Gonioctena fornicata* Brüggemann, 1873 (Coleoptera: Chrysomelidae) (Atay, 2018).

***Macquartia tessellum*** (Meigen, 1824)

Material examined. Silifke, N 36°26'11", E 34°5'43", 7.04.2021, 16m, ♂.

Distribution in Türkiye. Erzurum (Doğanlar, 1982b), Tokat (Kara, 1999a), Muğla (Lutovinovas et al., 2018); Çorum (Uysal, 2018; Uysal & Atay, 2021).

***Anthomyiopsis plagioderae*** (Mesnil, 1972)

Material examined. Toroslar, N 36°49'28", E 34°35'23", 02.10.2021, 78m, ♀.

Distribution in Türkiye. Sivas (Atay, 2011; Kara & Atay, 2015).

Host in Türkiye. *Phaedon cochleariae* (Fabricius, 1792) (Coleoptera: Chrysomelidae) (Atay, 2011; Kara & Atay, 2015).

Remarks. Tschorsnig & Herting (1994) reported two pairs of setae (basal and apical) on the scutellum. In the examined specimen, 3 pairs of setae (basal, lateral and apical) were observed (Figure 2).


Figure 2. *Anthomyiopsis plagioderae* ♀: a) General view, b) scutellum.

**Tribe: Megaprosopini**

***Microphthalma europaea*** (Egger, 1860)

Material examined. Erdemli, N 36°43'37", E 34°17'54", 29.09.2021, 678m, ♀.

Distribution in Türkiye. Aydın, Eskişehir, Diyarbakır (Karagöz et al., 2011); Sakarya (Balkan, 2014; Balkan et al., 2015), Aydın, Muğla (Lutovinovas et al., 2018), Çorum (Uysal, 2018; Uysal & Atay, 2021).

Host in Türkiye. *Polyphylla fullo* (L., 1758) (Coleoptera: Scarabaeidae) (Karagöz et al., 2011).

**Subfamily: Dexiinae**

**Tribe: Dexiini**

***Billaea adelpha*** (Loew, 1873)

Material examined. Toroslar, N 36°51'28", E 34°33'23", 04.06.2021, 154m, ♂, collected from *Ruta angustifolia* Pers. (Rutaceae); Yenişehir, N 36°53'48", E 34°30'22", 14.06.2021, 429m, ♂; N 36°49'50", E 34°28'19", 01.10.2021, 194m, ♂; Erdemli, N 36°41'16", E 34°19'25", 29.09.2021, 166m, ♂, collected from *Drimia maritima* (L.) Stearn (Asparagaceae); Silifke, N 36°27'6", E 34°6'10", 13.10.2021, 156m, ♂.

Distribution in Türkiye. Tokat (Kara, 2001a).

***Estheria nigripes*** (Villeneuve, 1920)

Material examined. Toroslar, N 37°1'52", E 34°35'22", 27.09.2021, 990m, 2♀♀; N 37°2'0", E 34°34'40", 27.09.2021, 1012m, ♀; N 36°57'25", E 34°31'37", 05.10.2021, 907m, ♀.

Distribution in Türkiye. Locality information is not provided (Herting, 1984; Cerretti & Tschorsnig, 2012). İzmir (Öncüer, 1991; Herting & Dely-Draskovits, 1993), Muğla (Lutovinovas et al., 2018).

****Estheria hertingi*** Cerretti & Tschorsnig, 2012

Material examined. Silifke, N 36°26'11", E 34°5'44", 19.05.2020, 65m, ♂; N 36°26'10", E 34°5'42", 18.06.2020, 24m, ♀; N 36°26'10", E 34°5'43", 22.06.2021, 6m, 2♀♀; N 36°25'34", E 33°39'49", 22.06.2021, 223m, 2♀♀; N 36°25'33", E 33°39'49", 22.06.2021, 195m, ♂, 3♀♀; Gülnar, N 36°26'13", E 33°31'26", 22.06.2021, 419m, 3♂♂; Tarsus, N 37°5'18", E 34°38'10", 15.07.2021, 837m, 2♀♀, ♂; N 37°4'37", E 34°37'1", 15.07.2021, 1052m, 3♂♂; Çamlıyayla, N 37°5'46", E 34°42'4", 15.07.2021, 987m, 2♀♀, ♂; N 37°7'27", E 34°37'44", 15.07.2021, 877m, ♀; Toroslar, N 36°52'27", E 34°33'21", 28.07.2020, 132m, 2♂♂, 13♀♀; N 36°51'28", E 34°33'23", 4.06.2021, 154m, ♂, collected from *Ruta angustifolia* Pers. (Rutaceae); N 37°1'52", E 34°35'22", 27.09.2021, 990m, ♀; N 37°2'25", E 34°33'45", 27.09.2021, 974m, ♀; N 36°58'0", E 34°31'12", 5.10.2021, 978m, 2♀♀, collected from *Dittrichia viscosa* (L). Greuter (Asteraceae); N 36°57'25", E 34°31'37", 5.10.2021, 907m, ♀; Erdemli, N 36°43'42", E 34°17'23", 16.06.2021, 679m, ♂, collected from *Pallenis spinosa* (L.) Cass. (Asteraceae).

Distribution in Türkiye. Recorded for the first time from Türkiye.

***Zeuxia tricolor*** (Portschinsky, 1881)

Material examined. Toroslar, N 36°52'32", E 34°33'58", 09.05.2020, 338m, ♀; N 36°53'11", E 34°34'9", 30.05.2020, 466m, 2♀♀.

Distribution in Türkiye. Konya (Herting, 1984), Tokat (Kara, 1999b; Lekin, 2014; Lekin et al., 2016) Amasya (Kara, 2001b), Eskişehir (Kara & Aksu, 2007), Manisa (Soykan, 2021; Soykan & Atay, 2022).

**Tribe: Voriini**

***Eriothrix rufomaculatus*** (De Geer, 1776)

Material examined. Yenişehir, N 36°49'50", E 34°28'19", 01.10.2021, 194m, ♂; Erdemli, N 36°46'31", E 34°0'1", 07.10.2021, 1395m, 4♂♂, 3♀♀; Silifke, N 36°29'47", E 33°54'32", 13.10.2021, 826m, ♀, collected from *Eryngium campestre* L. (Apiaceae).

Distribution in Türkiye. Erzurum (Doğanlar, 1982b), Tokat (Kara, 1999b; Lekin, 2014; Lekin et al., 2016), Kastamonu, Bartın, Zonguldak (Korkmaz, 2007), Sakarya (Balkan, 2014; Balkan et al., 2015), Muğla (Lutovinovas et al., 2018), Çorum (Uysal, 2018; Uysal & Atay, 2021).

***Voria ruralis*** (Fallén, 1810)

Material examined. Silifke, N 36°27'45", E 33°53'32", 13.10.2021, 566m, ♀, collected from *Mentha longifolia* L. (Lamiaceae).

Distribution in Türkiye. İzmir (Kavut et al., 1974), Erzurum (Avcı & Özbek, 1990), Tokat (Kara, 1999b), Adana (Anay, 2000), Niğde (Kara & Özdemir, 2000), Amasya (Kara, 2001b), Karabük (Korkmaz, 2007), Hatay (Kaya & Kornoşor, 2008), Tokat (Lekin, 2014; Lekin et al., 2016), Çorum (Uysal, 2018; Uysal & Atay, 2021), Aydın, Muğla (Lutovinovas et al., 2018), Manisa (Soykan, 2021; Soykan & Atay, 2022).

Host in Türkiye. *Spodoptera exigua* (Hübner, 1808) (Steiner, 1937), *Autographa gamma* (L., 1758) (Kavut et al., 1974; Avcı & Özbek, 1990; Anay, 2000; Kara & Özdemir, 2000), *Helicoverpa armigera* (Hübner, 1808) (Anay, 2000); Plusiinae sp. (Lepidoptera: Noctuidae) (Kaya & Kornoşor, 2008).

****Stomina calvescens*** Herting**,** 1977

Material examined. Mezitli, N 36°49'31", E 34°26'58", 01.10.2021, 524m, ♂, 2♀♀, collected from *Drimia maritima* (L.) Stearn (Asparagaceae); Toroslar, N 36°58'0", E 34°31'12", 05.10.2021, 978m, ♂; N 36°56'56", E 34°33'34", 05.10.2021, 718m, ♂; Silifke, N 36°27'45", E 33°53'32", 13.10.2021, 566m, ♀, collected from *Mentha longifolia* L. (Lamiaceae).

Distribution in Türkiye. Recorded for the first time from Türkiye.

Remarks. Herting (1977), reported the number of hairs under the last frontal seta as 1-5 in males. However, the number of hairs was more in the examined specimens (Figure 3 a,b). He also reported that the surstyli of *Stomina calvencens* similar to those of *Stomina caliendrata* (Rondani, 1862), but the basal part of the surstyli of *S. calvencens* was more developed (Figure 3 c,d).

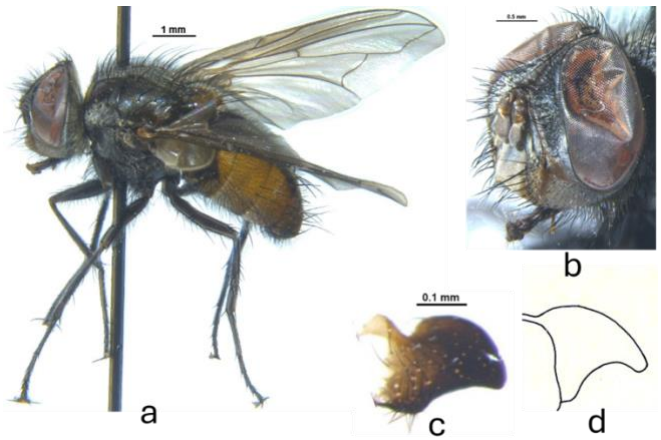


Figure 3. *Stomina calvescens* ♂:a) General view, b) head, c) surstyli, d) *Stomina caliendrata* ♂: surstyli (Herting, 1977).

***Stomina tachinoides*** (Fallén, 1817)

Material examined. Silifke, N 36°27'45", E 33°53'32", 13.10.2021, 566m, ♀, collected from *Mentha longifolia* L. (Lamiaceae).

Distribution in Türkiye. Eskişehir (Kara, 2001a).

**Subfamily: Phasiinae**

**Tribe: Phasiini**

***Gymnosoma rotundata*** (L., 1758)

Material examined. Toroslar, N 37°1'59", E 34°36'0", 04.06.2021, 886m, ♂, collected from *Galium odaratum* (L.) Scop. (Rubiaceae).

Distribution in Türkiye. Eastern Black Sea Region (Kurt, 1975), Tokat (Kara, 1998; Lekin, 2014; Lekin et al., 2016), Karabük, Kastamonu, Zonguldak (Korkmaz, 2007; Atay, 2017), Sakarya (Balkan, 2014; Balkan et al., 2015), Çorum (Uysal, 2018; Atay & Uysal, 2021), Manisa (Soykan, 2021; Soykan & Atay, 2022).

Host in Türkiye. *Aelia rostrata* Boheman, 1852 (Dikyar, 1981), *Palomena prasina* (L., 1761) (Hemiptera: Pentatomidae) (Kurt, 1975).

***Phasia mesnili*** (Draber-Monko, 1965)

Material examined. Mezitli, N 36°49'31", E 34°26'58", 01.10.2021, 524m, ♀, *Drimia maritima* (L.) Stearn (Asparagaceae).

Distribution in Türkiye. Tokat (Kara, 1998; Kara & Alaoğlu, 1999), Karabük (Korkmaz, 2007; Atay, 2017), Kastamonu, Zonguldak (Korkmaz, 2007), Bolu (Atay, 2017), Aydın, Burdur and Muğla (Lutovinovas et al., 2018).

**Tribe: Leucostomatini**

***Leucostoma crassa*** (Kugler, 1966)

Reared specimens. 11.10.2021, ♂; 12.10.2021, ♀, ♂; 14.10.2021, ♂ [host details. *Spilostethus pandurus* (Scopoli, 1763) (Hemiptera: Lygaeidae) specimens were collected in Erdemli, 24.09.2021, N 36°41'16", E 34°19'25", 166m, on *Drimia maritima* (L.) Stearn (Asparagaceae)]; 11.10.2021, ♂; 12.10.2021, ♀ (host details. *S. pandurus* were collected in Erdemli, 29.09.2021, N 36°43'9", E 34°20'16", 312m, on *D. maritima*).

Distribution in Türkiye. Locality information is not provided (Herting & Dely-Draskovits, 1993), Tokat (Kara, 1998).

Hosts in Türkiye. *Lygaeus equestris* (L., 1758) (Hemiptera: Lygaeidae) (Kara, 1998; Kara & Tschorsnig, 2003).

**Tribe: Cylindromyiini**

***Cylindromyia rubida*** (Loew, 1854)

Material examined. Toroslar, N 36°52'55", E 34°33'0", 26.09.2021, 185m, ♂ collected from *Mentha longifolia* L. (Lamiaceae).

Remarks. Herting (1983) reported that the ratio of the apical seta on the scutellum to the subapical seta was only 0.25 times. In the examined specimen, this ratio was measured as 0.49 times (Figure 4).

Distribution in Türkiye. İzmir (Çerçi, 2017), Adana (Tarla et al., 2023).

Hosts in Türkiye. *Piezodorus lituratus* (Fabricius, 1794) (Hemiptera: Pentatomidae) (Tarla et al., 2023).

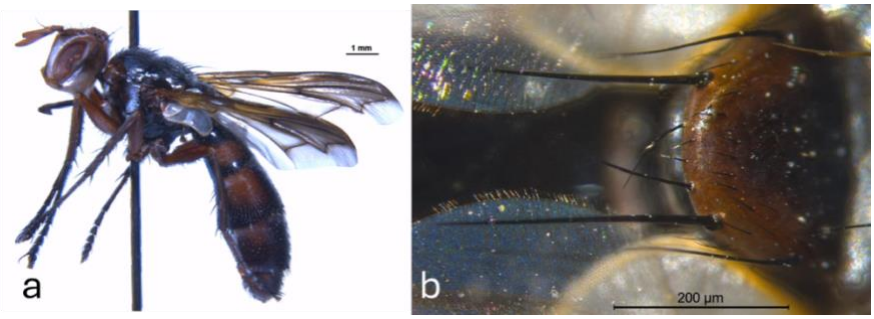


Figure 4. *Cylindromyia rubida* ♂: a) General view, b) scutellum.

***Cylindromyia gemma*** (Richter, 1972*)*

Material examined. Toroslar, N 37°2'45", E 34°33'36", 15.07.2021, 884m, 2♂♂, collected from *Xeranthemum inapertum* (L.) Mill. (Asteraceae).

Distribution in Türkiye. Manisa (Soykan, 2021; Soykan & Atay, 2022).

***Cylindromyia bicolor*** (Oliver, 1812)

Material examined. Toroslar, N 36°52'55", E 34°33'0", 26.09.2021, 185m, ♂, collected from *Mentha longifolia* L. (Lamiaceae); Mezitli, N 36°49'31", E 34°26'58", 05.10.2021, 526m, ♂, collected from *Drimia maritima* (L.) Stearn (Asparagaceae); Silifke, N 36°29'47", E 33°54'32", 13.10.2021, 826m, ♂, collected from *Eryngium campestre* L. (Apiaceae).

Distribution in Türkiye. Samsun (Herting, 1983), Black Sea Region (Işık et al., 1987), Tokat (Kara, 1998; Kara & Alaoğlu, 1999; Lekin, 2014; Lekin et al., 2016), Zonguldak (Korkmaz, 2007), Bartın, Karabük (Atay, 2017), Çorum (Uysal, 2018; Uysal & Atay, 2021), Aydın, Muğla (Lutovinovas et al., 2018), Manisa (Soykan, 2021; Soykan & Atay, 2022).

Host in Türkiye. *Rhaphigaster nebulosa* (Poda, 1761) (Hemiptera: Pentatomidae) (Herting, 1983).

***Cylindromyia brassicaria*** (Fabricius, 1775)

Material examined. Toroslar, N 36°50'24", E 34°33'46", 28.04.2021, 102m, ♀.

Distribution in Türkiye. Erzurum (Doğanlar, 1982b), İzmir (Karsavuran, 1986), Tokat (Kara, 1998; Kara & Alaoğlu, 1999; Atay, 2011; Atay & Kara, 2014; Lekin, 2014; Lekin et al., 2016), Eskişehir (Aksu, 2005), Antalya, Burdur (Keçeci et al., 2007; Kastamonu (Atay, 2017); Çorum (Uysal, 2018; Uysal & Atay, 2021); Aydın, Muğla (Lutovinovas et al., 2018), Manisa (Soykan, 2021; Soykan & Atay, 2022), Adana and Uşak (Tarla et al., 2023).

Host in Türkiye. *Dolycoris baccarum* (L., 1758) (Hemiptera: Pentatomidae) (Karsavuran, 1986; Kara & Tschorsnig, 2003; Keçeci et al., 2007; Atay, 2011; Atay & Kara, 2014; Tarla et al., 2023), *Holcostethus vernalis* (Wolff, 1804) (Hemiptera: Pentatomidae) (Kara, 1998; Kara & Alaoğlu, 1999).

***Cylindromyia pilipes*** (Loew, 1844)

Material examined. Toroslar, N 36°50'1", E 34°33'55", 26.09.2021, 73m, ♀, collected from *Symphyotrichum squamatum* (Spreng.) G.L.Nesom (Compositae).

Distribution in Türkiye. Bursa, İstanbul (Herting, 1984; Herting & Dely-Draskovits, 1993), Bartın, Kastamonu (Atay, 2017), Burdur (Lutovinovas et al., 2018), Çorum (Uysal, 2018; Uysal & Atay, 2021), Adana (Tarla et al., 2023).

Host in Türkiye. *Holcostethus vernalis* (Wolff, 1804) (Hemiptera: Pentatomidae) (Tarla, et al., 2023).

***Cylindromyia pusilla*** (Meigen, 1824)

Material examined. Toroslar, N 36°50'1", E 34°33'55", 26.09.2021, 73m, ♂, collected from *Symphyotrichum squamatum* (Spreng.) G.L.Nesom (Compositae).

Distribution in Türkiye. Locality information is not provided (Herting & Dely-Draskovits, 1993), Antalya (Herting, 1984), Zonguldak (Korkmaz, 2007), Karabük (Atay, 2017), Muğla (Lutovinovas et al., 2018), Manisa (Soykan, 2021; Soykan & Atay, 2022).

***Cylindromyia auriceps*** (Meigen, 1838)

Material examined. Toroslar, N 36°52'55", E 34°33'0", 26.09.2021, 185m, ♂, collected from *Mentha longifolia* L. (Lamiaceae).

Distribution in Türkiye. Tokat (Kara, 1998; Kara & Alaoğlu, 1999; Lekin, 2014; Lekin et al., 2016), Eskişehir (Aksu, 2005), Kastamonu (Korkmaz, 2007; Atay, 2017), Zonguldak (Korkmaz, 2007), Sakarya (Balkan, 2014; Balkan et al., 2015); Aydın, Muğla (Lutovinovas et al., 2018), Manisa (Soykan, 2021; Soykan & Atay, 2022).

Host in Türkiye. *Aelia acuminata* (L., 1758) (Het: Scutelleridae) (Kara & Tschorsnig, 2003).

During the study, the plants visited by the tachinids were determined and the names and families of the plants are given in Table 1.

Table 1 Plants visited by tachinids (Diptera)

| **Tachinids** | **Visited Plants** | |
|---|---|---|
| | **Species** | **Family** |
| ***Cylindromyia bicolor* (Olivier, 1812),**<br>***Eriothrix rufomaculata* (De Geer, 1776)** | *Eryngium campestre* L. | Apiaceae |
| ***Cylindromyia pusilla* (Meigen, 1824)**<br>***Cylindromyia pilipes* (Loew, 1844)** | *Symphyotrichum squamatum* (Spreng.) G.L.Nesom | Compositae |
| ***Voria ruralis* (Fallén, 1810)**<br>***Stomina calvescens* Herting, 1977**<br>***Stomina tachinoides* (Fallén, 1817)**<br>***Cylindromyia auriceps* (Meigen, 1838)**<br>***Cylindromyia bicolor* (Olivier, 1812)**<br>***Cylindromyia rubida* (Loew, 1854)** | *Mentha longifolia* L. | Lamiaceae |
| ***Billaea adelpha* (Loew, 1873)**<br>***Stomina calvescens* Herting, 1977**<br>***Phasia mesnili* (Draber-Monko, 1965)**<br>***Cylindromyia bicolor* (Olivier, 1812)** | *Drimia maritima* (L.) Stearn | Asparagaceae |
| ***Peleteria rubescens* (Robineau-Desvoidy, 1830)** | *Melissa officinalis* L. | Lamiaceae |
| ***Cylindromyia gemma* (Richter, 1972)** | *Xeranthemum inapertum* (L.) Mill. | Asteraceae |
| ***Spallanzania hebes* (Fallén, 1820)** | *Teucrium* sp. | Lamiaceae |
| ***Gymnosoma rotundata* (L., 1758)** | *Galium odaratum* (L.) Scop. | Rubiaceae |
| ***Macquartia tenebricosa* (Meigen, 1824),**<br>***Pales pavida* (Meigen, 1824)** | *Euphorbia helioscopia* L. | Euphorbiaceae |
| ***Macquartia praefica* (Meigen, 1824)** | *Glebionis coronaria* (L.) Cass. ex Spach | Asteraceae |
| ***Estheria hertingi* Cerretti & Tschorsnig, 2012** | *Dittrichia viscosa* (L). Greuter | Asteraceae |
| ***Billaea adelpha* (Loew, 1873)**<br>***Estheria hertingi* Cerretti & Tschorsnig, 2012** | *Ruta angustifolia* Pers. | Rutaceae |
| ***Estheria hertingi* Cerretti & Tschorsnig, 2012** | *Pallenis spinosa* (L.) Cass. | Asteraceae |

The study was conducted in 8 districts in order to reveal the Tachinidae fauna of Mersin province, as a result of which a total of 32 species were determined. Of the identified species, 3 species are new records for Türkiye and 31 species for the Mersin insect fauna. Also, 7 of the determined species were the second record from Türkiye. When looking at the number of species at the subfamily level, Phasiinae had the highest number of species, followed by Dexiinae. Tachininae and Exoristinae subfamilies had an equal number of species and ranked third. The distribution of Tachinidae subfamilies in Mersin province differed from the countrywide ranking. In Türkiye, the order was Exoristinae, Tachininae, Phasiinae, and Dexiinae (Kara et al., 2020). This difference may be attributed to the host insect and plant diversity specific to the Mersin province. As a result of this study, the number of known species belonging to the Tachinidae family has reached 39 in Mersin. These findings contribute to the understanding of the Tachinidae fauna in the Mersin province and provide valuable information about the diversity and distribution of these parasitic flies in the region. Furthermore, *L. crassa* was reared from *S. pandurus*, and this host-parasitoid coupling was confirmed as a new record for Türkiye.

During the field study, it was found that tachinids visited plants from the Apiaceae, Compositae, Asparagaceae, Rubiaceae, Euphorbiaceae, and Rutaceae families, particularly Asteraceae and Lamiaceae. As a result of the identification, it was determined that 13 plant species belonging to these families were visited by tachinids (Table 1). These plants likely serve as nectar sources for the tachinid flies, which feed on nectar and pollen. In other studies, it has been revealed that tachinids frequently visit plants belonging to the Asteraceae family in a similar manner (Sathe et al., 2014; Soykan & Atay, 2022).

Tachinids parasitize a variety of hosts, the majority of which are plant pests. As natural enemies of these important phytophagous groups, tachinids have been regarded as one of the most important groups of biological control agents both in natural and managed habitats. Their effectiveness as biological control agents depend on a comprehensive understanding of their diversity, behavior, and interactions with host insects and plants. Thus, we can contribute to sustainable pest management by supporting their natural populations.

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*Original article (Orijinal araştırma)*

# Occurrence and distribution of cyst nematodes, *Heterodera* spp. (Tylenchida: Heteroderidae) associated with black cabbage, *Brassica oleracea* var. *acephala* L. (Brassicales: Brassicaceae) in the Eastern Black Sea Region of Türkiye[1]

Türkiye'nin Doğu Karadeniz Bölgesi'nde karalahana, *Brassica oleracea* var. *acephala* L. (Brassicales: Brassicaceae) üretim alanlarındaki kist nematodları, *Heterodera* spp. (Tylenchida: Heteroderidae) ve dağılımları

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## Abstract

This study was conducted during 2021-2022 to detect and determine distribution and population of cyst nematodes, *Heterodera* spp. (Tylenchida: Heteroderidae) in black cabbage *Brassica oleracea* var. *acephala* L. (Brassicales: Brassicaceae) production areas of the Eastern Black Sea Region of Türkiye. For it, a total of 77 samples were taken from 53 districts belonging to the Artvin, Giresun, Ordu, Rize, and Trabzon provinces in the region. Soil samples were taken from around the root of the kale plants. Nematodes were extracted by using the centrifugal flotation technique. The nematodes were identified using morphological features and molecular analysis based on Polymerase Chain Reaction (PCR) method. For molecular analysis, the ribosomal DNA region including the gene region of 28S ribosomal RNA (rRNA) (ITS1, 5.8S, ITS2) was amplified using primer sets TW81/AB28. Additionally, a species-specific primer set (Car-F/Car-R) covering the Cytochrome Oxidase I (cox1) region of mitochondrial DNA (mtDNA) was used. As a result of the analysis, cyst nematodes *Heterodera cruciferae* Franklin, 1945*, Heterodera carotae* Jones, 1950 and *Heterodera fici* Kirjanova, 1954 species were identified in the kale production areas in the region. *Heterodera carotae* is the first record of the cyst nematode species in Türkiye. *Heterodera cruciferae, H. carotae,* and *H. fici* were detected from the total collected soil samples at 16.9%, 3.9%, and 1.3% relative frequency, respectively. Among all, Giresun was the most infected province with 35.3% infection rate, followed by Trabzon with 26.3%, Ordu with 21.1% and Rize with 13.3%.

**Keywords:** Black cabbage, *Heterodera*, ITS, PCR, taxonomy, Türkiye

## Öz

Bu çalışma, Türkiye'nin Doğu Karadeniz Bölgesi lahana, *Brassica oleracea* var. *acephala* L. (Brassicales: Brassicaceae) üretim alanlarında kist nematodlarını *Heterodera* spp. (Tylenchida: Heteroderidae) tespit etmek ve dağılımları ve popülasyonlarını belirlemek amacıyla 2021-2022 yıllarında yürütülmüştür. Bu amaçla, bölgedeki Artvin, Giresun, Ordu, Rize ve Trabzon illerine ait 53 ilçeden toplam 77 örnekleme gerçekleştirilmiştir. Toprak örnekleri karalahana bitkilerinin kök çevresinden alınmıştır. Nematodlar santrifüj yöntemi kullanılarak elde edilmiştir. Nematodlar, morfolojik özellikler ve Polimeraz Zincir Reaksiyonu (PCR) yöntemine dayanan moleküler analiz kullanılarak tanımlanmıştır. Moleküler analiz için, 28S ribozomal RNA (rRNA) gen bölgesini (ITS1, 5.8S, ITS2) içeren ribozomal DNA bölgesi TW81/AB28 primer setleri kullanılarak çoğaltılmıştır. Ayrıca, mitokondriyal DNA'nın (mtDNA) Sitokrom Oksidaz I (cox1) bölgesini kapsayan türe özgü primer seti (Car-F/Car-R) kullanılmıştır. Analiz sonucunda bölgedeki karalahana üretim alanlarında *Heterodera cruciferae* Franklin, 1945*, Heterodera carotae* Jones, 1950 ve *Heterodera fici* Kirjanova, 1954 kist nematod türleri teşhis edilmiştir. *Heterodera carotae* türü Türkiye için ilk kayıt niteliğindedir. Toplanan toplam toprak örneklerinde *H. cruciferae, H. carotae* ve *H. fici* sırasıyla %16.9, %3.9 ve %1.3 oranlarında tespit edilmiştir. Çalışmada, %35.3 ile Giresun ili en çok bulaşık olan il olurken, bunu %26.3 ile Trabzon, %21.1 ile Ordu ve %13.3 ile Rize illeri takip etmiştir.

**Anahtar sözcükler:** Karalahana, *Heterodera*, ITS, PCR, taksonomi, Türkiye

[1] This study was derived from the first authors' MSc thesis.

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## Introduction

Black cabbage, scientifically classified as *Brassica oleracea* var. *acephala* L., is a prominent member of the Brassicaceae family (Öztürk, 2005). A biennial vegetable, its cultivation spans the entire year in European nations, with exceptions during one or two months in specific locales (Vural, 2008). Globally, cabbage production yields a substantial 104 million tons, with China commanding a notable one-third of this output. The other important production countries are India, Russia, South Korea, Ukraine, Indonesia, Japan, Vietnam, the United States of America, Poland, and Kenya (FAO, 2020). Notably, Türkiye has registered an annual black cabbage production of 819.000 tons, cultivated across 4939.8 hectares as of 2021. Within Türkiye, the epicenter of this cultivation lies in the Black Sea region, spanning an impressive 4104.9 hectares. Among the provinces in the region, Giresun ranks first, followed by Samsun, Trabzon and Ordu (TUIK, 2021).

Despite its esteemed status as a globally significant crop, black cabbage cultivation is not resistant to losses from diseases, pests, and invasive vegetation. Cyst nematodes is one of the most important plant parasitic nematodes negatively affecting cabbage production (Pehlivan et al., 2020). Cyst nematodes are species of the *Heterodera* and *Globodera* genera that are extremely resistant to adverse conditions and cause economic losses in many cultivated plants. It is known that among these species, only *Heterodera cruciferae* Franklin 1945 and *Heterodera schachtii* Schmidt 1871 can feed on cabbage plants. Cabbage infected with *H. cruciferae*, also known as the cabbage cyst nematode, usually shows wilting, chlorosis between the veins, or a reddish color on the leaves (Thorne, 1961). It is stated that the presence of 20 cysts/100 g of soil is sufficient to cause severe wilt in cabbage plants (McCann, 1981). Jensen (1972) and McCann (1981) indicated that *H. schachtii* and *H. cruciferae* generally occur together in cabbage production areas.

A few researchers have performed studies on cyst nematodes in cabbage in Türkiye, but sufficiently comprehensive studies on these issues are still needed. In a study conducted by Muşdağı & Gözel (2015) on cabbage in Türkiye in Çanakkale province, 76 soil samples were taken on 5 different cabbage varieties to determine the prevalence and density of cyst nematodes. As a result of the survey, they reported that *Heterodera avenae* Wollenweber, 1924 (7.9%), *H. cruciferae* (7.9%), and *H. schachtii* (2.7%) were among the cyst nematodes detected. In addition, Mennan & Aydınlı (2007) found that approximately 45% of cabbage cultivated areas in Samsun province were infected with *H. cruciferae*. In another study, Mennan et al. (2009) determined that 45 of 101 fields were infected with cyst nematodes in their surveys conducted in cabbage cultivation areas in Samsun between 2002 and 2006. They reported that the most common species were *H. cruciferae* (77.70%) and *Heterodera mediterranea* Inserra, Vovlas & Stone, 1981 (20.00%). In another study, Aydınlı (2009) aimed to reveal the effects of *H. cruciferae* on the development of cabbage plants in cabbage production areas in Samsun. As a result of the research on the factors affecting larval emergence from *H. cruciferae* cysts, it was reported that the optimum temperature for egg opening was 10ºC and leaf cabbage root secretions promoted egg opening. Aydınlı & Mennan (2012), found that sixty percent of *acephala* (Kale) varieties were partially susceptible, while 40% were resistant. The studies conducted generally include other cabbage varieties, and it seems that not enough studies have been conducted on kale. There are no studies on cyst nematode populations in the Eastern Black Sea region, where kale production is intense. For this reason, the study aimed to reveal the cyst nematode species and their distribution in the cabbage cultivation areas of Artvin, Giresun, Ordu, Rize and Trabzon provinces.

The first objective of this study is to detect cyst nematodes in kale production areas within the provinces of Artvin, Giresun, Ordu, Rize and Trabzon in the Eastern Black Sea region of Türkiye, based on morphological and molecular characteristics. Secondly, the study aims to reveal the distribution and population of the nematodes obtained in the region.

## Materials and Methods

### Description of study sites

The Black Sea region is located in the north of Türkiye. It covers 18% of the Turkish territory and extends east-west for 1.400 km resembling a strip. The Eastern Black Sea region, which is the most mountainous and receives the highest amount of rainfall (average annual 842.6 mm) among the regions of the Black Sea, is characterized by humidity levels. There are significant climatic differences between the coastal and inland areas, leading to variations in the types of crops cultivated. In the Eastern Black Sea region, the highest rainfall occurs in autumn, while the lowest rainfall is observed in spring. The average yearly temperature ranges from 13 to 15ºC. Due to its geographical location and mostly rainy days, the Black Sea region has the lowest sunshine time. The soil structure in the provinces of the region is generally fine textural class, acidic reaction, non-saline, low lime content, and sufficient organic matter content (Ay & Kızılkaya, 2021). The primary crop in the region, particularly in its eastern areas, is hazelnuts. In addition, black cabbage (kale), corn, kiwi, rice, beans, and potatoes are among the important agricultural products in the region. Among these, kale is a cold climate plant. It is resistant to drought and difficult production conditions and has a wide production area in the world. It has dark green and broad leaves surrounding the stem and veins. Its leaves contain chlorophyll pigment, beta carotene, ascorbic acid and calcium. It contains plenty of vitamins and minerals (Anonymous, 2024a). In this study, seventy-seven black cabbage production fields from five provinces were surveyed during the September-November of 2021-2022.

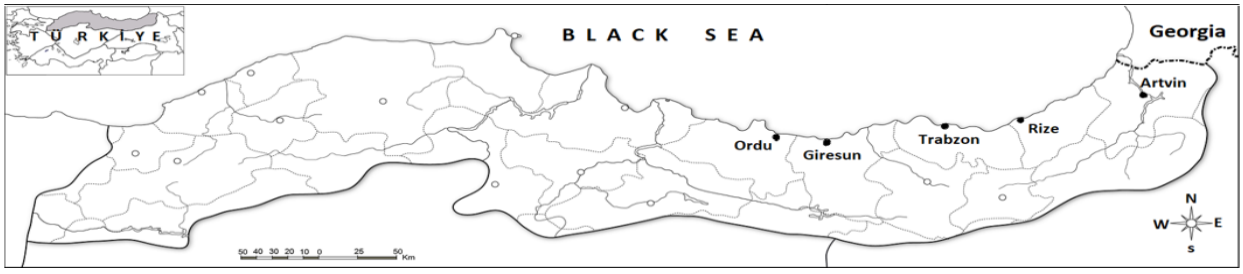


Figure 1. Map indicating location of sample locations within the five Eastern Black Sea region provinces (Anonymous, 2023a).

### Soil sampling

During the September-November of 2021-2022, surveys were conducted in53 districts, including 5 from Artvin, 15 from Ordu, 10 from Giresun, 11 from Rize, and 12 from Trabzon provinces in the Eastern Black Sea region of Türkiye. Samples were taken from a total of 77 locations including 7, 17, 19, 15, 19 from Artvin, Giresun, Ordu, Rize, and Trabzon provinces, respectively (Table 1). Soil-root samples were collected from the rhizosphere of black cabbage plants to a depth of approximately 20 cm (Figure 7a). Sampling was taken to represent the field, according to the field size. Soil samples were taken using a hand shovel and were obtained by combining samples from 5 places within 1 da area in each field. The latitude and longitude of each sampling field were recorded using the global positioning system (GPS) (Table 1). All subsamples were mixed well and a sample of 1kg of soil and roots. The collected samples were immediately placed in labeled plastic bags and transported to the laboratory. The samples were kept in the refrigerator at +6ºC until examined.

### Extraction of nematodes

Infective second stage juveniles ($J_2$) were extracted from the soil using the centrifugal flotation technique (Jenkins, 1964). Cysts were extracted from each soil sample using the sieving and flotation method (Shepherd, 1986). Cysts remaining on the 60-mesh sieve were collected with a brush using a stereomicroscope (Leica, S8APO) at 40x magnification on Whatman filter paper. A total of 17 cyst-forming nematode populations were collected from 77 samples. All cysts were preserved in laboratory conditions for molecular and morphological identification in this study.

Table 1. Locations and coordinates of surveys for the detection of cyst nematodes in kale plants in the Eastern Black Sea region in this study

| | | Districts | latitude | longitude | | | Districts | latitude | longitude |
|---|---|---|---|---|---|---|---|---|---|
| Ordu | 1 | Perşembe 1 | 41º02'22.6"N | 37º41'41.3"E | Giresun | 39 | Tirebolu 1 | 40º58'28.2"N | 38º45'31.7"E |
| | 2 | Perşembe 2 | 41º00'58.7"N | 37º49'41.2"E | | 40 | Tirebolu 2 | 40º57'25.6"N | 38º47'42.0"E |
| | 3 | Kabataş | 40º44'49.2"N | 37º23'56.8"E | | 41 | Tirebolu 3 | 40º57'27.0"N | 38º48'18.0"E |
| | 4 | Çatalpınar | 41º06'28.6"N | 37º15'10.1"E | | 42 | Tirebolu 4 | 40º57'15.5"N | 38º48'54.0"E |
| | 5 | Kumru | 40º53'00.5"N | 37º16'50.5"E | | 43 | Tirebolu 5 | 40º57'16.6"N | 38º48'44.3"E |
| | 6 | Gürgentepe 1 | 40º46'46.0"N | 37º36'37.7"E | | 44 | Güce | 40º54'50.4"N | 38º47'29.4"E |
| | 7 | Gürgentepe 2 | 40º46'46.2"N | 37º36'50.7"E | | 45 | Dereli | 40º44'16.7"N | 38º27'21.8"E |
| | 8 | Ulubey | 40º53'01.0"N | 37º46'48.4"E | | 46 | Eynesil 1 | 41º03'23.8"N | 39º08'42.0"E |
| | 9 | Gölköy | 40º41'36.5"N | 37º36'33.2"E | | 47 | Eynesil 2 | 41º02'23.5"N | 39º09'03.0"E |
| | 10 | Çaybaşı | 41º01'23.6"N | 37º06'51.3"E | | 48 | Görele | 40º55'07.7"N | 38º57'28.1"E |
| | 11 | Mesudiye | 40º27'14.4"N | 37º46'28.6"E | | 49 | Görele 2 | 41º01'49.9"N | 39º00'52.0"E |
| | 12 | Korgan | 40º51'53.6"N | 37º27'12.2"E | | 50 | Keşap 1 | 40º54'57.8"N | 38º31'15.3"E |
| | 13 | Altınordu 1 | 40º58'28.9"N | 37º57'51.8"E | | 51 | Keşap 2 | 40º53'51.5"N | 38º31'30.7"E |
| | 14 | Altınordu 2 | 40º58'35.5"N | 37º57'35.5"E | | 52 | Çanakçı | 40º55'48.4"N | 39º01'15.6"E |
| | 15 | Gülyalı | 40º58'03.4"N | 38º03'04.7"E | | 53 | Espiye | 40º56'34.4"N | 38º45'25.9"E |
| | 16 | Fatsa 1 | 40º54'21.2"N | 37º31'28.6"E | | 54 | Piraziz | 40º57'00.0"N | 38º09'06.5"E |
| | 17 | Fatsa 2 | 40º58'00.5"N | 37º30'16.6"E | | 55 | Bulancak | 40º56'09.5"N | 38º11'23.3"E |
| | 18 | Ünye | 41º07'09.7"N | 37º16'08.3"E | Rize | 56 | Çamlıhemşin | 41º04'58.1"N | 41º02'01.0"E |
| | 19 | Aybastı | 40º42'22.6"N | 37º24'43.1"E | | 57 | Güneysu | 40º59'46.0"N | 40º35'52.4"E |
| Trabzon | 20 | Çarşıbaşı | 41º05'32.6"N | 39º23'33.7"E | | 58 | Çayeli 1 | 41º03'18.0"N | 40º37'10.6"E |
| | 21 | Arsin 1 | 40º57'12.2"N | 39º54'27.7"E | | 59 | Çayeli 2 | 41º03'49.0"N | 40º43'02.3"E |
| | 22 | Arsin 2 | 40º57'13.1"N | 39º55'39.6"E | | 60 | Fındıklı | 41º14'59.3"N | 41º06'49.7"E |
| | 23 | Beşikdüzü | 41º02'48.8"N | 39º14'37.7"E | | 61 | Pazar | 41º10'15.6"N | 40º50'08.9"E |
| | 24 | Yomra | 40º57'16.9"N | 39º52'16.3"E | | 62 | Merkez 1 | 41º01'29.6"N | 40º32'33.7"E |
| | 25 | Vakfıkebir 1 | 41º02'49.6"N | 39º15'10.1"E | | 63 | Merkez 2 | 41º01'50.9"N | 40º33'32.4"E |
| | 26 | Vakfıkebir 2 | 41º00'23.0"N | 39º19'59.5"E | | 64 | Merkez 3 | 41º02'59.3"N | 40º36'32.8"E |
| | 27 | Araklı | 40º54'18.4"N | 40º03'21.2"E | | 65 | Derepazarı | 41º01'15.6"N | 40º25'20.6"E |
| | 28 | Sürmene 1 | 40º54'33.8"N | 40º06'39.6"E | | 66 | Kalkandere 1 | 40º57'06.1"N | 40º25'21.4"E |
| | 29 | Sürmene 2 | 40º54'45.4"N | 40º09'32.4"E | | 67 | Kalkandere 2 | 40º56'00.6"N | 40º26'07.8"E |
| | 30 | Hayrat | 40º54'43.9"N | 40º20'58.9"E | | 68 | Hemşin | 41º03'19.4"N | 40º53'58.6"E |
| | 31 | Yomra 2 | 40º57'26.4"N | 39º51'05.4"E | | 69 | İyidere | 40º59'20.4"N | 40º20'00.2"E |
| | 32 | Of 1 | 40º55'35.4"N | 40º13'40.8"E | | 70 | Ardeşen | 41º11'15.4"N | 40º59'06.0"E |
| | 33 | Of 2 | 40º54'00.0"N | 40º16'37.9"E | Artvin | 71 | Arhavi | 41º21'02.5"N | 41º18'00.0"E |
| | 34 | Of 3 | 40º49'39.7"N | 40º15'55.1"E | | 72 | Borçka | 41º26'51.0"N | 41º42'11.9"E |
| | 35 | Dernekpazarı | 40º47'32.3"N | 40º16'19.6"E | | 73 | Hopa | 41º23'31.2"N | 41º25'37.2"E |
| | 36 | Akçaabat 1 | 41º05'29.4"N | 39º28'48.0"E | | 74 | Merkez 1 | 41º10'44.4"N | 41º49'26.4"E |
| | 37 | Akçaabat 2 | 41º02'07.5"N | 39º33'24.0"E | | 75 | Merkez 2 | 41º10'56.5"N | 41º49'43.2"E |
| | 38 | Çaykara | 40º45'10.6"N | 40º14'53.7"E | | 76 | Kemalpaşa 1 | 41º29'33.4"N | 41º32'02.8"E |
| | | | | | | 77 | Kemalpaşa 2 | 41º28'34.8"N | 41º32'21.7"E |
| | Total | | | | | **77** | | | |

**Morphological studies**

For microscopical examination of morphological characters and using them in diagnosis, second stage juveniles ($J_2$), males and cysts were used. Nematodes transferred to a drop of pure water on a clean glass slide on the hot plate were killed in 4-6 seconds at 60ºC. The head structures, stylet and tail structures of the second instar larvae were examined. The morphological characters and preparing the microphotographs were performed using a light microscope (Carl Zeiss Axio) equipped with a ZEISS Axiocam 105 digital camera. The vulval cones region of cysts were examined on permanent slides including the main characters as vulval slit, underbridge, and fenestra structures. For the permanent preparations, the vulval cone regions of cyst were cut with 45% lactic acid and cleaned with a fine tip brush, then transferred into glycerin, and mounted on slides under a Leica S8APO stereo microscope (Taylor & Netscher, 1974; Hartman & Sasser, 1985).

**Molecular analyses**

**DNA extraction**

In this investigation, the genomic DNA extraction procedure adhered to the protocol elucidated by Pagan et al. (2015). Specifically, five second-stage nematode samples obtained from hatched eggs in the cysts were collected and transferred to 1.5 ml Eppendorf tubes., each containing 10 µl of extraction buffer (1M Tris, 0.1M EDTA, pH 8), composed of 10 mM Tris-HCl (pH 8.8), 1 mM EDTA, 0.1% Triton X-100 (v/v), and 20 mg/ml Proteinase K. Subsequently, the tubes were subjected to overnight storage at a temperature of −20ºC. Following this, each sample underwent grinding using a micropestle and was incubated at a temperature of 56ºC for a duration of 1 hour, followed by an additional incubation at 95ºC for 10 minutes. This extraction process yielded genomic DNA from the five specimens, which subsequently served as the template for the ensuing PCR reaction.

**PCR amplification**

Polymerase Chain Reaction (PCR) amplification of the Internal Transcribed Spacer (ITS1, 5.8S, ITS2) gene was undertaken utilizing the designated primers TW81 (5'-GTT TCC GTA GGT GAA CCT GC-3´) and AB28 (5´-ATA TGC TTA AGT TCA GCG GGT-3´) (Joyce et al., 1994). Additionally, the Cytochrome Oxidase I (cox1) region of mitochondrial DNA (mtDNA) was targeted with the species-specific primer set Car-F (5´-CTTTGGTTTAATTAGTTTAAGAG-3´) Car-R (5´-GAAAAATATCTAAACTAGCG-3´) for the purpose of *Heterodera carotae* Jones 1950 identification (Madani et al., 2018). The PCR reactions were executed in a final volume of 25 µl, comprising 8.5 µl of distilled water, 12.5 µl of DreamTaq Green Master mix (2X) (Thermo Fisher Scientific, Waltham, Massachusetts, USA), 1.25 µl of each primer (10 pMol/µl), and 1.5 µl of DNA template. For the ITS primers, PCR was conducted using a thermal cycler (96-Well, Veriti™ Singapore), employing the following program: denaturation at 95ºC for 4 min, followed by 40 cycles of 30 s at 95ºC, 45 s at 56ºC, 2 min at 72ºC, with a final extension at 72ºC for 10 min. The thermocycling reactions for the species-specific primer set (Car-F/Car-R) were performed following the protocol recommended by Madani et al. (2018).

The amplification products were subsequently segregated through electrophoresis in a 1% TAE (Tris-acetate-EDTA) buffer, 1.5% agarose gel, under a voltage of 100 V for a duration of 28 minutes. Following electrophoresis, the products were treated with ethidium bromide staining, and subsequently visualized through UV illumination using ErBiyotek GEN-BOX imageER Fx, employing the methodology as described by Sambrook et al. (1989). For the purpose of sequence analysis, the PCR products were forwarded to the STAB VIDA company located in Portugal. Sequencing was conducted using an ABI 3730xl DNA Analyzer. The acquired sequences were BLASTed to ascertain sequence similarity with those archived within the National Center for Biotechnology Information (NCBI) database.

## Results and Discussion

In this study conducted on cyst nematodes in the kale production areas of the Eastern Black Sea region in 2021-2022, 77 samples covering Artvin, Giresun, Ordu, Rize and Trabzon provinces were examined. As a result of the morphological characteristics and molecular analysis of the cyst nematode populations obtained from the surveyed areas, their species were determined. The cyst nematode species *Heterodera carotae* Jones, 1950, *H. cruciferae*, and *Heterodera fici* Kirjanova, 1954 belonging to the *Heterodera* genera were identified from the soil samples in the study. The consequence of this survey indicated that cyst nematode *H. cruciferae* was found to be the common species (Figure 2).


Figure 2. Map of soil sampling points and distribution of cyst nematode *Heterodera* spp. on the Eastern Black Sea region of Türkiye in this study (Placemarks are marked on google earth) (Anonymous, 2024b).

### Survey studies

In the region, only 17 of the 77 sampling areas surveyed were found to be infected (22%) with cyst nematodes species. Soil samples collected from four provinces including Ordu, Giresun, Trabzon and Rize were found as contaminated with cyst nematodes species. However, cyst nematodes were not found in the soil samples taken from Artvin province. *Heterodera cruciferae, Heterodera carotae* and *Heterodera fici* were detected from the total infected soil samples at 16.9%, 3.9%, and 1.3% relative frequency, respectively. Of the 17 soils detected as infected, 13 (76.5%) were found to be infected with *H. cruciferae*, 3 (17.6%) with *H. carotae* and 1 (5.9%) with *H. fici*. The most common species was *H. cruciferae* present in all provinces except Artvin; The highest population density of *H. cruciferae* was detected in Altınordu district with 38 cysts/100 $cm^3$ soil and 18 J2s/100 $cm^3$ soil. *Heterodera carotae* was found in Ordu and Giresun provinces. The highest density was 48 cysts/100 $cm^3$ soil in Gülyalı district and 40 J2s/100 $cm^3$ soil in Fatsa district in Ordu (Table 2). *Heterodera fici* was found only in Ordu. It was only detected in Mesudiye district with 2 cysts/100 $cm^3$ soil and 8 J2s/100 $cm^3$ soil. In the study, as a result of the surveys conducted in Ordu province, 4 out of 19 soil samples taken from 15 districts were found to be infected (21.1%) with cyst nematodes. The detection of second stage juvenile and cysts from the soil was found only in 4 districts. In these districts of Ordu province, 29 J2s/ 100 $cm^3$ soil and 48 cysts/100 $cm^3$ soil were obtained and the highest population was found in Gülyalı district (Figure 3). Additionally, white females and brown cysts were found on kale root samples taken from Gülyalı (Figure 7 b,c). This was followed by Fatsa district with 20 infective puppies and 16 cysts/100 cm3 soil. In Altınordu district, 9 J2s and 19 cyst/100 $cm^3$ soil populations were detected. The minimum density was 2 J2s and 8 cysts/100 $cm^3$ soil populations in Mesudiye district. Cyst nematodes were not found in the soils taken from other 11 surveyed districts. In Giresun province, cyst nematode was found in 6 of 17 soil samples taken from 10 districts.

As a result of the evaluation, 35.3% of the soils taken from Giresun province where kale is grown were found to be contaminated with cyst nematode. The detection of second stage juveniles and cysts from the soil was found only in 5 districts. The highest population was found in Dereli district with 12 J2s and 16 cysts / 100 $cm^3$ soil, which was followed by Eynesil district with 7 J2s and 5 cysts / 100 $cm^3$ soil (Figure 3). In Keşap district, 2 J2s and 5 cysts were detected in 100 $cm^3$ soil. In Tirebolu district, 3 J2s and 3 cyst/ 100 $cm^3$ soil were detected. The lowest density was found in Piraziz district, where only 2 cysts /100 $cm^3$ soil were detected. Cyst nematodes were not found in the soils taken from other 6 surveyed districts. In Trabzon Province, cyst nematode was found in 5 of 19 soil samples taken from 13 districts. As a result of the evaluation, 26.3% of the black cabbage grown soils from Trabzon province were found to be contaminated with cyst nematode. The detection of second stage larvae and cysts from the soil was found only in 4 districts. In these districts of Trabzon province, the highest population was found in Arsin district with 10 J2s and 6 cysts per 100 $cm^3$ soil. Arsin district was followed by Akçaabat district and 9 J2s and 4 cysts/100 $cm^3$ soil were found (Figure 3). In Vakfıkebir district, 4 J2s and 6 cysts were detected in 100 $cm^3$ soil. The lowest density was detected in Yomra district, where only 2 cysts populations were detected in 100 $cm^3$ soil. Cyst nematodes were not found in the soils taken from other 9 surveyed districts. In Rize province, cyst nematode was found in 2 of 15 soil samples taken from 10 districts. As a result of the evaluation, 13.3% of the black cabbage cultivated soils taken from Rize province were found to be contaminated with cyst nematode. The detection of second stage juvenile and cysts from the soil was found only in 2 districts. In these districts of Rize province, 1 infective juvenile and 5 cysts were obtained in 100 $cm^3$ soil and the highest population was found in Çamlıhemşin district. The lowest density was detected in Ardeşen district with only 3 cysts per 100 $cm^3$ soil (Figure 3). Cyst nematodes were not found in the soils taken from other 8 surveyed districts.

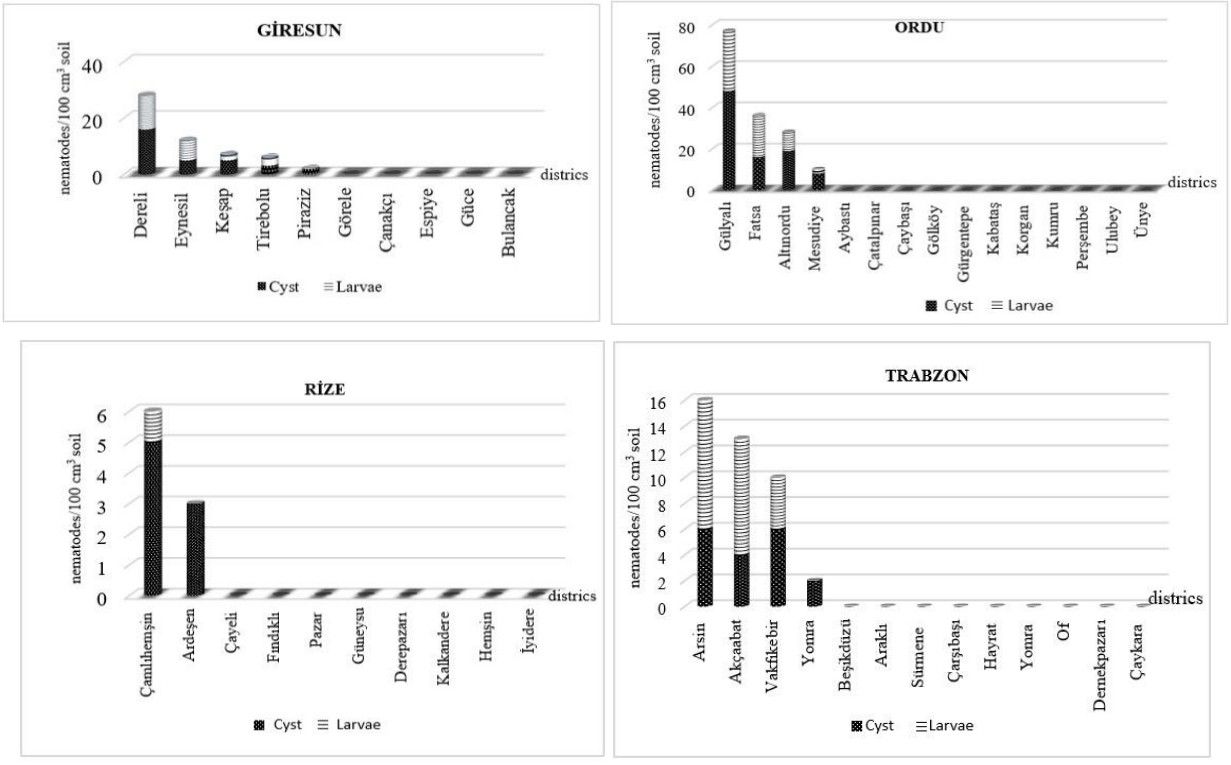


Figure 3. Population abundance of *Heterodera* spp. cysts and larvae in 100 $cm^3$ soil in districts of Giresun, Ordu, Rize and Trabzon, provinces in this study.

Table 2. Detected cyst nematode species and their abundance and incidence of the cyst and infective juveniles in kale production areas in Artvin, Giresun, Ordu, Rize, and Trabzon provinces in the Eastern Black Sea region

| Provinces | Districts | Number of positive samples | Cysts/100 $cm^3$ soil | Infective Juvenile /100 $cm^3$ soil | Incidence (%) | Species |
|---|---|---|---|---|---|---|
| | | | Abundance | | | |
| Ordu | Altınordu | 1 | 38 | 18 | 50 | H. *cruciferae* |
| | Fatsa | 1 | 31 | 40 | 50 | H. *carotae* |
| | Gülyalı | 1 | 48 | 29 | 100 | H. *carotae* |
| | Mesudiye | 1 | 8 | 2 | 100 | H. *fici* |
| Giresun | Tirebolu-5 | 1 | 18 | 18 | 16.6 | H. *cruciferae* |
| | Dereli | 1 | 16 | 12 | 100 | H. *cruciferae* |
| | Keşap-2 | 1 | 9 | 3 | 50 | H. *cruciferae* |
| | Eynesil-1 | 1 | 4 | 13 | 100 | H. *carotae* |
| | Eynesil-2 | 1 | 5 | 2 | 100 | H. *cruciferae* |
| | Piraziz | 1 | 2 | 0 | 100 | H. *cruciferae* |
| Trabzon | Arsin | 1 | 6 | 10 | 50 | *H. cruciferae* |
| | Yomra | 1 | 2 | 0 | 100 | *H. cruciferae* |
| | Vakfıkebir-1 | 1 | 8 | 5 | 100 | *H. cruciferae* |
| | Vakfıkebir-2 | 1 | 3 | 3 | 100 | *H. cruciferae* |
| | Akçaabat | 1 | 7 | 18 | 50 | *H. cruciferae* |
| Rize | Çamlıhemşin | 1 | 5 | 1 | 100 | *H. cruciferae* |
| | Ardeşen | 1 | 3 | 0 | 100 | *H. cruciferae* |
| Artvin | Arhavi | 1 | 0 | 0 | 0 | Not found |
| | Borçka | 1 | 0 | 0 | 0 | Not found |
| | Hopa | 1 | 0 | 0 | 0 | Not found |
| | Merkez 1 | 1 | 0 | 0 | 0 | Not found |
| | Merkez 2 | 1 | 0 | 0 | 0 | Not found |
| | Kemalpaşa 1 | 1 | 0 | 0 | 0 | Not found |
| | Kemalpaşa 2 | 1 | 0 | 0 | 0 | Not found |

In Artvin province, no cyst nematode was found in any of the 7 soil samples taken from 5 districts. Considering the districts of the other provinces where cyst nematodes were found in the study, it is noteworthy that they are districts located on the coastline, but cyst nematodes are not found in high-altitude districts. As a result of the study, it was determined that the soils taken from Artvin province where kale is grown are not found with cyst nematodes. The absence of cyst nematode in the soils of this province, even though it is a host, highlights the effect of soil conditions. Several studies have established a correlation between nematode population densities and environmental conditions, particularly variations in soil properties. Chowdhury et al. (2020) stated that soil properties like soil texture, pH, and organic matter are considered the main variables of the nematode. Similarly, one of the most influential environmental factors affecting nematode development is soil temperature. It is also, key factors such as soil texture, moisture levels, and temperature have been identified as important in influencing the presence of plant parasitic nematodes (Wallace, 1959; Schmidt et al., 1993; Avendaño et al., 2004). Fenwick (1951) reported that environmental factors such as soil temperature influence the number of eggs and larvae in cysts of some species. Abd-Elgawad (2021) stated that soil organic matters have presented as an important suppressor of plant-parasitic nematodes. Hbirkou et al. (2011) stated that soil texture has an indirect effect on the living conditions of nematodes. In the light of these explanations, the reasons why cyst nematodes are not encountered in kale fields in Artvin province can be listed.

**Morphological characters**

The morphological details of cysts nematodes obtained from black cabbage fields in Türkiye were observed using second stage juveniles, males and cysts. The morphological characteristics of cyst nematode stages were examined using light microscope in this study (Figure 4).

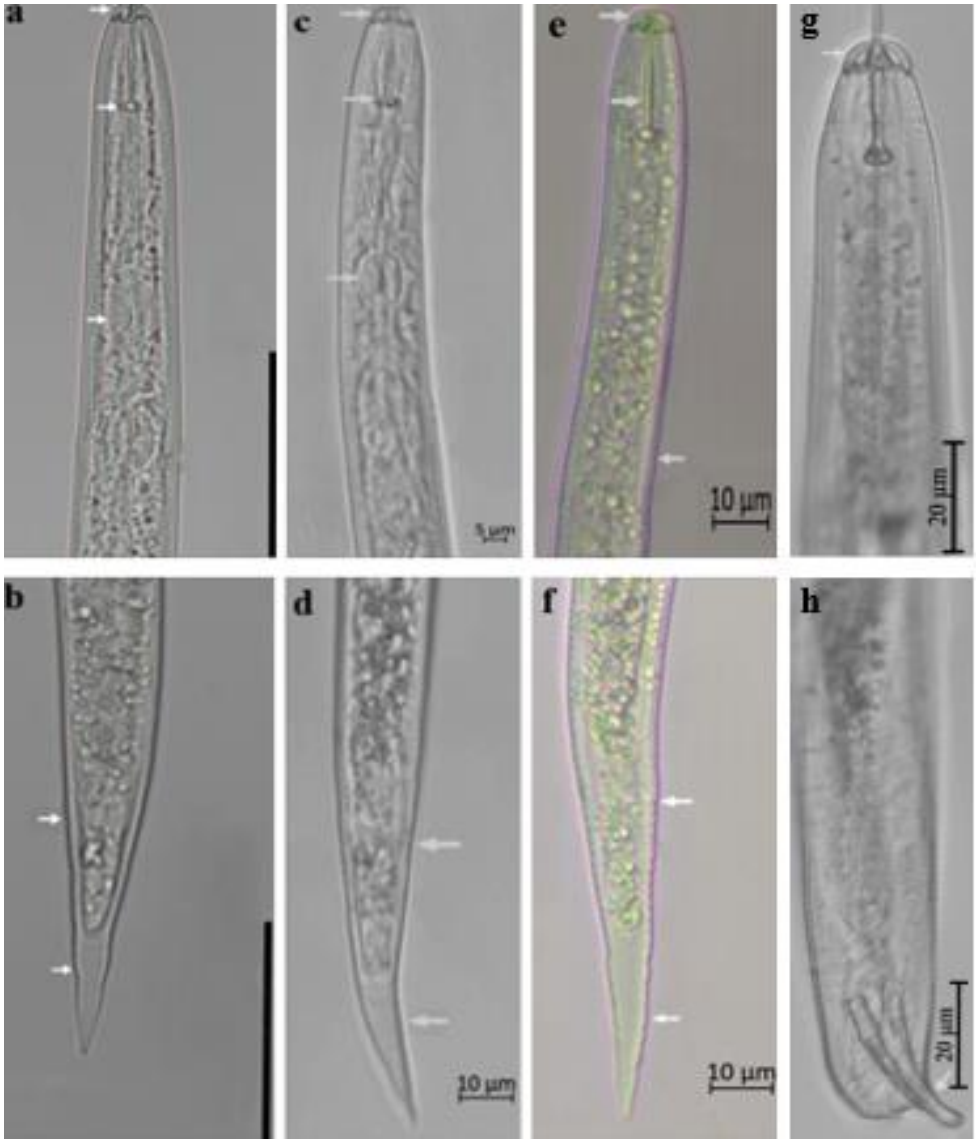


Figure 4. Photomicrographs of second stage juvenile structure of: a, b) *Heterodera carotae,* c, d) *Heterodera cruciferae,* e, f) *Heterodera fici* and male of g, h) *Heterodera carotae* isolated from kale production areas in Eastern Black Sea region of Türkiye. a, c, e) anterior regions showing head, stylet, and median bulb, b, d, f) posterior regions showing tail, anus, hyaline portion, g) anterior region showing head framework and stylet of *H. carotae* male, h) posterior region showing spicula and tail.

*Heterodera carotae:* Second stage juvenile body structure was vermiform. The head is slightly offset, and cephalic framework is well developed and heavily sclerotized (Figure 4a). The stylet is remarkably robust, with round stylet knobs (Figure 4a). The median bulb is oval, featuring a distinct valve. The pharyngeal glands are elongated, tapering posteriorly, and overlapping the intestine ventrally (Figure 4a). The tail is conical and has a prominent terminal hyaline part (Figure 4b). Male body is vermiform, the head is offset, and cephalic framework is robust (Figure 4g). The stylet is strong, characterized by well-developed knobs. The spicules are arcuate, the gubernaculum is slightly curved (Figure 4h). The tail short. Cysts lemon-shaped with distinct neck and color changes from white to russet brown (Figure 5a). Vulval bridge broken in some specimens (Figure 6a). Bullae absent. Underbridge poorly developed, vulval slit long. *H. carotae* is most closely related to *H. crucifera*. It has been identified as a belonging to the *Goettingiana* group. It differs from *H. cruciferae* by a longer average hyaline part of tail region in J2 and a longer average vulval slit in cysts (Subbotin et al., 2010).

*Heterodera cruciferae:* Second-stage juveniles body vermiform, head rounded. Cephalic framework strongly developed (Figure 4c). Stylet well developed and stylet knobs rounded (Figure 4c). Cysts slightly lemon shaped, lighyt to dark brown (Figure 5b). Body has zigzag cuticular surface pattern. The vulva semifenestrate ambifenestrate without bullae (Figure 6b). Underbridge a very weak. Male not found. *Heterodera cruciferae* is placed in the Goettingiana group (Handoo & Subbotin, 2018) and has been detected in various regions of Türkiye.

*Heterodera fici:* Second-stage juveniles' body vermiform, head slightly set off, rounded. Cephalic framework moderate, stylet well developed, basal knobs rounded in second stage larvae (Figure 4e). Esophageal lobe overlaps anterior part of intestine. Hyaline terminal about 1/2 tail length (Figure 4f). Cysts lemon shaped (Figure 5c), the fenestrae in some cysts are small and appearing biffenestrate. Ballue small and dome-shaped scattered about the level of underbridge. Underbridge weakly developed (Figure 6c), with furcate ends. Vulval slit about same length as bridge (Figure 6d). Male not found. Golden et al. (1988) stated that *H. fici* properly belongs in the "schachtii group" of species. *H. fici* is most closely related to *H. schachtii*, *Heterodera glycies* Ichiohe, 1952, and *Heterodera cajani*, 1967. It differs from these species by the presence of a weakly developed underbridge and small, scattered bullae (Golden et al.,1988).

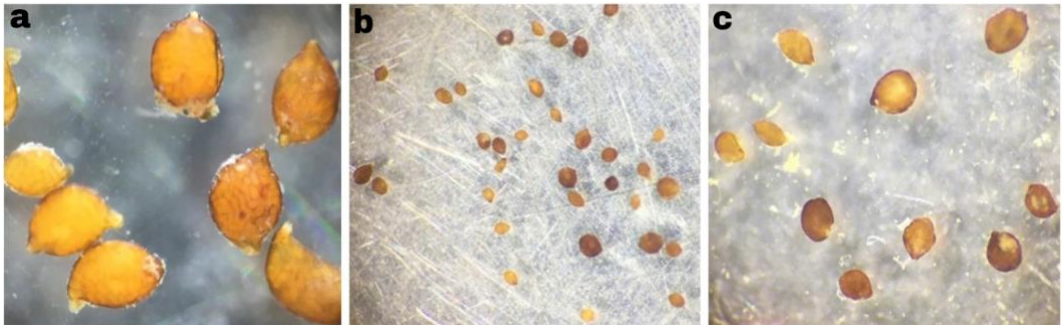


Figure 5. Photomicrograph of cyst forming females of *Heterodera* spp. extracted from kale production areas in Eastern Black Sea region of Türkiye: a) *Heterodera carotae,* b) *Heterodera cruciferae,* c) *Heterodera fici*.

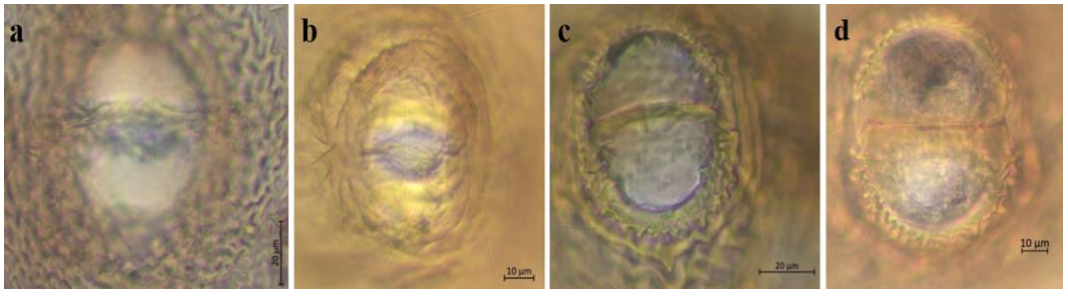


Figure 6. Photomicrograph of perineal pattern structure of *Heterodera* spp. isolated from kale production areas in Eastern Black Sea region of Türkiye: a) *Heterodera carotae,* b) *H*eterodera *cruciferae* and c, d) *Heterodera fici.*

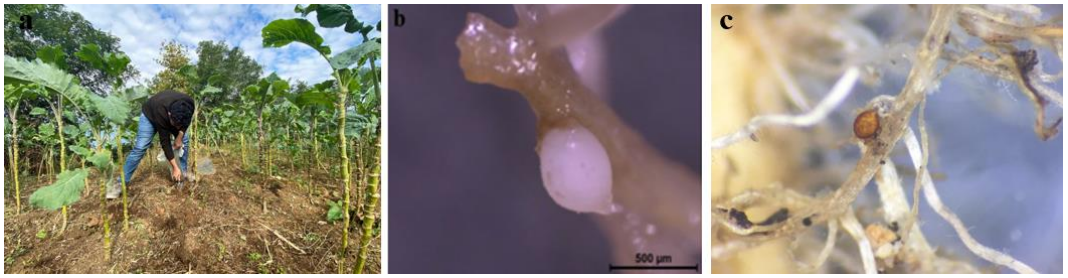


Figure 7. Photomicrograph of *Heterodera carotae*: a) soil sampling rhizosphere of kale, b) white females on kale (black cabbage) roots, c) brown cyst on the kale roots.

**Molecular analyses**

Genomic DNAs of the populations obtained in this study were amplified by PCR and then visualized by gel electrophoresis. Internal transcribed spacer (ITS) gene expansion segments produced a single 1020 bp fragment for *all* three species (Figure 8 a). The amplification of the expansion segments utilizing the specific primer Car-F/Car-R for *H. carotae* yielded a fragment measuring 350 base pairs, as determined through gel electrophoresis analysis (Figure 8 b). The sequences of the ribosomal region spanning the ITS gene obtained from PCR products of *Heterodera* populations in this study were compared with those present in the GenBank database using BLAST revealed a high similarity. The sequence results obtained were found to be similar to *H. carotae* (e.g., GenBank accession nos. MG976790.1), *H. cruciferae* (e.g., GenBank accession nos. MG848393.1) and *H. fici* (e.g., GenBank accession nos. KY635987.1) with a similarity rate of over 99% in the similarity query in the NCBI gene bank.

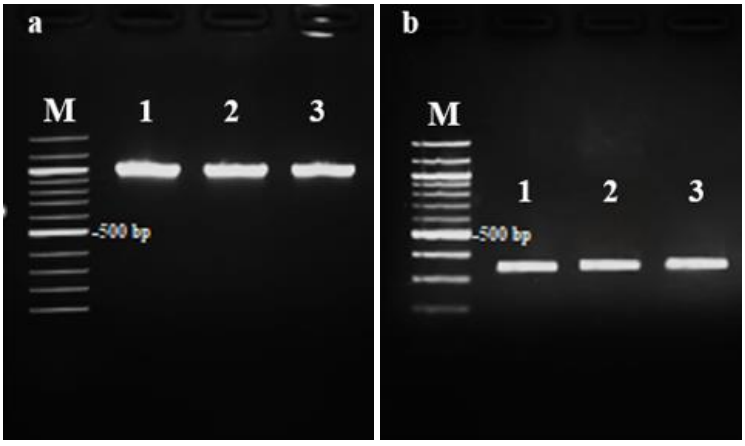


Figure 8. PCR products of *Heterodera carotae, H. cruciferae* and *H. fici* species. a: Fragments of internal-transcribed spacer (ITS) (ITS1-5.8S) region of rRNA using TW81/AB28 primer pair (Line1-4); b: Fragments of cytochrome oxidase I of mitochondrial DNA (coxI) using Car F/Car R primer pair for H. *carotae* (Line1-3); M, 100 bp DNA marker ladder.

## Discussion

Molecular approaches are increasingly used in nematode diagnosis as they provide accurate diagnosis. For this reason, ribosomal DNA has become the preferred option for nematode diagnosis. Ribosomal ITS regions of nematodes are highly variable and consequently useful for diagnosis (Subbotin et al. 2011). Most *Heterodera* species identified to date have been identified using morphological and molecular data, particularly based on the rDNA-ITS region (ITS + 5.8 S + ITS2). Accurate identification of plant parasitic nematode species that cause plant yield losses is important for effective control against them. This research aims to conduct a comprehensive review focusing on the taxonomic identification of *Heterodera* species cultivating kale. During this research study, it was determined that kale cultivation areas harbor a community of *Heterodera* species such as *H. carotae, H. cruciferae* and *H. fici*. The scope of nematode parasitism on kale covers a spectrum of three *Heterodera* species. The most prominent among these is *H. cruciferae*, which occurs in twelve separate locations, followed by *H. carotae*, which occurs in four different locations. In contrast, the relatively rare *H. fici* was detected in a single location.

*Heterodera carotae*, a member of the Heteroderidae family, was originally described by Jones (1950). The present study identifies *H. carotae* in kale plants, indicating a novel host association. This discovery underscores the adaptability of *H. carotae* to kale. Notably, this nematode exhibits a restricted host range, primarily impacting carrots, *Daucus carota* (L.) (Apiales: Apiaceae) and *Daucus pulcherrimus (*Willd.) W. D. Koch ex DC.1830 (Apiales: Apiaceae)). Its detrimental effects on plants include uneven growth, yellowing leaves, chlorosis, stunted growth, wilting, taproot rot, and premature lignification, rendering affected carrots

unfit for market (Anonymous, 2023b). In the context of carrot production in Italy, *H. carotae* is responsible for considerable yield losses, ranging from 20% to 90% (Greco et al., 1993). While Jones (1950) initially documented *H. carotae* in carrot soils in Spain, subsequent reports have expanded its host range to include *Daucus carota* (Yu et al., 2017), *Daucus pulcherrimus* (Goodey et al., 1965), *Torilis arvensis (*Huds.) Link, 1821 *(*Apiales: *Apiaceae*) and *Torilis leptophylla* (L.) Reichb. (Apiales: Apiaceae) (Escobar-Avila et al., 2018). Beyond Spain, *H. carotae* has been reported in various regions, encompassing Europe, India, Cyprus, South Africa, North America, and Mexico, with documented occurrences on carrots by Berney & Bird (1992), Subbotin et al. (2010), Escobar-Avila et al. (2018), and Shubane et al. (2021). Taxonomically, *H. carotae* is classified within the Goettingiana group. Its distinctions from *H. cruciferae* include a longer average hyaline region in the tail of J2 and a lengthier average vulval slit in cysts (Subbotin et al., 2010).

*Heterodera cruciferae* represents a distinct species within the taxonomic confines of the Heteroderidae family, an attribution initially proposed by Franklin in 1945, as chronicled by Winslow in 1955. The first recorded instance of identifying *H. cruciferae* on cabbage dates back to the year 1963, within the locale of Erzurum in Türkiye (Yüksel, 1973). In the context of the broader *Heterodera* genus, *H. cruciferae* stands apart for its comparatively circumscribed geographic distribution, which has been documented across diverse global locales. The species' occurrence spans multiple regions, encompassing Europe, the United States most notably California-Australia, Iran, and Azerbaijan (Franklin, 1945; Stone & Rowe, 1976; Sturhan & Lišková, 2004; Jabbari & Niknam, 2008; Chizhov et al., 2009; Mennan & Handoo, 2012). Regarded as a prominent taxon within the realm of *Heterodera*, *H. cruciferae* assumes a position of economic salience due to its capacity to impose substantial agrarian detriment, with a pronounced predilection for cruciferous crops, notably cabbage and Brussels sprouts (Ravichandra, 2014; Mennan & Handoo, 2012). The ecological imprint of this species reverberates across an array of crops, embracing cabbage, broccoli, cauliflower, radish, turnip, pea, and rapeseed. Empirical findings by Turner and Subbotin underscore that *H. cruciferae*'s maturation trajectory culminates within 30 days at a temperature of 20ºC, facilitating the succession of up to three discrete generations. Moreover, its ubiquity persists seamlessly throughout the seasonal panorama in the European milieu (Turner & Subbotin, 2013).

Toktay et al. (2022) used ribosomal DNA region (rDNA-ITS) and cytochrome oxidase subunit 1 (mtDNA-COI) sequences to identify cyst nematodes in cabbage production areas in Niğde province with molecular methods. For the first time in Türkiye, *H. cruciferae* were used for identification. Jabbari and Niknam (2008) investigated plant parasitic nematode biodiversity in vegetable fields in Tabriz city of East Azerbaijan province of Iran between 2004 and 2005. They identified 25 species of 16 nematode genera from 88 soil and root samples, including a large population of cyst nematodes, *H. cruciferae*, in most of the sampling sites. During a nematological survey conducted in Russia, *H. cruciferae* was detected in cabbage-growing areas along the Oka River, Ozery and Serpukhov regions in the Moscow region of Russia. They recorded the first report of this nematode in the Moscow region. Rapeseed, rutabaga and radish have been identified as additional host plants for this nematode (Chizhov et al., 2009).

*Heterodera fici*, a constituent of the Heteroderidae family, was originally characterized by Kirjanova (1954). Fig cyst nematode, *H. fici*, was first described by Kirjanova in 1954 from rubber plant (*Ficus elastica* Roxb. Ex Hernem (Rosales: Moraceae) roots in Harbin, People's Republic of China (Kirjanova, 1954). A study conducted in the Aegean region of Türkiye reported for the first time that *H. fici* parasitized *Ficus carica* (L.) (Rosales: Moraceae) and *F. domestica* (Yuksel, 1981). Later, Mulvey and Golden identified this cyst nematode from California, Florida and Virginia in the United States; They summarized its known spread from Brazil, Australia, Germany, Italy, Poland, South Africa, Spain, Türkiye, USSR and Yugoslavia. During a study conducted in 1986 in an orchard in Saryab, Quetta, Pakistan, it was reported that *H. fici* was heavily parasitized on the roots of fig (*F. carica*) plants, and these plants showed signs of growth retardation and yellowing of the leaves (Mulvey, 1972; Mulvey et al., 1983). *Heterodera fici* is a harmful species on fig plants and heavy infestation has been reported to cause growth retardation and yellowing of leaves (Maqbool et al., 1987).

Di Vito & Sasanelli (1990) investigated the emergence of offspring and cysts of *H. fici* in a growth chamber at 24ºC for a period of 7 weeks in 2% natural and artificial incubation materials. Cysts were collected from commercial fig roots and incubated in batches of 100 each in ornamental or commercial fig roots, picrolonic acid, sodium metavanadate, zinc chloride, zinc sulfate or distilled water. They reported that more juvenile cysts appeared in commercial fig root juice (97%) compared to ornamental fig root juice (45%). They reported that the yield in sodium metavanadate was 64%, in zinc chloride 40% and in zinc sulfate 27%, and in picrolonic acid the yield was very low (5%).

## Conclusion

The objectives of this study are to understand the yield losses caused by the *Heterodera* genus in the Eastern Black Sea region and to focus on reducing these losses, especially in kale cultivation. Consequently, there is an imperative for further investigations to formulate effective strategies aimed at the control of *Heterodera* species, ultimately augmenting yield in cabbage fields. It is crucial to underscore that ongoing and future research endeavors directed towards the *Heterodera* genus remain imperative for the prevention of yield losses specifically in black cabbage cultivation.

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*Original article (Orijinal araştırma)*

# The effect of sublethal doses of flupyradifurone on the life table and esterase enzyme of *Myzus persicae* (Sulzer, 1776) (Hemiptera: Aphididae)[1]

Flupyradifurone'nun subletal dozlarının *Myzus persicae* (Sulzer, 1776) (Hemiptera: Aphididae)'nin yaşam çizelgesi ve esteraz enzimi üzerine etkisi

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**Ali Kemal BİRGÜCÜ[2]** **Sibel YORULMAZ[2]**

## Abstract

The aim of this study was to determine the effect of two different sublethal doses ($LC_{10}$ and $LC_{30}$) of flupyradifurone on the life table and esterase enzyme of *Myzus persicae* (Sulzer, 1776) (Hemiptera: Aphididae). The experiments were conducted in 2022 in Isparta University of Applied Sciences laboratory and climate rooms in 2022 as 1 control + 2 sublethal doses. For the life table, control, $LC_{10}$ and $LC_{30}$ doses were established as 30, 25 and 30 replications, respectively. Female and total lifespan of *M. persicae* adults exposed to $LC_{10}$ concentrations of flupyradifurone were significantly shortened. Daily and total numbers of the offsprings decreased at both $LC_{10}$ and $LC_{30}$ concentrations. Furthermore, these negative effects on the aphid were revealed as a lower intrinsic rate of increase ($r$), net reproductive rate ($R_0$), finite rate of increase ($\lambda$) and fecundity ($F$). Based on the obtained data, flupyradifurone seems to suppress the population growth of *M. persicae*. It was determined that esterase enzyme activity involved in pesticide detoxification did not change in populations exposed to two different sublethal doses of flupyradifurone and unexposed (control). It is thought that this study facilitates the understanding of the lethal and sublethal effects of flupyradifurone on aphid performance.

**Keywords:** Aphid, detoxification, insecticide, life table, sublethal effect

## Öz

Bu çalışmanın amacı, flupyradifurone etken maddesinin iki farklı subletal dozunun ($LC_{10}$ ve $LC_{30}$) *Myzus persicae* (Sulzer, 1776) (Hemiptera: Aphididae)'nin yaşam çizelgesi ve esteraz enzimi üzerine etkisini belirlemektir. Denemeler, 2022 yılında Isparta Uygulamalı Bilimler Üniversitesi laboratuvar ve iklim odalarında 1 kontrol+ 2 sublethal doz olacak şekilde yürütülmüştür. Yaşam çizelgesi için kontrol, $LC_{10}$ ve $LC_{30}$ dozları sırasıyla 30, 25 ve 30 tekerrür olarak kurulmuştur. Flupyradifurone'nun $LC_{10}$ konsantrasyonuna maruz kalan *M. persicae* erginlerinin dişi ömrü ve toplam yaşam süreleri önemli ölçüde kısalmıştır. Günlük ve toplam yavru sayıları hem $LC_{10}$ hem de $LC_{30}$ konsantrasyonlarında azalmıştır. Ayrıca yaprakbiti üzerindeki bu olumsuz etkiler daha düşük bir kalıtsal üreme yeteneği ($r$), net üreme gücü ($R_0$), içsel artış oranı ($\lambda$) ve üreme oranları ($F$) olarak ortaya çıkmıştır. Elde edilen verilere göre, flupyradifurone'nun *M. persicae*'nin popülasyon büyümesini baskıladığı görülmektedir. Pestisit detoksifikasyonunda rol alan esteraz enzim aktivitesinin flupyradifurone'nun iki farklı subletal dozları uygulanmış ve uygulanmamış (kontrol) popülasyonlarında değişmediği belirlenmiştir. Çalışmanın, flupyradifurone'nun yaprakbiti performansı üzerindeki letal ve subletal etkilerinin anlaşılmasını kolaylaştırdığı düşünülmektedir.

**Anahtar sözcükler:** Yaprakbiti, detoksifikasyon, insektisit, yaşam çizelgesi, subletal etki

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## Introduction

Aphids belong to the superfamily Aphidoidea and have a very high number of species (Erol et al., 2018). It is known that there are approximately 5000 species belonging to 510 genera in the world (Blackman & Eastop, 2023). In Turkey, 532 species belonging to approximately 142 genera have been identified (Şenol et al., 2015). The green peach aphid, *Myzus persicae* (Sulzer, 1776) (Hemiptera: Aphididae), is the most economically important aphid pest in the world due to its high host diversity, the damage mechanism it causes to the plant, its life cycle, its ability to spread rapidly, its vectoring of virus diseases and its ability to easily develop resistance to insecticides (Foster et al., 2000; van Emden, 2007; Boukhris-Bouhachem et al., 2017). *Myzus persicae* causes damage by sucking the sap of the plant during the whole development period of the plant. This pest, which has a very wide distribution area in the world, causes damage on a variety of plants in Türkiye. Under ideal conditions, it can continue its activity and reproduction in every month of the year (Lodos, 1986).

Acute toxicity testing of pesticides to insects is largely done by lethal dose or concentration determination studies. Median lethal dose ($LD_{50}$) or median lethal concentration ($LC_{50}$) is used to determine the effects of pesticides on both pests and natural enemies. These values are parameters used to compare the effects of different active substances or formulations on the test organism. In addition to the direct lethal effects of pesticides, it is also important to determine the effects of low pesticide concentrations on the physiology and behavior of insects (Desneux et al., 2007). Sublethal effects can be defined as physiological, demographic or behavioral effects on individuals or populations that survive exposure to lethal or sublethal doses or concentrations of a toxicant (De França et al., 2017). Sublethal dose means non-lethal; below lethal dose. Sublethal effects in insects can occur in the form of changes in lifespan, development time, population growth, egg production, sex ratios and behavior, deformations, search for food and reproductive sites, shortening of feeding and reproductive time (Lee, 2000). Therefore, the effects of sublethal doses and concentrations on insect physiology, behavior, demographic parameters and natural enemies are crucial in the selection of insecticides for use in integrated pest control programs (De França et al., 2017). It has been reported that low doses (sublethal) of pesticides have stimulatory effects on pests, while higher doses have inhibitory or toxic effects on pests (Calabrese & Baldwin, 2003). Luckey used the term "hormoligosis", which comes from the Greek words "hormo" (excite) and "oligo" (in small quantities), to describe the mild stimulating effects of toxic or non-toxic stress effects on an organism under suitable conditions, such as pesticides, temperature, light, etc. (Luckey, 1968; Cohen, 2006). In entomology, the term hormoligosis is known as sublethal doses of a pesticide on pest or natural enemy species to stimulate fertility or egg production.

Flupyradifurone is the first member of the new class of butanolide insecticides grouped as 4D according to the IRAC classification (Colares et al., 2017). Flupyradifurone can provide rapid and systemic protection with xylem mobility (Barbosa et al., 2017). By reversibly binding to post-synaptic nicotinic acetylcholine receptors (nAChRs), it mimics acetylcholine in the nervous system of insects by keeping them open and eventually causing uncontrolled axonal excitation (Nauen et al., 2015; Colares et al., 2017). Although flupyradifurone targets the nAChR, it differs from other nAChR agonists based on structure-activity relationships (Jeschke et al., 2015). nAChR has been an insecticide molecular target site of increasing importance for many years, playing a central role in mediating fast excitatory synaptic transmission in the insect central nervous system (CNS). The active ingredient, flupyradifurone is a newly licensed product for the control against whitefly in Türkiye. Although this active ingredient is not licensed against *M. persicae*, it is thought to have an effect on aphids somehow in the same environment due to its extensive use in whitefly control, especially in greenhouse production.

Esterases are a large and heterogeneous group of enzymes that metabolize internal and external substrates with ester bonds. Also, esterases; It also plays a role in processes such as insect development,

behavior (by breaking down odors, etc.), reproduction, digestion and pesticide detoxification (Montella et al., 2012). Many groups of insecticides, such as organic phosphorus, benzoylphenyl ureases, organic chlorinates, carbamates, pyrethroids and juvenile hormone analogues, are susceptible to esterase hydrolysis. Although some esterases involved in insecticide resistance have limited catalytic effect, they can be produced in large numbers and bind to the insecticide before reaching their target, reducing availability (Field et al., 1988). This process is known as “sequestration” (Bass & Field 2011).

In this study, the effect of two different sublethal doses ($LC_{10}$ and $LC_{30}$) of flupyradifurone on *M. persicae* was investigated. The effects of these doses on average lifespan, total number of offsprings, pre-reproductive, reproductive, and post-reproductive periods were calculated for female *M. persicae* individuals using life tables. Additionally, the effects of two different sublethal doses of flupyradifurone on the esterase enzyme, which plays an important role in pesticide detoxification, were also examined.

## Materials and Methods

### Aphid culture

*Myzus persicae* population used in the study was obtained from Ankara Pest Control Research Institute in 2018. To date, the aphid population is produced in the climate rooms without exposure to any pesticide application. Radish, *Raphanus sativus* L. (Brassicales: Brassicaceae) was used as the host plant because it is easy to grow in climate rooms. *Myzus persicae* population was grown on clean radish plants in water-filled tubs covered with tulle and in climate rooms with 26±1°C temperature, 60-65% humidity and 16:8 (L/D) hour photoperiod conditions.

### Insecticide

It is the first member of the new class of butenolide insecticides classified by IRAC as flupyradifurone 4D. Sivanto SL 200 (Bayer), a commercial preparation with the active ingredient flupyradifurone, was used in the study.

### Determination of LC values

The study was conducted in Isparta University of Applied Sciences, Faculty of Agriculture, Acarology Laboratory between 2022-2023. The leaf dipping method was used to determine LC values for the flupyradifurone. To determine the LC against flupyradifurone in the aphid population, 1 control + 6 doses (100, 50, 25, 12.5, 6.25, 3.125 µl/100ml) were used, with each dose consisting of 3 replicates. In each replicate, 25±5 adult aphid individuals were used. Flupyradifurone doses were prepared using the 50% dilution method. Only pure water was applied to the control group. First of all, 1% agar powder was mixed with distilled water, boiled and allowed to cool. After cooling, the agar medium was poured into a 9 cm petri dish at a height of approximately 4 mm and the medium was waited for it to freeze. The main purpose of using agar medium in the study is to ensure that the trial leaf meets its moisture need from the environment. After the radish leaves were cut into 3 cm disk shapes, they were dipped into the doses for 10 seconds and the leaves were placed in petri dishes and *M. persicae* adults were transferred onto them with the help of a binocular. Petri dishes were placed in climate rooms with 26±1°C temperature, 60-65% humidity and 16:8 h (L/R) photoperiodic conditions. Dead and alive counts were made at the end of the 72nd hour. The results obtained from the dead alive counts were analyzed and evaluated with the POLO computer package program (LeOra Software, 1994). As a result of the study, in addition to the $LC_{50}$ value for flupyradifurone, $LC_{10}$ and $LC_{30}$ values used as sublethal doses were also determined.

### Sublethal dose applications and determination of biological parameters

Generally, pests are exposed to low concentrations of pesticides due to degradation, etc. in the field (Desneux et al., 2007, Biondi et al., 2012). This leads to various physiological and behavioral sublethal effects

in individuals (He et al., 2013, Chen et al., 2016, Zeng et al., 2016). Therefore, in this study, $LC_{10}$ and $LC_{30}$ sublethal doses were used to determine the effects of flupyradifurone on biological parameters of *M. persicae*. The experiments were established as 1 control + 2 sublethal doses ($LC_{10}$ and $LC_{30}$). In the life table, 30 repetitions were established in the control and $LC_{30}$ groups and 25 repetitions in the $LC_{10}$ group. For each replicate, one *M. persicae* female was transferred to the radish leaf. It was checked after 1 day and the mother and other aphids were removed so that 1 newborn aphid was left in each replication. Thus, individuals of the same age were used for each dose and control group throughout the entire experiment. After the mother and other aphids were removed flupyradifurone sublethal doses were prepared and 2 mL insecticide concentration was applied into the petri dish under 1 atm pressure with the help of spray tower (Burkard Manufacturing Co Ltd). Only pure water was applied to the control group. All replicates were checked daily and the reproductive periods of aphid individuals that reached the adult stage and total number of offspring daily were observed. The observations in the experiment continued until the repetitions in all applications died.

**Life table studies**

In order to determine the effects of flupyradifurone sublethal doses on the life cycle of *M. persicae*, parameters were calculated according to Age-stage, two-sex life table (Chi et al., 2020, 2023). The parameters and formulas for the calculated life tables are as follows.

Survival rate depending on age and period: $s_{xj}$

Age-specific survival rate: $l_x$

Age-specific fecundity: $m_x$ (female/female/day)

Net reproductive rate, $R_0$ (nymphs /individual): $\sum_{x=0}^{\infty} lxmx$

Intrinsic rate of increase: $r$ (day $^{-1}$): $\sum_{x=0}^{\infty} e^{-r(x+1)}\, lxmx = 1$

Fecundity: $F$ (nymphs/female): $\frac{\sum_{x=1}^{Nf} Ex}{Nf}$

Finite rate of increase ($\lambda$, (day $^{-1}$)): $\lambda = e^{r}$

Mean generation time ($T$,days): $T = \frac{\ln R_0}{r}$

Population-doubling time ($T_2$,day): $T_2 = \frac{\ln 2}{r}$

To compute the differences and SEs, 100,000 bootstrap replicates were performed (Efron & Tibshirani, 1993; Huang & Chi, 2012; Akca et al., 2015; Akköprü et al., 2015). At a 5% significant level, the paired bootstrap test was used to evaluate the differences in demographic parameters between the flupyradifurone sublethal doses - exposed groups and the control group based on the confidence interval of the difference (Wei et al., 2020).

**Esterase activity**

This study was conducted to determine whether sublethal doses of flupyradifurone caused changes in the esterase enzyme activity of *M. persicae*. First of all, $LC_{10}$ and $LC_{30}$ doses of flupyradifurone were applied to *M. persicae* individuals and two different populations were created. Esterase enzyme activities were determined in three different populations of *M. persicae*. The method developed by (Devonshire, 1975) to determine total esterase activity was rearranged by (Devonshire et al., 1992) by adapting it to a 96-well microplate. 50 µL of 20 mM phosphate buffer (pH: 7.0) containing 0.1% Triton X-100 (Boehringer Mannheim, especially purified) was placed in each well of the microplate with a multichannel micropipette. Adult aphids belonging to the populations to be tested were transferred to each well using a brush. Aphids were homogenized using a multiple homogenizer and 15 minutes were waited for the tissues to dissolve thoroughly.

30 mg of Fast Blue RR Salt was weighed and completed with phosphate buffer (pH: 6.0) to 50 mL, and after filtering through Whatman filter 1, 500 L of 100 mM 1-naphthyl acetate solution was added. 200 µL of the prepared dye-substrate solution was taken and placed into all wells with a multi-channel micropipette. "Optical density" (O.D.) values were obtained by making "kinetic" readings on a Molecular Devices brand microplate reader at 450 nm wavelength with 10-second intervals for a total of 5 minutes.

**Data analysis**

The logarithmic-probit model was used to calculate the $LC_{10}$, $LC_{30}$, $LC_{50}$ values, slopes and their 95% confidence limits of flupyradifurone against *M. persicae* using POLO computer program (LeOra Software Inc., Berkely, CA). Non-overlapping 95% confidence limits were used to determine statistical differences between populations. The esterase enzyme values in *M. persicae* individuals exposed to $LC_{10}$ and $LC_{30}$ sublethal doses of flupyradifurone and individuals in the control group were statistically analyzed by one-way analysis of variance with Tukey's post hoc test with significance set at $p < 0.05$ (IBM, SPSS Statistics, version 22).

## Results and Discussion

$LC_{10}$, $LC_{30}$ and $LC_{50}$ values against flupyradifurone in *M. persicae* are given in Table 1.

Table 1. LC values against flupyradifurone in *Myzus persicae*

| Insecticide | $n^a$ | $x^2$ /df/ $P^b$ | Slope±SE | $LC_{10}$ (mga.i. $L^{-1}$) (95% $CL^c$) | $LC_{30}$ (mga.i. $L^{-1}$) (95% $CL^c$) | $LC_{50}$ (mga.i. $L^{-1}$) (95% $CL^c$) |
|---|---|---|---|---|---|---|
| *Flupyradifurone* | 578 | 0.535/4/0.179 | 1.497±0.142 | 1.219 (0.661-1.882) | 3.908 (2.678-5.186) | 8.756 (6.810-10.851) |

a: number of individuals used in the experiment; b: chi-square/degrees of freedom /p-value; c: confidence limits.

Developmental stages and life span of *M. persicae* individuals exposed to $LC_{10}$ and $LC_{30}$ doses of flupyradifurone are given in Table 2.

Table 2. Development stages and life span of *Myzus persicae* individuals exposed to $LC_{10}$ and $LC_{30}$ sublethal doses of Flupyradifurone (Days)*

| Biological Period | Type | n | Mean | |
|---|---|---|---|---|
| I. Nymph Stage | *Control* | 30 | 1.80±0.07 | a |
| | *$LC_{10}$* | 25 | 1.68±0.16 | a |
| | *$LC_{30}$* | 30 | 1.73±0.10 | a |
| II. Nymph Stage | *Control* | 30 | 1.66±0.10 | a |
| | *$LC_{10}$* | 25 | 1.36±0.12 | a |
| | *$LC_{30}$* | 30 | 1.56±0.14 | a |
| III. Nymph Stage | *Control* | 30 | 1.50±0.09 | a |
| | *$LC_{10}$* | 25 | 1.44±0.11 | a |
| | *$LC_{30}$* | 30 | 1.76±0.11 | a |
| IV. Nymph Stage | *Control* | 30 | 1.63±0.11 | a |
| | *$LC_{10}$* | 25 | 2.04±0.15 | a |
| | *$LC_{30}$* | 30 | 1.76±0.13 | a |
| Development time (born to from adult) | *Control* | 30 | 6.60±0.09 | a |
| | *$LC_{10}$* | 25 | 6.52±0.10 | a |
| | *$LC_{30}$* | 30 | 6.83±0.12 | a |
| Life span of Adult Female (to adult to died) | *Control* | 30 | 17.26±0.26 | a |
| | *$LC_{10}$* | 25 | 14.80±0.42 | b |
| | *$LC_{30}$* | 30 | 16.46±0.42 | a |
| Total Life Time (to born from died) | *Control* | 30 | 23.86±0.26 | a |
| | *$LC_{10}$* | 25 | 21.32±0.47 | b |
| | *$LC_{30}$* | 30 | 23.30±0.42 | a |

* The difference between the means (± standard errors) marked with the same letter for each parameter is statistically insignificant. Standard errors were estimated by using the bootstrap technique with 100,000 resampling. Difference was compared using the paired bootstrap test ($p < 0.05$).

The first nymphal stage of *M. persicae* individuals exposed to sublethal doses and individuals in the control group varied between 1.68 days and 1.80 days, and statistically they were all in the same group. Similarly, in the second, third, fourth nymphal stages and development stages, all of them were statistically in the same group. When the adult female life span data were analyzed, it was seen that the $LC_{10}$ dose was in a different statistical group compared to the control and $LC_{30}$ values with 14.80 days. Also, total life span was found to be in a different statistical group compared to the control and $LC_{30}$ values with 21.32 days at $LC_{10}$ sublethal dose and the difference between them was found to be significant.

Prereproductive, reproductive, postreproductive period (days), daily and total offspring numbers of *M.persicae* individuals exposed to $LC_{10}$ and $LC_{30}$ sublethal doses of flupyradifurone and in the control group are given in Table 3. The prereproductive period of *M. persicae* individuals varied between 1.20 days and 3.06 days and each of them were statistically in separate groups. Reproductive periods were determined as 13.66, 12.08 and 14.00 days for control, $LC_{10}$ and $LC_{30}$, respectively. In postreproductive periods, all groups were statistically in the same group. The daily and total number of offsprings of individuals exposed to $LC_{10}$ and $LC_{30}$ doses were in the same statistical group, while the control group was in a different class in both cases. Daily and total offspring numbers were highest in the control groups and the difference was statistically significant compared to $LC_{10}$ and $LC_{30}$ doses of flupyradifurone (Table 3).

Table 3. Prereproductive, reproductive, postreproductive periods (Days), daily and total offspring numbers of *Myzus persicae* individuals exposed to $LC_{10}$ and $LC_{30}$ sublethal doses of flupyradifurone*

| Parameter | Type | n | Mean | |
|---|---|---|---|---|
| Prereproductive Period | *Control* | 30 | 3.06±0.16 | a |
| | $LC_{10}$ | 25 | 1.72±0.14 | b |
| | $LC_{30}$ | 30 | 1.20±0.13 | c |
| Reproductive Period | *Control* | 30 | 13.66±0.35 | ab |
| | $LC_{10}$ | 25 | 12.08±0.73 | b |
| | $LC_{30}$ | 30 | 14.00±0.56 | a |
| Postreproductive Period | *Control* | 30 | 0.53±0.15 | a |
| | $LC_{10}$ | 25 | 1.00±0.33 | a |
| | $LC_{30}$ | 30 | 1.26±0.29 | a |
| Daily number of offspring per Day | *Control* | 30 | 2.45±0.12 | a |
| | $LC_{10}$ | 25 | 1.18±0.10 | b |
| | $LC_{30}$ | 30 | 1.34±0.11 | b |
| Total number of offspring | *Control* | 30 | 42.40±2.02 | a |
| | $LC_{10}$ | 25 | 17.84±1.65 | b |
| | $LC_{30}$ | 30 | 22.53±2.07 | b |

* The difference between the means (± standard errors) marked with the same letter for each parameter is statistically insignificant. Standard errors were estimated by using the bootstrap technique with 100,000 resampling. Difference was compared using the paired bootstrap test ($p < 0.05$).

Life table parameters of *M. persicae* exposed to $LC_{10}$ and $LC_{30}$ doses of flupyradifurone and control *M. persicae* individuals are given in Table 4. The differences between the intrinsic rate of increase ($r$), net reproductive rate ($R_0$) and finite rate of increase $\lambda$ ($day^{-1}$) values of both sublethal doses-exposed and control *M. persicae* individuals separately were statistically significant. The longest mean generation time ($T$) was 15.51 days in *M. persicae* individuals in the control group and the shortest was 13.89 days in individuals exposed to $LC_{30}$ sublethal dose (Table 4). The highest fecundity was again observed in the control group. The shortest population doubling time was observed in the control group with 2.87 days and the longest with 3.42 days in individuals exposed to sublethal dose of $LC_{30}$ (Table 4).

Table 4. Life table parameters of *Myzus persicae* individuals exposed to $LC_{10}$ and $LC_{30}$ sublethal doses of flupyradifurone*

| Parameter | Type | n | Mean | |
|---|---|---|---|---|
| Intrinsic rate of increase, $r$ ($day^{-1}$) | Control | 30 | 0.2415±0.0002 | a |
| | $LC_{10}$ | 25 | 0.2020±0.0001 | c |
| | $LC_{30}$ | 30 | 0.2230±0.0002 | b |
| Net reproductive rate, $R_0$ (offspring/individual) | Control | 30 | 42.39±0.25 | a |
| | $LC_{10}$ | 25 | 17.84±0.11 | c |
| | $LC_{30}$ | 30 | 22.53±0.11 | b |
| Finite rate of increase, $\lambda$ ($day^{-1}$) | Control | 30 | 1.2729±0.0009 | a |
| | $LC_{10}$ | 25 | 1.2242±0.0003 | c |
| | $LC_{30}$ | 30 | 1.2495±0.0009 | b |
| Fecundity, $F$ (nymphs/female) | Control | 30 | 42.39±0.31 | a |
| | $LC_{10}$ | 25 | 17.84±0.12 | c |
| | $LC_{30}$ | 30 | 22.53±0.12 | b |
| Mean generation time, $T$ (day) | Control | 30 | 15.51±0.20 | a |
| | $LC_{10}$ | 25 | 14.22±0.15 | b |
| | $LC_{30}$ | 30 | 13.89±0.19 | b |
| Theoretical population-doubling time, DT (day) | Control | 30 | 2.87 | |
| | $LC_{10}$ | 25 | 3.42 | |
| | $LC_{30}$ | 30 | 3.09 | |

* The difference between the means (± standard errors) marked with the same letter for each parameter is statistically insignificant. Standard errors were estimated by using the bootstrap technique with 100,000 resampling. Difference was compared using the paired bootstrap test ($p < 0.05$).

Age and stage dependent survival rate ($s_{xj}$), age-specific survival rates ($l_x$) and fertility rates ($m_x$) curves of *M. persicae* individuals exposed to $LC_{10}$ and $LC_{30}$ sublethal doses of flupyradifurone and individuals in the control group are given in Figure 1-2.

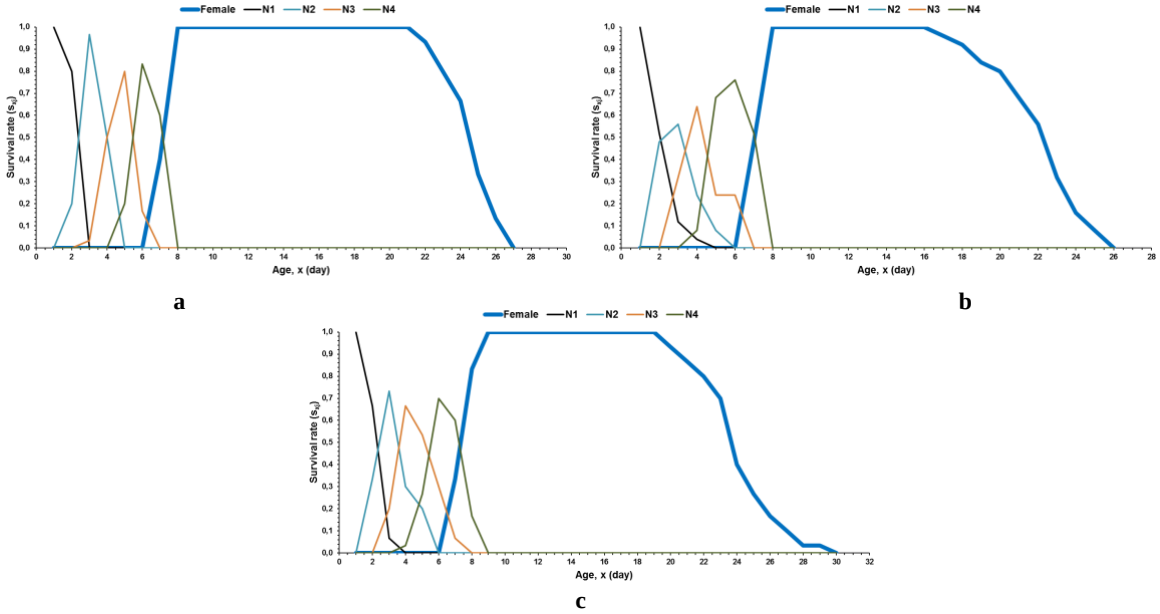


Figure 1. Age- and stage-dependent survival rates ($s_{xj}$) of *Myzus persicae* individuals (a: control, b: $LC_{10}$, c: $LC_{30}$) (Female: female, N1: 1st instar nymph, N2: 2nd instar nymph, N3: 3rd instar nymph, N4: 4th instar nymph).

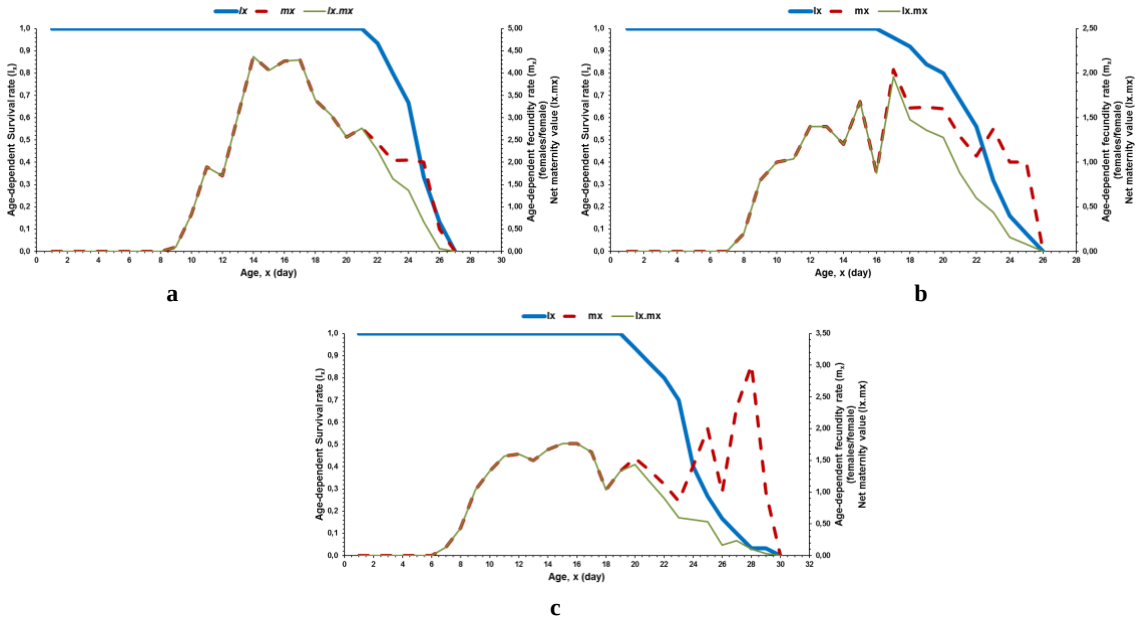


Figure 2. Age-specific survival rates ($l_x$) and fertility rates ($m_x$) of *Myzus persicae* individuals (a: control, b: $LC_{10}$, c: $LC_{30}$).

Esterase enzyme values in individuals exposed to $LC_{10}$ and $LC_{30}$ doses of flupyradifurone and in the control group were found to be 1.80, 2.05 and 1.55 mOD $min^{-1}$ $mg^{-1}$ protein, respectively (Table 5). According to these data, it was observed that esterase enzyme activity did not change with control, $LC_{10}$ and $LC_{30}$ sublethal doses and all of them were in the same statistical group ($p<0.05$).

Table 5. Esterase enzyme values in individuals exposed to $LC_{10}$, $LC_{30}$ doses of flupyradifurone and in the control group

| Population | n* | Total Esterase mOD/min/mg protein±SE | | R/S** |
|---|---|---|---|---|
| Control | 4 | 1.55± 0.28 | a*** | |
| $LC_{10}$ | 4 | 1.80± 0.65 | a | 1.16 |
| $LC_{30}$ | 4 | 2.05± 0.45 | a | 1.32 |

* Number of repetition;

** Enzyme activity of the tested population/ enzyme activity of the control;

*** Letters in each column show statistical differences according to Tukey test (F (2, 30): 13.07, p <0.05) for total esterase.

The extensive use of various insecticides in the control of aphids has led to resistance to many insecticides with different modes of action (Wei et al., 2017; Fouad et al., 2022). The development of new alternative insecticides such as flupyradifurone is a great necessity. The high toxicity of flupyradifurone has been determined for several sap-feeding pests, including *M. persicae, Aphis gossypii* Glover, 1877 (Hemiptera: Aphididae) and *Bemisia tabaci* (Gennadius,1889) (Hemiptera: Aleyrodidae) (Nauen et al., 2015; Tang et al., 2019). In this study, since the active ingredient flupyradifurone is not licensed in Türkiye for *M. persicae*, $LC_{50}$ determination studies were first carried out against this substance in aphids and it was found to be quite toxic as 8.756 mg/L. Similarly, in the study conducted by (Tang et al., 2019), the $LC_{50}$ analysis result of *M. persicae* in adult individuals at the end of 48 hours was 8.491 mg/L, indicating that it is very toxic. Sial et al. (2018), *M. persicae* individuals were exposed to deltamethrin and lambda cyhalothrin for 48 hours and as a result, $LC_{50}$ values were found to be 381 mg $L^{-1}$ and 1010 mg $L^{-1}$, respectively.

In addition to the lethal effects of insecticides, insect populations are often exposed to low concentrations of insecticides in the field due to the variable distribution and continuous degradation of insecticides (Bonmatin et al., 2005; Desneux et al., 2005). Therefore, sublethal effects of insecticides can increase or decrease insect populations (Desneux et al., 2007). Evaluation of development, survival, reproduction and

behavioral response is important for an overall understanding of the effects of flupyradifurone for IPM. Investigating different toxicity parameters, such as sublethal effects, is essential to delay the development of resistance (Liang et al., 2019). Sublethal effects of flupyradifurone have been reported in several pests such as *B. tabaci, A. gossypii, M. persicae, Diaphorina citri* Kuwayama, 1908 (Hemiptera: Liviidae) and *Lygus hesperus* (Knight, 1917) (Hemiptera: Miridae) (Smith & Giurcanu, 2013; Joseph & Bolda, 2016; Chen et al., 2017; Liang et al., 2019; Tang et al., 2019). In the study conducted for this purpose, the sublethal effects of $LC_{10}$ and $LC_{30}$ sublethal doses of flupyradifurone on life table characteristics in *M. persicae* were evaluated. Effects that reduce fecundity, longevity, and alter behavior have been observed in many pests, often after exposure to sublethal insecticide concentrations (Desneux et al., 2007; Han et al., 2012; Guo et al., 2013; Zeng et al., 2016; Tang et al., 2019). For example, sublethal concentrations of endosulfan significantly decreased the fecundity of *Apolygus lucorum* Meyer-Dür, 1843 (Hemiptera: Miridae) (Liu et al., 2008), while sublethal doses of buprofezin shortened the adult life span of *B. tabaci (*Sohrabi et al., 2011). In this study, female longevity and total life span of *M. persicae* adults were significantly shortened when exposed to leaf discs treated with a sublethal $LC_{10}$ concentration of flupyradifurone. However, no significant effect was found on nymph stage periods and development time. Daily and total offspring numbers decreased at both $LC_{10}$ and $LC_{30}$ concentrations. Moreover, these negative effects on the aphid were manifested as a lower intrinsic rate of increase ($r$), net reproductive ability ($R_0$), finite rate of increase ($\lambda$) and fecundity ($F$). This suggests that flupyradifurone suppresses population growth of *M. persicae*. Similarly, sublethal effects of insecticides on population growth have been reported in many pests such as *A. gossypii, A. lucorum*, *B. tabaci, Brevicoryne brassicae* (L., 1758) (Hemiptera: Aphididae), *Bradysia odoriphaga* Yang & Zhang, 1985 (Diptera: Sciaridae), *M. persicae* and *Lipaphis erysimi* (Kaltenbach, 1843) (Hemiptera: Aphididae) (Devine et al., 1996; Lashkari et al., 2007; Wang et al., 2008; Tan et al., 2012; Chen et al., 2016; Liang et al., 2019; Hosseini et al., 2020). Under laboratory conditions, as a result of sublethal doses of rotenone and abamectin application to the green peach aphid, its reproduction decreased by 44.29% and 54.01%, respectively; with fenvalerate application, the average daily reproduction per female decreased significantly compared to the control (Wang et al., 2008). In the study conducted by Wang et al. (2008), it was reported that the sublethal concentration ($LC_{25}$) of six different insecticides (Imidacloprid, Rotenone, Fenvalerate, Abamectin, Pirimicarb, Azadirachtin) did not have a significant effect on the reproduction of *M. persicae*. Another study showed that exposure to low concentrations of afidopyropen significantly decreased the lifespan and fecundity of *M. persicae*, and that the life parameters of the F1 progeny were also affected (Liu et al., 2022). The findings provide a basis for further investigation of the sublethal effects of afidopyropen and other insecticides on aphids (Liu et al., 2022).

Many studies show that one of the main reasons for insect resistance to pesticides is the increased detoxification capabilities of enzymes associated with pesticide metabolism (Cai et al., 2021). The role of acetylcholinesterase, carboxylesterase or other esterase enzymes in insecticide resistance in aphids has been studied (Gao et al., 1992; Song et al., 1995). Metabolic enzymes reported to provide resistance in *M. persicae* include esterase E4 (or its Mediterranean variant, FE4), which confers broad-spectrum resistance to organophosphates, carbamates, and pyrethroids, and cytochrome P450 CYP6CY3, which imparts resistance to neonicotinoids (Bass et al., 2014). In the study, it was determined that the difference between the control and the populations exposed to $LC_{10}$ and $LC_{30}$ sublethal doses was not statistically significant according to the activity of the esterase enzyme.

It is thought that this study facilitates the understanding of the lethal and sublethal effects of flupyradifurone on aphid performance. However, additional studies are needed to fully evaluate the sublethal effects of this new insecticide on *M. persicae* under field conditions. In addition, the effects on natural enemies should be investigated in order to preserve the natural balance.

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*Original article (Orijinal araştırma)*

# Faunistic contributions and zoogeographical and ecological evaluations on species belonging to the genus *Philonthus* Stephens, 1829 (Coleoptera: Staphylinidae: Staphylininae: Staphylinini: Philonthiina) from the Aegean Region (Türkiye)[1]

Ege Bölgesi (Türkiye)'nden *Philonthus* Stephens, 1829 (Coleoptera: Staphylinidae: Staphylininae: Staphylinini: Philonthiina) cinsine bağlı türler üzerine faunistik katkılar ile zoocoğrafik ve ekolojik değerlendirmeler

**Osman SERT[2*] Senem ÖZDEMİR TÜRKMEN[2] Burcu ŞABANOĞLU ŞİMŞEK[2]**

## Abstract

*Philonthus* Stephens, 1829 is the most speciose genus of the tribe Staphylini in the world. In this present study, faunistic contributions to the *Philonthus* fauna of Türkiye were made along with additional records. A total of 30 species were recorded from the examined material which was composed of specimens collected from the Aegean Region between 2019-2022. Among them, 13 species are new records for the region and new province records for 15 species are also provided. Additionally, first detailed locality records are provided for the widely distributed *Philonthus viridipennis* Fauvel, 1875. Besides several ecological properties, general distribution of species in Türkiye and other zoogeographical regions are presented and discussed. Previous records from the study region are given and evaluated with our results, zoogeographic status of species is discussed. As a result of the study, composition of the collected species represent regional characters, as they mostly belong to the European and Asian fauna.

**Keywords:** Fauna, new records, *Philonthus*, Staphylininae, Staphylinini, Türkiye

## Öz

*Philonthus* Stephens, 1829, Staphylinini tribüsünün dünyada türce en zengin cinsidir. Bu çalışmada, Türkiye *Philonthus* faunasına yeni kayıtlar aracılığı ile faunistik katkılar yapılmıştır. 2019-2022 yılları arasında Ege Bölgesinden toplanan örneklerden oluşan inceleme materyalinden, toplamda 30 tür kaydedilmiştir. Bunların arasından 13 tür bölge için yeni kayıttır ve 15 tür için de yeni il kayıtları sağlanmıştır. Ayrıca, geniş bir dağıla sahip olan *Philonthus viridipennis* Fauvel, 1875 türü için ilk ayrıntılı dağılım kayıtları verilmiştir. Birkaç ekolojik özelliğin yanısıra, türlerin Türkiye ve diğer zoocoğrafik bölgelerdeki genel dağılımları sunulmuş ve tartışılmıştır. Bölgeden daha önceki kayıtlar verilerek kendi sonuçlarımızla değerlendirilmiş, türlerin zoocoğrafik durumları tartışılmıştır. Çalışmanın sonucu olarak, toplanan türlerin kompozisyonu, türlerin çoğunlukla Avrupa ve Asya faunasına ait olmasından ötürü, bölgesel karakterleri yansıtmaktadır.

**Anahtar sözcükler:** Fauna, yeni kayıtlar, *Philonthus*, Staphylininae, Staphylinini, Türkiye

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## Introduction

The subfamily Staphylininae, including the tribe Stapylinini, is the third largest subfamily of Staphylinidae comprising over 9.000 species in more than 400 genera worldwide (Newton, 2022). *Philonthus* Stephens, 1829, as the most speciose genus belonging to the tribe Staphylinini, has 1.333 species worldwide and 67 species/subspecies in Türkiye (Anlaş, 2009; Schülke & Smetana, 2015; Fırat & Sert, 2016a, b; Özgen et al., 2016; Özdemir, 2021). Staphylinines are one of the most widely distributed animals on earth and found in various kinds of humid habitats (Demirsoy, 2003; Frank & Thomas, 2010). As most of the staphylinines, *Philonthus* species are defined predators and coprophiles, and adults are generally found on riverbanks, under leaf debris and in dung and carrion/carcasses (Coiffait, 1972).

The study area, Aegean Region comprises Türkiye's fifth biggest region, covering 10.1% of the country's lands. It stretches along the shores of the Aegean Sea and neighbours the Marmara, Central Anatolian and Mediterranean Regions. Along the coastal part, mediterranean climate type is dominant and because of the depression plains, it reaches almost 100-150 km inland through shores. Further inland, a transitional state between mediterranean and continental climate is seen. Decreased precipitation on inner part relative to the coastal part shifts towards spring season. Therefore, summer drought is much less than coastal part (Atalay & Mortan, 2011).

## Materials and Methods

Material used in the study was collected from the Aegean Region of Türkiye, including the southern part of Balıkesir province (Figure 1), between April 2019 and October 2022. Specimens were collected by conventional collection methods using aspirator on dung and riverbanks-understones, sifting debris and sweeping herbaceous plants. Collected specimens are preserved in ethanol-acetic acid (%10) solution in order to keep them soft until examination. Coordinates were recorded by using GPS. Material is deposited in the Hacettepe University Zoology Museum (Ankara, Türkiye) (HUZOM). Identifications were done by using identification keys from Coiffait (1974) and Schillhammer (2011). For the examinations, Nikon SMZ-U and Euromex Nexius Zoom binocular stereomicroscopes were used. Catalogue of Löbl & Löbl (2015) were used for taxonomic classification and zoogeographical distributions. Species were organized in Table 1 as common species with Europe (E), common species with Asia (A), common species with North Africa (N), and Afrotropical (AFR), Nearctic (NAR), and Oriental (ORR) regions, and also cosmopolitan species (COS).

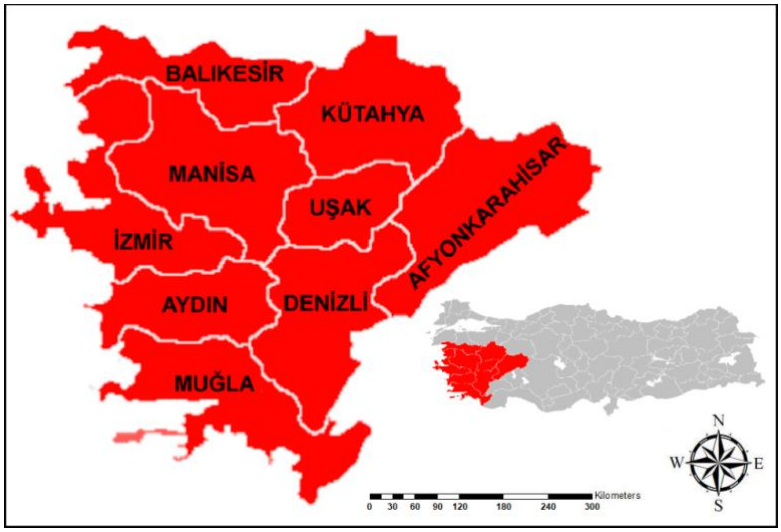


Figure 1. Map of research area (ArcMap 10.6.1).

## Results and Conclusion

A total of 30 species belonging to *Philonthus* were detected. Among all *Philonthus* species found in Türkiye, 24 species were previously recorded from the study region. In the present study, 13 species are detected as new for the region and together with these records, a total of 37 species are now distributed in Aegean region including the widely distributed *Philonthus viridipennis*. Ecological collecting data regarding number of specimens, collecting months, vertical distribution and collecting habitat-methods are given in Table 1 along with their zoogeographical distribution. Previous records from the study region are given in comparison with our results in Table 2.

**Tribe Staphylinini** Latreille, 1802

**Subtribe Philonthina** Kirby, 1837

**Genus *Philonthus*** Stephens, 1829

***Philonthus carbonarius*** (Gravenhorst, 1802)

Material examined. Afyonkarahisar: Sultandağı, 1138m., 1.V.2019, ♀, leg. S. Özdemir.

Distribution in Türkiye. Afyonkarahisar, Aksaray, Antalya, Ardahan, Bingöl, Bursa, Elazığ, Erzurum, Kahramanmaraş, Kars, Konya, Manisa (Bodemeyer, 1906; Anlaş & Rose, 2009; Kesdek et al., 2009; Özgen & Anlaş, 2010; Assing, 2013; Fırat & Sert, 2016a; Özgen et al., 2016; Daşdemir & Tozlu, 2022).

***Philonthus cognatus*** Stephens, 1832

Material examined. Afyonkarahisar: Sandıklı, 1483m., 21.X.2019, ♂, leg. Y. Turan; Kütahya: Central province, 1164m., 14.VI.2022, ♂, leg. S. Özdemir.

Distribution in Türkiye. Afyonkarahisar, Ankara, Ardahan, Artvin, Balıkesir (Kaz Mountain), Erzurum, Eskişehir, Gaziantep, Giresun, İzmir, Kars, Konya, Manisa, Mersin, Trabzon, Yozgat (Bodemeyer, 1900; Smetana, 1953; Fagel, 1963; Coiffait, 1978; Kesdek et al., 2009; Abacıgil et al., 2013; Çiftçi & Hasbenli, 2016; Fırat & Sert, 2016a; Özgen et al., 2016; Özdemir, 2021).

***Philonthus concinnus*** (Gravenhorst, 1802)

Material examined. Afyonkarahisar: Başmakçı, 1110m., 26.VII.2021, 3♀♀, ♂, leg. S. Özdemir, B. Şabanoğlu; Bayat, 1388m., 16.VII.2021, ♀, leg. B. Şabanoğlu; Bolvadin, 1282m., 19.VII.2021, 10♀♀, 5♂♂, leg. S. Özdemir, O. Sert; Bolvadin, 1175m., 19.VII.2021, ♂, leg. S. Özdemir; Çay, 1029m., 24.IV.2021, ♀, leg. M. Kabalak; Çay, 1920m., 17.VII.2021, ♀, ♂, leg. S. Özdemir, B. Şabanoğlu; Sultandağı, 1310m., 05.VII.2019, 4♀♀, 3♂♂, leg. S. Özdemir, O. Sert; Sultandağı, 1215m., 05.VII.2019, 3♀♀, ♂, leg. S. Özdemir, Y. Turan; Emirdağ, 1252m., 01.VI.2019, ♀, leg. S. Özdemir; Emirdağ, 1511m., 06.VII.2019, 5♀♀, ♂, leg. O. Sert, S. Özdemir; Emirdağ, 982m., 05.VI.2021, ♀, leg. M. Kabalak; İscehisar, 1360m., 19.VII.2021, ♀, leg. S. Özdemir; Sandıklı, 1528m., 18.VII.2021, 3♀♀, leg. S. Özdemir, B. Şabanoğlu; Sandıklı, 1654m., 18.VII.2021, ♀, leg. S. Özdemir; Sandıklı, 1428m., 18.VII.2021, ♀, leg. S. Özdemir; Aydın: Bozdoğan, 384m., 02.VI.2022, ♂, leg. O. Sert; Karacasu, 470m., 28.VI.2021, 2♀♀, leg. S. Özdemir, O. Özdil; Koçarlı, 750m., 19.V.2022, 2♂♂, leg. S. Özdemir, E C. Ceylan; Nazilli, 665m., 20.V.2022, ♀, leg. S. Özdemir; Nazilli, 434m., 20.V.2022, 8♀♀, 8♂♂, leg. S. Özdemir, O. Sert; Nazilli, 975m., 22.VII.2022, ♂, leg. S. Özdemir; Balıkesir: Burhaniye, 562m., 03.VII.2022, ♀, leg. S. Özdemir; Edremit, 1723m., 26.V.2022, ♀, ♂, leg. S. Özdemir, U. Özfuçucu; Denizli: Acıpayam, 1346m., 13.VI.2021, ♂, leg. O. Sert; Bekilli, 799m., 18.VII.2022, ♀, ♂, leg. S. Özdemir, O. Özdil; Çal, 1198m., 28.VII.2021, 2♀♀, leg. S. Özdemir, O. Özdil; Çal, 697m., 12.VI.2021, 6♀♀, leg. S. Özdemir, O. Özdil; Çardak, 1403m., 13.VI.2021, 2♀♀, leg. O. Sert, S. Özdemir; İzmir: Aliağa, 195m., 27.VI.2019, 3♂♂, leg. S. Özdemir, Y. Turan; Aliağa, 120m., 27.VI.2019, ♀, leg. S. Özdemir; Bergama, 473m., 23.V.2022, 3♀♀, 3♂♂, leg. S. Özdemir, B. Şabanoğlu; Bergama,

605m., 23.V.2022, ♀, leg. S. Özdemir; Kemalpaşa, 879m., 29.VI.2019, ♀, leg. S. Özdemir; Tire, 358m., 12.V.2019, 2♀♀, 2♂♂, leg. S. Özdemir, Y. Turan; Tire, 952m., 12.V.2019, ♀, leg. B. Şabanoğlu; Kütahya: Central province, 1164m., 14.VI.2022, 2♀♀, leg. S. Özdemir, B. Şabanoğlu; Domaniç, 873m., 14.VI.2022, ♀, 1♂, leg. S. Özdemir, B. Şabanoğlu; Domaniç, 1204m., 06.VIII.2022, ♀, leg. O. Sert; Emet, 972m., 23.VI.2019, ♀, ♂, leg. S. Özdemir, O. Sert; Gediz, 742m., 29.V.2021, 2♀♀, leg. S. Özdemir, B. Şabanoğlu; Simav, 719m., 23.VI.2021, ♀, leg. S. Özdemir; Tavşanlı, 967m., 21.VI.2019, ♀, ♂, leg. S. Özdemir, B. Şabanoğlu; Manisa: Salihli, 759m., 16.IX.2019, ♀, ♂, leg. S. Özdemir, O. Sert; Salihli, 141m., 25.V.2022, ♂, leg. S. Özdemir; Saruhanlı, 775m., 07.VI.2021, 2♀♀, ♂, leg. S. Özdemir, O. Özdil; Muğla: Milas, 66m., 26.VI.2021, ♀, leg. B. Şabanoğlu; Uşak: Banaz, 1498m., 17.VI.2019, 4♀♀, ♂, leg. S. Özdemir, O. Sert; Banaz, 1804m., 13.VII.2021, 7♀♀, 2♂♂, leg. S. Özdemir, O. Özdil; Central province, 465m., 15.VI.2019, ♀, leg. B. Şabanoğlu; Central province, 934m., 14.VII.2021, ♀, leg. S. Özdemir; Eşme, 881m., 17.VI.2019, 4♀♀, ♂, leg. S. Özdemir, B. Şabanoğlu; Eşme, 950m., 30.VI.2019, ♀, leg. O. Sert; Eşme, 767m., 15.VI.2019, ♀, leg. B. Şabanoğlu; Eşme, 488m., 12.VII.2021, 2♂♂, leg. S. Özdemir, O. Özdil; Karahallı, 1077m., 25.VII.2022, ♂, leg. O. Sert; Ulubey, 832m., 18.V.2019, ♀, leg. O. Sert; Ulubey, 565m., 25.VII.2022, 4♀♀, leg. S. Özdemir, O. Sert.

Distribution in Türkiye. Adana, Afyonkarahisar, Aksaray, Ankara, Antalya, Ardahan, Balıkesir (Kaz Mountain), Bilecik, Bingöl, Bolu, Bursa, Çankırı, Denizli, Diyarbakır, Elazığ, Erzincan, Erzurum, Eskişehir, Gaziantep, Gümüşhane, Isparta, Iğdır, İzmir, Karaman, Kayseri, Kırıkkale, Kırşehir, Konya, Kütahya, Malatya, Manisa, Mardin, Mersin, Muğla, Muş, Nevşehir, Niğde, Siirt, Sivas, Tunceli, Uşak, Yozgat (Bodemeyer, 1906; Sahlberg, 1913; Smetana, 1953, 1967; Anlaş, 2009; Anlaş & Rose, 2009; Kesdek et al., 2009; Özgen & Anlaş, 2010; Özgen et al., 2010, 2015, 2016; Abacıgil et al., 2013; Assing, 2013; Anlaş et al., 2014; Çiftçi & Hasbenli, 2016; Fırat & Sert, 2016a; Özgen, 2017; Tanyeri et al., 2017; Tezcan et al., 2019; Özdemir, 2021).

Remarks. This species is a widely distributed species and together with records from Aydın in this study, it is now distributed in whole Aegean Region.

***Philonthus coprophilus*** Jarrige, 1949

Material examined. Afyonkarahisar: Sultandağı, 1134m., 01.V.2019, ♀, 4♂♂, leg. S. Özdemir, B. Şabanoğlu; Kütahya: Central province, 1164m., 21.VI.2019, ♀, leg. S. Özdemir; Uşak: Ulubey, 873m., 18.V.2019, ♀, leg. S. Özdemir.

Distribution in Türkiye. Aksaray, Balıkesir (Kaz Mountain), Erzurum, Kırıkkale, Konya, Muğla, Nevşehir, Sinop (Assing, 2007, 2010; Abacıgil et al., 2013; Fırat & Sert, 2016a, Tezcan et al., 2019).

***Philonthus cruentatus*** (Gmelin, 1790)

Material examined. Afyonkarahisar: Bayat, 1388m., 16.VII.2021, ♀, leg. B. Şabanoğlu; Balıkesir: Sındırgı, 465m., 25.V.2022, 2♀♀, 3♂♂, leg. S. Özdemir, U. Özfuçucu; İzmir: Bergama, 517m., 28.IV.2022, ♀, leg. S. Özdemir; Kütahya: Central province, 1164m., 21.VI.2019, 3♀♀, ♂, leg. S. Özdemir, O. Sert; Tavşanlı, 996m., 22.VI.2019, 2♀♀, leg. S. Özdemir, Y. Turan; Manisa: Central province, 1373m., 24.V.2022, 2♀♀, ♂, leg. S. Özdemir, E.C. Ceylan; Uşak: Banaz, 1066m., 16.VI.2019, ♀, 1♂, leg. S. Özdemir, O. Sert; Central province, Ovacık, 976m., 16.VI.2019, ♂, leg. O. Sert; Ulubey, 834m., 18.V.2019, 3♀♀, 2♂♂, leg. S. Özdemir, O. Sert, B. Şabanoğlu.

Distribution in Türkiye. Antalya, Balıkesir (Kaz Mountain), Denizli, Erzurum, Eskişehir, Gümüşhane, İstanbul, Konya, Manisa, Mersin, Nevşehir (Apfelbeck, 1902; Bodemeyer, 1906, 1927; Sahlberg, 1913; Öncüer, 1991; Anlaş & Rose, 2009; Anlaş et al., 2014; Çiftçi & Hasbenli, 2016; Fırat & Sert, 2016a; Özgen et al., 2016; Daşdemir & Tozlu, 2022).

***Philonthus debilis*** (Gravenhorst, 1802)

Material examined. Afyonkarahisar: Emirdağ, 1719m., 26.VI.2019, ♂, leg. M. Kabalak; Aydın: Nazilli, 383m., 22.VII.2022, ♀, ♂, leg. S. Özdemir, U. Özfuçucu; Denizli: Tavas, 1060m., 01.VII.2019, ♀, leg. S. Özdemir; İzmir: Aliağa, 120m., 27.VI.2019, 2♀♀, ♂, leg. S. Özdemir, B. Şabanoğlu; Kemalpaşa, 879m., 29.VI.2019, ♀, leg. S. Özdemir; Kütahya: Aslanapa, 1351m., 23.VI.2019, ♂, leg. S. Özdemir; Manisa: Salihli, 380m., 30.VI.2019, 2♀♀, 3♂♂, leg. S. Özdemir, O. Sert; Uşak: Sivaslı, 957m., 14.VII.2021, ♂, leg. S. Özdemir.

Distribution in Türkiye. Adana, Afyonkarahisar, Ankara, Balıkesir (Kaz Mountain), Bilecik, Denizli, Erzincan, Eskişehir, Konya, Mersin, Rize (Peyron, 1858; Fauvel, 1874; Bodemeyer, 1900; Sahlberg, 1913; Smetana, 1953; Öncüer, 1991; Abacıgil et al., 2013; Assing, 2013; Çiftçi & Hasbenli, 2016; Fırat & Sert, 2016a; Özgen et al., 2016; Özdemir, 2021).

***Philonthus dimidiatipennis*** Erichson, 1840

Material examined. Afyonkarahisar: Çay, 1028m., 05.VII.2019, ♂, leg. S. Özdemir; Çobanlar, 1037m., 17.VII.2021, ♀, leg. S. Özdemir.

Distribution in Türkiye. Adana, Ankara, Isparta, Konya (Smetana, 1953, 1967; Scheerpeltz, 1958; Fırat & Sert, 2016a).

Remarks. This species is here reported for the first time from the Aegean Region.

***Philonthus ebeninus*** Gravenhorst, 1802

Material examined. Kütahya: Tavşanlı, 967m., 21.VI.2019, 2♀♀, leg. S. Özdemir, B. Şabanoğlu; Uşak: Ulubey, 834m., 18.V.2019, ♂, leg. S. Özdemir.

Distribution in Türkiye. Adana, Aksaray, Antalya, Bursa, Çankırı, Elazığ, Eskişehir, İzmir, Kırşehir, Manisa, Mersin, Muş, Nevşehir (Peyron, 1858; Smetana, 1953; Anlaş, 2009; Anlaş & Rose, 2009; Anlaş et al., 2014; Çiftçi & Hasbenli, 2016; Fırat & Sert, 2016a; Özgen et al., 2016).

***Philonthus frigidoides*** Coiffait, 1963

Material examined. Afyonkarahisar: Çay, 1215m., 05.VII.2019, ♀, ♂, leg. S. Özdemir, Y. Turan; Çay, 1689m., 05.VII.2019, ♀, leg. Y. Turan.

Distribution in Türkiye. Isparta, Ordu (Coiffait, 1963, 1978).

Remarks. This species is here reported for the first time from the Aegean Region.

***Philonthus frigidus frigidus*** Märkel & Kiesenwetter, 1848

Material examined. Afyonkarahisar: Çay, 1920m., 17.VII.2021, ♀, leg. S. Özdemir.

Distribution in Türkiye. Aksaray, Bayburt, Giresun, Rize (Fırat & Sert, 2016a; Özdemir, 2021).

Remarks. This species is here reported for the first time from the Aegean Region.

***Philonthus fumarius*** (Lacordaire, 1835)

Material examined. Kütahya: Central province, 1043m., 24.VI.2019, ♀, leg. S. Özdemir.

Distribution in Türkiye. İstanbul, Kırşehir, Muş (Apfelbeck, 1902; Horion, 1965; Fırat & Sert, 2016a; Özgen et al., 2016).

Remarks. This species is here reported for the first time from the Aegean Region.

***Philonthus intermedius*** (Lacordaire, 1835)

Material examined. Afyonkarahisar: Emirdağ, 1719m., 26.VI.2019, ♂, leg. M. Kabalak; Denizli: Tavas, 1115m., 11.VI.2021, 2♂♂, leg. S. Özdemir, O. Özdil; İzmir: Kemalpaşa, 256m., 05.V.2019, ♀, ♂, leg. O. Sert, B. Şabanoğlu; Tire, 781m., 12.V.2019, ♀, leg. O. Sert; Kütahya: Central province, 1164m., 14.VI.2022, ♂, leg. U. Özfuçucu; Manisa: Salihli, 141m., 25.V.2022, 2♀♀, 2♂♂, leg. S. Özdemir, E.C. Ceylan; Muğla: Kavaklıdere, 805m., 02.VI.2022, ♀, leg. S. Özdemir; Uşak: Central province, 976m., 16.VI.2019, ♀, 3♂♂, leg. O. Sert, B. Şabanoğlu; Central province, 946m., 16.VI.2019, ♂, leg. S. Özdemir; Eşme, 767m., 15.VI.2019, ♀, ♂, leg. S. Özdemir, B. Şabanoğlu; Ulubey, 873m., 18.V.2019, ♀, leg. S. Özdemir; Ulubey, 834m., 18.V.2019, ♀, 3♂♂, leg. S. Özdemir, O. Sert, B. Şabanoğlu.

Distribution in Türkiye. Adıyaman, Aksaray, Ankara, Antalya, Balıkesir (Kaz Mountain), Bilecik, Denizli, Elazığ, Erzincan, Eskişehir, Gümüşhane, İzmir, Kahramanmaraş, Kayseri, Kırıkkale, Kırşehir, Kocaeli, Konya, Malatya, Mardin, Manisa, Mersin, Muğla, Muş, Nevşehir, Sivas (Peyron, 1858; Sahlberg, 1913; Öncüer, 1991; Anlaş, 2009; Anlaş & Rose, 2009; Özgen & Anlaş, 2010; Abacıgil et al., 2013; Anlaş et al., 2014; Özgen et al., 2015, 2016; Çiftçi & Hasbenli, 2016; Fırat & Sert, 2016a; Tanyeri et al., 2017).

***Philonthus juvenilis*** Peyron, 1858

Material examined. Afyonkarahisar: Sandıklı, 1702m., 18.VII.2021, ♀, leg. B. Şabanoğlu; Aydın: Nazilli, 665m., 20.V.2022, ♀, leg. S. Özdemir; Balıkesir: Havran, 242m., 05.VI.2021, ♀, leg. O. Özdil; İzmir: Bergama, 220m., 28.IV.2022, 2♀♀, leg. S. Özdemir, B. Şabanoğlu; Central province, 691m., 04.V.2019, ♀, ♂, leg. S. Özdemir, Y. Turan; same locality, 29.IV.2022, ♀, leg. B. Şabanoğlu; Kemalpaşa, 879m., 29.VI.2019, ♂, leg. O. Sert; Ödemiş, 1376m., 20.V.2022, 2♂♂, leg. S. Özdemir, B. Şabanoğlu; Kütahya: Dumlupınar, 1164m., 28.VII.2021, ♂, leg. S. Özdemir; Hisarcık, 772m., 23.VI.2019, ♀, leg. S. Özdemir; Uşak: Banaz, 957m., 19.V.2019, ♀, leg. O. Sert.

Distribution in Türkiye. Antalya, Bayburt, Erzurum, Konya, Mersin, Niğde (Peyron, 1858; Bodemeyer, 1900; Smetana, 1953; Coiffait, 1974; Anlaş & Rose, 2009; Fırat & Sert, 2016a; Özdemir, 2021).

Remarks. This species is here reported for the first time from the Aegean Region.

***Philonthus laminatus*** (Creutzer, 1799)

Material examined. Balıkesir: Sındırgı, 465m., 25.V.2022, 2♀♀, 3♂♂, leg. S. Özdemir, U. Özfuçucu; İzmir: Central province, 725m., 29.IV.2022, ♀, leg. S. Özdemir; Tire, 781m., 12.V.2019, ♀, leg. B. Şabanoğlu; Manisa: Central province, 1373m., 24.V.2022, 2♀♀, ♂, leg. S. Özdemir, B. Şabanoğlu; Kula, 596m., 09.VI.2021, ♀, leg. S. Özdemir.

Distribution in Türkiye. Ankara, Balıkesir, Bayburt, Erzurum, Eskişehir, Kırşehir, Manisa, Mersin, Muğla, Tunceli (Fauvel, 1874; Bodemeyer, 1906; Smetana, 1953; Horion, 1965; Coiffait, 1978; Schillhammer, 2003; Kesdek et al., 2009; Anlaş et al., 2014; Çiftçi & Hasbenli, 2016; Fırat & Sert, 2016a; Daşdemir & Tozlu, 2022).

***Philonthus longicornis*** Stephens, 1832

Material examined. Manisa: Salihli, 141m., 25.V.2022, ♀, ♂, leg. S. Özdemir, E.C. Ceylan; Uşak: Eşme, 939m., 12.VII.2021, ♀, leg. S. Özdemir; Ulubey, 565m., 25.VII.2022, ♀, leg. O. Sert.

Distribution in Türkiye. Adana, Ankara (Smetana, 1953).

Remarks. This is the first record from the Aegean Region.

***Philonthus nigrita*** (Gravenhorst, 1806)

Material examined. Kütahya: Central province, 1221m., 27.V.2021, ♀, leg. S. Özdemir.

Distribution in Türkiye. Bolu (Korge, 1971).

Remarks. This species is here reported for the second time from Türkiye and the first time from the Aegean Region.

***Philonthus nitidicollis*** (Lacordaire, 1835)

Material examined. Afyonkarahisar: Çay, 1230m., 05.VII.2019, ♀, leg. S. Özdemir; Aydın: Nazilli, 434m., 20.V.2022, ♀, leg. O. Sert; Balıkesir: Edremit, 1723m., 26.V.2022, ♀, leg. S. Özdemir; Sındırgı, 465m., 25.V.2022, ♀, leg. S. Özdemir; Denizli: Tavas, 183m., 03.VI.2022, ♂, leg. B. Şabanoğlu; İzmir: Bergama, 517m., 28.IV.2022, ♀, leg. S. Özdemir; Bergama, 473m., 23.V.2022, 3♀♀, ♂, leg. S. Özdemir, B. Şabanoğlu; Karaburun, 466m., 02.V.2019, ♀, leg. O. Sert; Manisa: Central province, 1373m., 24.V.2022, ♀, leg. S. Özdemir; Salihli, 141m., 25.V.2022, ♂, leg. E.C. Ceylan; Saruhanlı, 328m., 17.IX.2019, ♂, leg. S. Özdemir.

Distribution in Türkiye. Adana, Aksaray, Ankara, Antalya, Balıkesir (Kaz Mountain), Bingöl, Bursa, Çanakkale (Gökçeada-Bozcaada), Denizli, Diyarbakır, Eskişehir, Gaziantep, Isparta, İzmir, Karaman, Kayseri, Kırşehir, Konya, Manisa, Mardin, Mersin, Muğla, Nevşehir, Siirt, Şırnak (Sahlberg, 1913; Bodemeyer, 1927; Smetana, 1953; Anlaş, 2009; Anlaş & Rose, 2009; Özgen & Anlaş, 2010; Japoshvili & Anlaş, 2011; Abacıgil et al., 2013; Anlaş et al., 2014; Assing, 2014; Çiftçi & Hasbenli, 2016; Fırat & Sert, 2016a; Özgen et al., 2016; Sezer, 2018; Tezcan et al., 2019 Daşdemir & Tozlu, 2022).

***Philonthus parvicornis*** (Gravenhorst, 1802)

Material examined. Afyonkarahisar: Sultandağı, 1134m., 01.V.2019, 2♂♂, leg. S. Özdemir, O. Sert; İzmir: Aliağa, 195m., 27.VI.2019, ♂, leg. Y. Turan; Kütahya: Tavşanlı, 996m., 22.VI.2019, ♀, leg. S. Özdemir; Harmancık, 967m., 21.VI.2019, ♂, leg. O. Sert; Uşak: Central province, 976m., 16.VI.2019, ♀, leg. O. Sert; Ulubey, 873m., 18.V.2019, ♂, leg. B. Şabanoğlu; Ulubey, 834m., 18.V.2019, ♂, leg. O. Sert.

Distribution in Türkiye. Eskişehir, Isparta, Konya, Manisa, Muğla, Muş (Bodemeyer, 1900, 1927; Anlaş, 2009; Assing, 2013; Anlaş et al., 2014; Çiftçi & Hasbenli, 2016; Özgen et al., 2016).

***Philonthus punctus punctus*** (Gravenhorst, 1802)

Material examined. Afyonkarahisar: Çobanlar, 1037m., 17.VII.2021, 2♀♀, ♂, leg. S. Özdemir, B. Şabanoğlu; Kütahya: Central province, 1084m., 24.VI.2019, ♂, leg. S. Özdemir.

Distribution in Türkiye. Thrace (Türkiye-Bulgaria frontier), Mersin (Peyron, 1858; Smetana, 1953).

Remarks. This species is here reported for the first time from the Aegean Region.

***Philonthus quisquiliarius quisquiliarius*** (Gyllenhal, 1810)

Material examined. Afyonkarahisar: Central province, 1119m., 16.VII.2021, ♂, leg. B. Şabanoğlu; Çay, 1028m., 05.VII.2019, 2♂♂, leg. S. Özdemir, B. Şabanoğlu; Çobanlar, 1271m., 07.VII.2019, ♀, 4♂♂, leg. S. Özdemir, O. Sert; Aydın: Bozdoğan, 94m., 09.V.2019, ♀, leg. B. Şabanoğlu; Didim, 0m., 10.V.2019, 7♀♀, 7♂♂, leg. S. Özdemir, B. Şabanoğlu; Koçarlı, 14m., 10.V.2019, ♀, 4♂♂, leg. S. Özdemir, Y. Turan; Koçarlı, 65m., 26.IV.2022, 10♀♀, 8♂♂, leg. S. Özdemir, B. Şabanoğlu, U. Özfuçucu; Söke, 23m., 11.V.2019, 4♀♀, 5♂♂, leg. S. Özdemir, B. Şabanoğlu; Denizli: Buldan, 207m., 12.VI.2021, ♂, leg. B. Şabanoğlu; Çal, 697m., 12.VI.2021, ♀, leg. O. Özdil; Tavas, 1036m., 01.VII.2019, 2♂♂, leg. S. Özdemir, O. Sert; Tavas, 1077m., 01.VII.2019, 11♀♀, 9♂♂, leg. S. Özdemir, O. Sert; İzmir: Foça, 0m., 27.VI.2019, 7♂♂, leg. S. Özdemir, O. Sert; Yenifoça, 112m., 27.VI.2019, ♀, ♂, leg. S. Özdemir, Y. Turan; Kemalpaşa, 143m., 28.VI.2019, ♂, leg. S. Özdemir; Kütahya: Altıntaş, 1231m., 28.V.2021, ♀, leg. B. Şabanoğlu; Aslanapa, 1138m., 08.VIII.2022, ♂, leg. S. Özdemir; Central province, 1164m., 21.VI.2019, ♀, leg. S. Özdemir; Central province, 1046m., 29.VII.2021, 4♀♀, 2♂♂, leg. S. Özdemir, O. Özdil; Manisa: Kula,

343m., 09.VI.2021, ♀, leg. S. Özdemir; Salihli, 126m., 16.IX.2019, 3♀♀, ♂, leg. S. Özdemir, B. Şabanoğlu; Saruhanlı, 144m., 07.VI.2021, ♂, leg. S. Özdemir; Muğla: Central province, 15m., 31.V.2022, 2♀♀, ♂, leg. S. Özdemir, O. Sert; Uşak: Eşme, 777m., 18.V.2019, ♀, leg. S. Özdemir.

Distribution in Türkiye. Adana, Aksaray, Ankara, Bayburt, Diyarbakır, Eskişehir, İzmir, Kırıkkale, Kırşehir, Konya, Mersin, Nevşehir, Siirt (Peyron, 1858; Sahlberg, 1913; Smetana, 1953, 1967; Öncüer, 1991; Özgen et al., 2010, 2016; Assing, 2013; Çiftçi & Hasbenli, 2016; Fırat & Sert, 2016a; Özdemir, 2021).

***Philonthus rectangulus*** Sharp, 1874

Material examined. Afyonkarahisar: İhsaniye, 1160m., 07.VII.2019, ♀, 3♂♂, leg. S. Özdemir, B. Şabanoğlu; Aydın: Çine, 464m., 28.VI.2021, ♀, leg. S. Özdemir; Balıkesir: Savaştepe, 568m., 06.VI.2021, ♀, ♂, leg. S. Özdemir, B. Şabanoğlu; Denizli: Acıpayam, 1031m., 27.VII.2021, 5♀♀, 2♂♂, leg. S. Özdemir, O. Özdil; Kale, 1031m., 02.VI.2022, ♂, leg. S. Özdemir; Tavas, 1060m., 01.VII.2019, 3♀♀, ♂, leg. S. Özdemir, O. Sert; İzmir: Bergama, 390m., 09.VII.2021, ♀, 3♂♂, leg. O. Sert, S. Özdemir; Tire, 781m., 12.V.2019, 3♀♀, ♂, leg. S. Özdemir, B. Şabanoğlu; Muğla: Milas, 66m., 26.VI.2021, 2♀♀, 3♂♂, leg. S. Özdemir, B. Şabanoğlu; Uşak: Central province, 976m., 16.VI.2019, ♀, leg. O. Sert; Eşme, 939m., 12.VII.2021, ♀, leg. S. Özdemir; Sivaslı, 957m., 14.VII.2021, 2♀♀, leg. S. Özdemir, B. Şabanoğlu; Ulubey, 565m., 25.VII.2022, ♂, leg. O. Sert.

Distribution in Türkiye. Aksaray, Ankara, Erzurum, Eskişehir, Kayseri, Kırıkkale, Manisa, Şanlıurfa, Trabzon, Tunceli, Yozgat (Smetana, 1953; Anlaş, 2009; Kesdek et al., 2009; Anlaş et al., 2014; Çiftçi & Hasbenli, 2016; Fırat & Sert, 2016a).

***Philonthus rubripennis*** Stephens, 1832

Material examined. Balıkesir: Central province, 299m., 04.VI.2021, ♂, leg. S. Özdemir; Kepsut, 190m., 27.V.2022, ♀, leg. O. Özdil; İzmir: Bergama, 473m., 23.V.2022, ♀, leg. B. Şabanoğlu; Kütahya: Simav, 758m., 22.VI.2019, 2♀♀, ♂, leg. S. Özdemir, O. Sert.

Distribution in Türkiye. Ankara, Bayburt, Erzurum, Eskişehir, Gümüşhane, Konya, Mardin, Mersin, Rize, Tunceli, Uşak (Peyron, 1858; Fauvel, 1874; Smetana, 1953; Anlaş, 2009; Kesdek et al., 2009; Çiftçi & Hasbenli, 2016; Fırat & Sert, 2016a; Özgen et al., 2016; Özdemir, 2021).

***Philonthus rufimanus*** Erichson, 1840

Material examined. Balıkesir: Central province, 207m., 03.VII.2022, ♀, ♂, leg. S. Özdemir, B. Şabanoğlu; İvrindi, 314m., 06.VI.2021, 6♀♀, 3♂♂, leg. S. Özdemir, B. Şabanoğlu, O. Özdil; İvrindi, 284m., 03.VII.2022, ♂, leg. S. Özdemir; Sındırgı, 310m., 06.VI.2021, ♀, 2♂♂, leg. S. Özdemir, B. Şabanoğlu; İzmir: Kınık, 189m., 23.V.2022, ♀, leg. S. Özdemir; Kütahya: Simav, 758m., 22.VI.2019, ♀, leg. O. Sert; Tavşanlı, 1051m., 27.V.2021, ♂, leg. S. Özdemir; Manisa: Gördes, 384m., 25.V.2022, ♀, leg. S. Özdemir; Kula, 596m., 09.VI.2021, ♀, leg. S. Özdemir; Kula, 343m., 09.VI.2021, ♀, 2♂♂, leg. B. Şabanoğlu, O. Özdil; Muğla: Ula, 402m., 01.VI.2022, ♂, leg. O. Sert; Yatağan, 519m., 25.IV.2019, ♀, leg, Y. Turan; Uşak: Eşme, 524m., 23.VII.2022, ♀, leg. S. Özdemir.

Distribution in Türkiye. Aksaray, Ankara, Artvin, Aydın, Balıkesir (Kaz Mountain), Bursa, Bilecik, Bayburt, Elazığ, Erzurum, Eskişehir, Çankırı, Gümüşhane, İzmir, Kahramanmaraş, Kayseri, Kilis, Konya, Kütahya, Malatya, Manisa, Mardin, Mersin, Muğla, Muş, Siirt, Sivas, Thrace, Tunceli, Uşak, Yozgat (Peyron, 1858; Fauvel, 1874; Bodemeyer, 1900, 1927; Sahlberg, 1913; Smetana, 1953, 1967; Horion, 1965; Tezcan & Amiryan, 2003; Anlaş, 2009; Anlaş & Rose, 2009; Abacıgil et al., 2013; Özgen et al., 2015; 2016; Çiftçi & Hasbenli, 2016; Fırat & Sert, 2016a; Tanyeri et al., 2017; Özdemir, 2021).

***Philonthus salinus*** Kiesenwetter, 1844

Material examined. Afyonkarahisar: Çobanlar, 1037m., 17.VII.2021, ♂, leg. S. Özdemir; Aydın: Didim, 0m., 10.V.2019, ♂, leg. S. Özdemir.

Distribution in Türkiye. Balıkesir (Coiffait, 1974)

Remarks. This species was previously recorded from Manyas Lake, Balıkesir by Coiffait (1974), which belongs to Marmara region. It is here recorded from the Aegean Region for the first time.

***Philonthus sanguinolentus*** (Gravenhorst, 1802)

Material examined. Afyonkarahisar: Sultandağı, 1134m., 01.V.2019, ♂, leg. S. Özdemir; Kütahya: Aslanapa, 1351m., 23.VI.2019, 2♂♂, leg. S. Özdemir, O. Sert; Uşak: Banaz, 1066m., 16.VI.2019, ♀, leg. O. Sert; Central province, 976m., 16.VI.2019, ♀, leg. B. Şabanoğlu.

Distribution in Türkiye. Aksaray, Ankara, Konya, Nevşehir (Fırat & Sert, 2016a).

Remarks. This species is here reported for the first time from the Aegean Region.

***Philonthus spinipes kabardensis*** Bolov & Kryzhanovskij, 1969

Material examined. Afyonkarahisar: Sultandağı, 1134m., 01.V.2019, 2♂♂, leg. S. Özdemir, B. Şabanoğlu; Kütahya: Central province, 1164m., 14.VI.2022, ♂, leg. B. Şabanoğlu; Manisa: Salihli, 141m., 25.V.2022, ♀, ♂, leg. S. Özdemir, E.C. Ceylan; Muğla: Kavaklıdere, 805m., 02.VI.2022, ♂, leg. S. Özdemir; Uşak: Central province, 976m., 16.VI.2019, ♀, leg. S. Özdemir; Sivaslı, 871m., 19.V.2019, ♀, leg. S. Özdemir.

Distribution in Türkiye. Antalya, Konya, Mersin, Nevşehir (Assing, 2006; Fırat & Sert, 2016a).

Remarks. This species is here reported for the first time from the Aegean Region.

***Philonthus tenuicornis*** Mulsant & Rey, 1853

Material examined. Afyonkarahisar: Sultandağı, 1134m., 01.V.2019, ♀, 3♂♂, leg. S. Özdemir, O. Sert; Kütahya: Tavşanlı, 1092m., 22.VI.2019, ♀, leg. S. Özdemir; Uşak: Banaz, 1066m., 16.VI.2019, 3♀♀, ♂, leg. S. Özdemir, O. Sert.

Distribution in Türkiye. Kırklareli, Manisa, Rize (Özgen et al., 2016; Özdemir, 2021).

***Philonthus umbratilis*** (Gravenhorst, 1802)

Material examined. Afyonkarahisar: Çay, 1600m., 17.VII.2021, ♂, leg. S. Özdemir; Çobanlar, 1037m., 17.VII.2021, 2♂♂, leg. S. Özdemir, B. Şabanoğlu; Aydın: Söke, 23m., 11.V.2019, 2♂♂, leg. S. Özdemir, B. Şabanoğlu; Balıkesir: Dursunbey, 518m., 02.VII.2022, ♀, leg. S. Özdemir; Denizli: Çal, 1198m., 28.VII.2021, ♀, leg. S. Özdemir; Kütahya: Hisarcık, 772m., 23.VI.2019, ♀, ♂, leg. S. Özdemir, B. Şabanoğlu; Uşak: Ulubey, 565m., 25.VII.2022, ♂, leg. S. Özdemir.

Distribution in Türkiye. Tunceli (Özgen et al., 2016).

Remarks. This species is here reported for the first time from the Aegean Region.

***Philonthus varians*** (Paykull, 1789)

Material examined. Aydın: Karpuzlu, 592m., 19.V.2022, ♂, leg. B. Şabanoğlu; Muğla: Dalaman, 86m., 01.VI.2022, ♂, leg. O. Sert; Uşak: Banaz, 1066m., 16.VI.2019, ♂, leg. S. Özdemir; Eşme, 767m., 15.VI.2019, ♂, leg. S. Özdemir.

Distribution in Türkiye. Ankara, Eskişehir, Gümüşhane, Manisa, Mersin, Sinop (Peyron, 1858; Assing, 2010; Anlaş et al., 2014; Çiftçi & Hasbenli, 2016; Özgen et al., 2016; Altunsoy et al., 2017).

***Philonthus viridipennis*** Fauvel, 1875

Material examined. Afyonkarahisar: Çobanlar, 1037m., 17.VII.2021, 4♀♀, 7♂♂, leg. S. Özdemir, B. Şabanoğlu; İhsaniye, 1111m., 16.VII.2021, ♀, leg. B. Şabanoğlu; Aydın: Çine, 75m., 28.VI.2021, 2♀♀, 2♂♂, leg. S. Özdemir, O. Sert; Çine, 737m., 28.VI.2021, 5♂♂, leg. S. Özdemir, B. Şabanoğlu; Çine, 723m., 28.VI.2021, 2♀♀, ♂, leg. S. Özdemir, B. Şabanoğlu; Koçarlı, 65m., 26.IV.2022, 2♀♀, ♂, leg. S. Özdemir, E.C. Ceylan; Köşk, 105m., 26.IV.2022, ♀, leg. S. Özdemir; Balıkesir: Sındırgı, 310m., 06.VI.2021, ♀, leg. S. Özdemir; Denizli: Tavas, 1077m., 01.VII.2019, 4♀♀, 2♂♂, leg. S. Özdemir, O. Sert; Kütahya: Central province, 16084m., 24.VI.2019, ♀, leg. S. Özdemir; Central provicne, 1093m., 29.VII.2021, ♀, leg. S. Özdemir; Manisa: Kula, 596m., 09.VI.2021, ♀, leg. S. Özdemir; Uşak: Eşme, 777m., 18.V.2019, ♀, 2♂♂, leg. S. Özdemir, O. Sert.

Distribution and remarks. Although its presence in Türkiye was reported by Coiffait (1967, 1974), Herman (2001) and Schülke & Smetana (2015), a detailed locality record is given for the first time with this study.

## Discussion

According to the data, *Philonthus* species were collected by aspirator on river/water/lake edges under stones and from dung, as they are predators and recurring habitants of dung feeding on Diptera larvae. *Philonthus concinnus* is the most abundant species with 155 specimens and it is also the only species occuring in the entire altitude range of the study. *Philonthus quisquiliarius quisquiliarius* is the second abundant species with 112 specimens, which is also the only species continuously found between April and September. With respect to their phenology, within the study period of April-October, they can be found mostly between May-July (Table 1).

It is determined that all of the species, except three cosmopolitan species, are shared with the European fauna, while 25 of species with Asian and 16 of them with the North Africa subsections of the Palaearctic fauna. Since 11 detected species are introduced to Nearctic fauna, origin of species mostly belongs to Asiatic-European and this composition reflects the location of the country which is an intersection area for all three subsections of Palaearctic region (Table 1). When regional records were compared with previous records (Table 1), the most number of species are shared with Central Anatolian Region (22 species), followed by Mediterranean (19 species) and Aegean Regions (17 species). Following this, 15 species both for Marmara and Eastern Anatolian, and 12 species are shared with Black Sea Region. The fact that only seven species are shared with the Southeastern Anatolian Region may be explained by a less thorough and less frequent sampling activity.

According to the results, 44.7% of all the species reported from Türkiye and 70.8% of the previously reported species from the Aegean Region were collected in this study (Table 2). Thirteen of the species are new records for the region. Together with this result, it can be said that, 81% of the recorded *Philonthus* species are determined by the study. Besides the 13 new species records for the region, new province records are provided for most of the species.

Table 1. Collected species from research area

| Species | Sp. | Vertical Distribution | Collecting Months | Collecting habitat-method | Distribution in Türkiye | Zoogeographical Distributions |
|---|---|---|---|---|---|---|
| *Philonthus carbonarius* | 1 | E | May | III | MR, AR, MDR, CAR, EAR | Eu, N, As, NARi |
| *Philonthus cognatus* | 2 | E, F | Jun, Oct | II | MR, AR, MDR, CAR, BSR, EAR, SEAR | Eu, N, As, NARi |
| *Philonthus concinnus* | 155 | A, B, C, D, E, F, G, H | Apr, May, Jun, Jul, Aug | I, II, III, IV | MR, AR, MDR, CAR, BSR, EAR, SEAR | Eu, N, As, NARi |
| *Philonthus coprophilus* | 7 | D, E | May, Jun | I | MR, AR, CAR, BSR, EAR | Eu, N, As |
| *Philonthus cruentatus* | 24 | B, C, D, E, F | Apr, May, Jun, Jul | I | MR, AR, MDR, CAR, BSR | Eu, N, As, NARi |
| *Philonthus debilis* | 15 | A, B, D, E, F, G | Jun, Jul | I, II, III, IV | MR, AR, MDR, CAR, EAR | Eu, N, As, NARi |
| *Philonthus dimidiatipennis* | 2 | E | Jul | II | MDR, CAR | Eu, N, As, ORR |
| *Philonthus ebeninus* | 3 | D | May, Jun | I | MR, AR, MDR, CAR, EAR | Eu, N, As |
| *Philonthus frigidoides* | 3 | E, G | Jul | II | MDR, BSR | Eu, As |
| *Philonthus frigidus frigidus* | 1 | H | Jul | II | CAR | Eu |
| *Philonthus fumarius* | 1 | E | Jun | III | MR, CAR, EAR | Eu |
| *Philonthus intermedius* | 24 | A, B, D, E, G | May, Jun | I, IV | MR, AR, MDR, CAR, BSR, EAR, SEAR | Eu, N, As |
| *Philonthus juvenilis* | 14 | A, C, D, E, F, H | Apr, May, Jun, Jul | II | MDR, CAR | Eu, As |
| *Philonthus laminatus* | 11 | B, C, D, F | May, Jun | II, III | MR, AR, MDR, CAR, BSR, EAR, | Eu, As |
| *Philonthus longicornis* | 4 | A, C, D | May, Jul | I, II | MDR, CAR | Eu, N, As, COS, NARi |
| *Philonthus nigrita* | 1 | E | May | II | BSR | Eu, As |
| *Philonthus nitidicollis* | 16 | A, B, C, E, F, G | Apr, May, Jun, Jul, Sep | I, II, III | MR, AR, MDR, CAR, EAR, SEAR | Eu, N, As |
| *Philonthus parvicornis* | 8 | A, D, E | May, Jun | I | AR, MDR, CAR, EAR | Eu, N, As, ORR |
| *Philonthus punctus punctus* | 4 | E | Jun, Jul | II | MR, MDR | Eu, N, As |
| *Philonthus quisquiliarius quisquiliarius* | 112 | A, C, E, F | Apr, May, Jun, Jul, Aug, Sep | II | AR | Eu, N, As, AFR |
| *Philonthus rectangulus* | 37 | A, B, C, E | May, Jun, Jul | II | AR, CAR, BSR, EAR, SEAR | Eui, Ni, As, COS, NARi |
| *Philonthus rubripennis* | 6 | A, B, D | May, Jun | II | AR, MDR, CAR, BSR, EAR, SEAR | Eu, N, As |
| *Philonthus rufimanus* | 26 | A, B, C, D, E | May, Jun, Jul | I, II | MR, AR, MDR, CAR, BSR, EAR, SEAR | Eu, As |
| *Philonthus salinus* | 2 | A, E | May, Jul | II | MR | Eu, As |
| *Philonthus sanguinolentus* | 5 | D, E, F | May, Jun | I | CAR | Eu, N, As, NARi |
| *Philonthus spinipes kabardensis* | 8 | A, D, E | May, Jun | I | MDR, CAR | Eu, As |
| *Philonthus tenuicornis* | 9 | B | May, Jun | I, II | MR, AR | Eu, As, NARi |
| *Philonthus umbratilis* | 10 | A, C, D, E, G | May, Jun, Jul | II | EAR | Eu, N, As, NARi |
| *Philonthus varians* | 4 | A, C, D, E | May, Jun | I | AR, MDR, CAR, BSR | Eu, N, As, COS, NARi |

Vertical distributions (A: 0-250 m; B: 251-500 m; C: 501-750 m; D: 751-1000 m; E: 1001-1250 m; F: 1251-1500 m; G: 1501-1750 m; H: 1751-2000 m); Collecting Months, Apr: April, May: May, Jun: June, Jul: July, Aug: August, Sep: September, Oct: October; Collecting habitat-methods, (I: on dung by aspirator, II: under stones by aspirator, III: sifting debris, IV: sweeping herbaceous plants), Distributions in Türkiye, MR: Marmara Region, AR: Aegean Region, MDR: Mediterranean Region, CAR: Central Anatolian Region, BSR: Black Sea Region, EAR: Eastern Anatolian Region, SEAR: South Eastern Anatolian Region; Zoogeographical Distributions, COS: Cosmopolitan, As: Asia, Eu: Europe, N: North Africa, AFR: Afrotropical, NAR: Nearctic, ORR: Oreintal, i: introduced (Schülke & Smetana, 2015).

Table 2. Comparison of record of *Philonthus* species which are previously recorded from the region and collected with this study

| Species | Previous provincial studies | This study |
|---|---|---|
| *Philonthus alberti*** | Afyonkarahisar | - |
| *Philonthus carbonarius* | Afyonkarahisar, Manisa | Afyonkarahisar |
| *Philonthus cognatus* | Afyonkarahisar, Balıkesir, İzmir, Manisa | Afyonkarahisar, Kütahya |
| *Philonthus concinnus* | Afyonkarahisar, Balıkesir, Denizli, Manisa, Muğla, İzmir, Kütahya, Uşak | Afyonkarahisar, Aydın, Balıkesir, Denizli, İzmir, Kütahya, Manisa, Uşak |
| *Philonthus coprophilus* | Balıkesir, Muğla | Afyonkarahisar, Kütahya, Uşak |
| *Philonthus corruscus*** | Balıkesir, İzmir, Manisa, Muğla | - |
| *Philonthus cruentatus* | Balıkesir, Denizli, Manisa | Afyonkarahisar, Balıkesir, İzmir, Kütahya, Uşak |
| *Philonthus debilis* | Afyonkarahisar, Balıkesir, Denizli | Afyonkarahisar, Aydın, Denizli, İzmir, Kütahya, Manisa, Uşak |
| *Philonthus dimidiatipennis** | - | Afyonkarahisar |
| *Philonthus ebeninus* | İzmir, Manisa | Kütahya, Uşak |
| *Philonthus frigidoides** | - | Afyonkarahisar |
| *Philonthus frigidus frigidus** | - | Afyonkarahisar |
| *Philonthus fumarius** | - | Kütahya |
| *Philonthus intermedius* | Balıkesir, Denizli, İzmir, Manisa, Muğla | Afyonkarahisar, Denizli, İzmir, Manisa, Muğla, Uşak |
| *Philonthus juvenilis** | - | Afyonkarahisar, Aydın, Balıkesir, İzmir, Kütahya, Uşak |
| *Philonthus laminatus* | Balıkesir, Manisa, Muğla | Balıkesir, İzmir, Manisa |
| *Philonthus longicornis** | - | Manisa, Uşak |
| *Philonthus mimus*** | Afyonkarahisar, Balıkesir (Manyas Lake) | - |
| *Philonthus minutus*** | Manisa | - |
| *Philonthus nigrita** | - | Kütahya |
| *Philonthus nitidicollis* | Balıkesir, Denizli, İzmir, Kütahya, Manisa, Muğla | Afyonkarahisar, Aydın, Balıkesir, Denizli, İzmir, Manisa |
| *Philonthus parvicornis* | Manisa, Muğla | Afyonkarahisar, İzmir, Kütahya, Uşak |
| *Philonthus politus*** | Manisa | - |
| *Philonthus punctus punctus** | - | Afyonkarahisar, Kütahya |
| *Philonthus quisquiliariformis* (E)*** | Aydın, Manisa | - |
| *Philonthus quisquiliarius* quisquiliarius | İzmir | Afyonkarahisar, Aydın, Denizli, İzmir, Kütahya, Manisa, Uşak |
| *Philonthus rectangulus* | Manisa | Afyonkarahisar, Aydın, Balıkesir, Denizli, İzmir, Muğla, Uşak |
| *Philonthus rubripennis* | Uşak | Balıkesir, İzmir, Kütahya, Muğla |
| *Philonthus rufimanus* | Aydın, Balıkesir, İzmir, Kütahya, Manisa, Muğla, Uşak | Balıkesir, İzmir, Kütahya, Manisa, Muğla, Uşak |
| *Philonthus salinus* | Balıkesir (Manyas Lake) | Afyonkarahisar, Aydın |
| *Philonthus sanguinolentus** | - | Afyonkarahisar, Kütahya, Uşak |
| *Philonthus spinipes kabardensis** | - | Afyonkarahisar, Kütahya, Manisa, Muğla, Uşak |
| *Philonthus splendens splendens*** | Manisa | - |
| *Philonthus tenuicornis* | Manisa | Afyonkarahisar, Kütahya, Uşak |
| *Philonthus umbratilis** | - | Afyonkarahisar, Aydın, Balıkesir, Denizli, Kütahya, Uşak |
| *Philonthus varians* | Manisa | Aydın, Muğla, Uşak |
| *Philonthus viridipennis** | First detailed locality | Afyonkarahisar, Aydın, Denizli, Kütahya, Manisa, Uşak |

Species which are recorded from the region for the first time are indicated by asterisk (*), species which were previously recorded but could not be found with this study are indicated by two asterisks (**). E: Endemic species.

In the study only seven species could not be collected from the region. It is thought that there would be some reasons for this situation. The first and simplest of all, previous records could have been misidentifications. Furthermore, male specimens are usually required for the exact identification of species. Thus, when there are only females, sometimes identifications are not reliable. Moreover, when previous records of not found species were examined, the most recent record from the region dates back to 2010. Even, *Philonthus mimus* was reported from region by Coiffait in year 1974. Having the purpose to determine the fauna, sufficient field studies have been done. Previous available locations of species were revisited. Even though just about ten years may not seem like a very long period of time, it is possible that species may have retracted from the region due to various reasons. For example, overuse of natural resources by humans is a known cause of biodiversity loss. Due to the population growth, this seems to be one of the possible reasons. Besides that, climate change could also be a potential reason, causing devastating results regarding habitat loss. As a result, although, this study was not designed to determine this, effects of these potential reasons can easily be seen.

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*Original article (Orijinal araştırma)*

# The importance of host weed species for root-knot nematodes, *Meloidogyne* spp. Göldi, 1897 (Tylenchida: Heteroderidae) in banana plantations[1]

## Muz üretim plantasyonlarında kök-ur nematodları, *Meloidogyne* spp. Göldi, 1897 (Tylenchida: Heteroderidae) için konukçu yabancı ot türlerinin önemi

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## Abstract

Banana is a significant economic source in Türkiye. Root-knot nematodes, *Meloidogyne* spp. Göldi, 1897 (Tylenchida: Heteroderidae) are the important pests in banana fields. This study was conducted from 2021 to 2022 to elucidate the relationship between root-knot nematodes and weed species in bananas in the Mediterranean Region. 2% of the banana production area in Adana, Antalya, Hatay and Mersin were surveyed regularly for this purpose. 1m$^2$ frames were placed within a 2m radius around banana plants in the sampled areas. The host of root-knot nematodes with weed species identified within the frames was examined. Survey results indicated that *Amaranthus retroflexus* L. (46.34%), *Portulaca oleracea* L. (40.63%), and *Solanum nigrum* L. (37.84%) were the weed species most infected with root-knot nematodes. Furthermore, molecular analyses revealed that *Abutilon theophrasti* Medik., *Amaranthus* spp., *Cucumis melo* var. *agrestis* Naudin., *Erodium cicutarium* (L.) L'Hér. ex Aiton, *Kickxia commutata* (Bernh. ex Rchb.) Fritsch, *Malva* spp., *Mercurialis annua* L., *P. oleracea*, *S. nigrum*, and *Sonchus oleraceus* L. were suitable hosts for root-knot nematodes. This study is an important step in understanding the interaction between root-knot nematodes and weeds in banana. The presence of weed species in agricultural fields should be considered as they may support nematode populations and pose a threat to subsequent crops. Therefore, the implementation of weed control strategies could help producers to control nematode populations.

**Keywords:** Banana, infection, Mediterranean basin, molecular, nematode-weed relationship

## Öz

Muz yetiştiriciliği Türkiye'de ekonomik açıdan önemli bir gelir kaynağıdır. Muz alanlarında kök-ur nematodları, *Meloidogyne* spp. Göldi, 1897 (Tylenchida: Heteroderidae) ise ana zararlıları konumundadır. Akdeniz Bölgesi'nde muz üretiminde kök-ur nematodları ile yabancı ot türleri arasındaki ilişkiyi ortaya koymak amacıyla 2021-2022 yılları arası planlanan bu çalışmada, periyodik çıkışlarla muz üretim alanlarının %2'si (Adana, Antalya, Hatay ve Mersin) gezilmiştir. Örnekleme yapılan alanlarda dikilen muz bitkilerinin 2m'lik çap çevresine 1m$^2$'lik çerçeveler atılmıştır. Çerçeve içerisinde saptanan yabancı ot türlerinin kök-ur nematodlarıyla olan konukçuluk durumu incelenmiştir. Sürveyler sonunda *Amaranthus retroflexus* L. (46.34%), *Portulaca oleracea* L. (40.63%) ve *Solanum nigrum* L. (37.84%) türlerinin en fazla kök-ur nematoduyla bulaşık olduğu belirlenmiştir. Dahası moleküler yöntemlerle yapılan analizlerde *Abutilon theophrasti* Medik., *Amaranthus* spp., *Cucumis melo* var. *agrestis* Naudin., *Erodium cicutarium* (L.) L'Hér. ex Aiton, *Kickxia commutata* (Bernh. ex Rchb.) Fritsch, *Malva* spp., *Mercurialis annua* L., *P. oleracea*, *S. nigrum* ve *Sonchus oleraceus* L. türlerinin kök-ur nematodları için uygun konukçular olduğu saptanmıştır. Bu çalışma muz üretiminde kök-ur nematodları ile yabancı otlar arasındaki etkileşimi anlamak için önemli bir adımdır. Tarım alanlarında yabancı ot türlerinin bulunması nematod popülasyonlarının yaşamlarını sürdürebileceği ve bir sonraki kültür bitkilerine zarar verebileceği göz önünde bulundurulmalıdır. Bu açıdan nematod popülasyonlarını kontrol altına almada, yabancı ot mücadele stratejilerini uygulamaları konusunda üreticilere rehberlik edebileceği söylenebilmektedir.

**Anahtar sözcükler:** Muz, bulaşma durumu, Akdeniz havzası, moleküler, nematod-yabancı ot ilişkisi

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## Introduction

The banana, *Musa* spp. L., belonging to the Musaceae family, is cultivated in subtropical regions and represents a monocotyledonous, perennial crop. Among non-grain crops worldwide, bananas are the second most produced commodity in terms of trade volume after coffee, cereals, sugar, and cocoa in terms of trade volume (Aurore et al., 2009; Singh et al., 2016). Banana production, an important component of major crop groups in Asia and Africa, serves as a crucial source of income for producers in Türkiye. Initially limited, production has gradually expanded to reach 12 827 hectares with a yield of 883 455 tones in Türkiye (TÜİK, 2022). Banana plantations in the Mediterranean Region occurs both in closed greenhouses and open fields along coastal areas. The varieties most favored by producers include Grand Nain and Azman varieties.

Plant-parasitic nematodes are obligate parasites that require a host plant to complete their life cycle. In addition to cultivated plants, weeds that pose challenges to crop production serve as alternative hosts for plant-parasitic nematodes (Bélair & Benoit, 1996; Castillo et al., 2008). Weeds that can act as alternative hosts can be either weak or strong hosts for plant-parasitic nematodes (Hogger & Bird, 1976; Griffin, 1982; Gast et al., 1984). Weeds that favour the development of nematode species can sustain harmful nematode populations, thus perpetuating their persistence and causing damage to crops (Hogger & Estey, 1976; Egunjobi & Bolaji, 1979).

Studies conducted on banana have reported that *Pratylenchus* species attack banana plants in East African countries such as Burundi, Ethiopia, Kenya, Rwanda, Tanzania, and Uganda, with the most prevalent species being *Pratylenchus coffeae* Goodey, 1951 (Tylenchida: Pratylenchidae), and *Pratylenchus goodeyi* Sher & Allen, 1953 (Tylenchida: Pratylenchidae) (Machon & Hunt, 1985; Bridge, 1988; Sarah, 1989; Gowen & Quénéhervé, 1990; Bridge, 1993; Kashaija et al., 1994). Additionally, *Helicotylenchus multicinctus* (Cobb, 1893) Golden, 1956 (Tylenchida: Hoplolaimidae) has been identified as a problem in banana, while *Radopholus similis* (Cobb, 1893) Thorne, 1949 (Tylenchida: Pratylenchidae) is reported to be rare (McSorley & Parrado, 1986). Previous studies in banana fields in Türkiye have found *H. multicinctus*, *Helicotylenchus dihystera* (Cobb, 1893) Sher, 1961 (Tylenchida: Hoplolaimidae), *Meloidogyne incognita* (Kofoid & White, 1919) Chitwood, 1949 (Tylenchida: Heteroderidae), and *Meloidogyne javanica* (Treub, 1885) Chitwood, 1949 (Tylenchida: Heteroderidae) (Gürdemir, 1979; Elekcioğlu, 1992; Elekçioğlu & Uygun, 1994; Özarslandan & Elekcioğlu, 2010; Nacar & Özarslandan, 2021; Kalay Sarı et al., 2023). Surveys in banana greenhouses in the Bozyazı district of Mersin have even shown that *H. multicinctus* has a higher population than *M. incognita* and *M. javanica* (Elekcioğlu et al., 2014). Similarly, Özarslandan & Dinçer (2015) have identified *Helicotylenchus* spp. and *Meloidogyne* spp. in banana fields in the provinces of Antalya, Mersin, and Hatay provinces and reported a higher total nematode count (*Helicotylenchus* spp. + *Meloidogyne* spp.) in August compared to May, based on root and soil samples collected from banana plants.

The identification of these nematode species in banana fields has raised the possibility of weed species acting as hosts. Worldwide, it has been determined that 24 weed species serve as hosts for *R. similis*, 23 for *Helicotylenchus* spp., 13 for *Pratylenchus* spp., 13 for *Haplolaimus seinhorsti* Luc, 1958 (Tylenchida: Hoplolaimidae), 29 for *Meloidogyne* spp., and 24 for *Rotylenchulus reniformis* Linford and Oliveira, 1940 (Tylenchida: Hoplolaimidae) in banana fields (Quénéhervé et al., 2006). Important weed species such as *Amaranthus* spp. (Caryophyllales: Amaranthaceae), *Cucumis* spp. (Cucurbitales: Cucurbitaceae), *Portulaca oleracea* L. (Caryophyllales: Portulacaceae), *Euphorbia* spp. (Malpighiales: Euphorbiaceae), *Solanum nigrum* L. (Solanales: Solanaceae) have been identified as both weak and strong hosts for root-knot nematodes (Kaur et al., 2007; Rich et al., 2008; Singh et al., 2010; Kokalis-Burelle & Rosskopf, 2012; Ntidi et al., 2016). In other studies, it has been revealed that nematodes thrive in *Amaranthus dubius* Mart. Ex Thell, *Colocasia esculenta* (L.) Schott (Alismatales: Araceae), and *Peperomia pellucida* Kunth (Piperales: Piperaceae), while they develop well in *Cleome aculeata* L. (Brassicales: Cleomaceae), *Cyperus* sp. (Poales: Cyperaceae), *Echinochloa colona* (L.) Link (Poales: Poaceae), *Eleusine indica (*L.) Gaertn. (Poales:

Poaceae), *Leptochloa filiformis* P. Beauv. (Poales: Poaceae), *Mimosa pudica* L. (Fabales: Fabaceae), *Phenax sonneratii* (Poir.) Wedd. (Rosales: Urticaceae), *Pilea microphylla* (L.) Liebm. (Rosales: Urticaceae), *Setaria barbata* (Lam.) Kunth (Poales: Poaceae), and *Solanum americanum* Mill. For *Amaranthus spinosus* L., *Cecropia* sp. (Rosales: Urticaceae), *Cleome rutidosperma* DC. (Brassicales: Cleomaceae), *Clidemia hirta* (L.) D.Don (Myrtales: Melastomataceae), *Commelina diffusa* Burm.f. (Commelinales: Commelinaceae), *Euphorbia heterophylla* L., *Laportea aestuans* (L.) Chew (Rosales: Urticaceae), *Mikania micrantha* Kunth (Asterales: Asteraceae), *Paspalum fasciculatum* Willd. Ex Flugge (Poales: Poaceae), *Passiflora* sp. (Malpighiales: Passifloraceae), *Phyllanthus amarus* Schumach. & Thonn. (Malpighiales: Phyllanthaceae), *Solanum torvum* Schltdl., *Urena lobata* L. (Malvales: Malvaceae), *Vernonia cinerea* (L.) Less. (Asterales: Asteraceae), and *Xanthosoma nigrum* (Vell.) Stellfeld (Alismatales: Araceae) are found to have weak nematode development (Quénéhervé et al., 2006). Araya & De Waele (2005) identified nematode species in weeds and banana roots at different soil depths in banana fields and found that weed management was associated with nematode distribution around the roots. Similarly, other studies have elucidated the ability of *Meloidogyne* spp., *H. multicinctus*, *R. similis*, *P. coffeae*, *R. reniformis*, and *H. seinhorsti* nematodes to act as hosts on weeds (Duyck et al., 2009).

Weeds therefore play a crucial role in the survival, development, reproduction, and establishment of plant-parasitic nematodes. Knowledge of alternative hosts is highly beneficial for effective control of plant-parasitic nematodes that cause yield losses in crops. Regular weed control has been reported as an effective technique in reducing nematode populations among various nematode control methods (Quénéhervé et al., 2006). The relationship between root-knot nematodes (*Meloidogyne* spp.) and weeds has been studied in citrus, wheat and vegetables in the Mediterranean region. It was determined that *Amaranthus viridis* L., *Amaranthus retroflexus* L., *Amaranthus albus* L., *Chenopodium album* L. (Caryophyllales: Amaranthaceae), *Cynodon dactylon* (L.) Pers. (Poales: Poaceae), *Cyperus rotundus* L., *Digitaria sanguinalis* (L.) Scop. (Poales: Poaceae), *E. indica*, *Malva sylvestris* L. (Malvales: Malvaceae), *Paspalum paspaloides* Scribn. (Poales: Poaceae), *Physalis angulata* L. (Solanales: Solanaceae), *P. oleracea*, *Setaria verticillata* (L.) P. Beauv. (Poales: Poaceae), *S. nigrum*, *Xanthium strumarium* L. (Asterales: Asteraceae), *Chenopodium* sp., and *Trifolium* sp. (Fabales: Fabaceae) weed species could serve as hosts for root-knot nematodes, *Meloidogyne arenaria* Neal, 1889 (Tylenchida: Heteroderidae); 8%, *M. incognita*; 44%, and *M. javanica*; 48% (Ercan, 2009).

There is no detailed study on the relationship between root-knot nematodes (Meloidogyne spp.), the main pests of banana fields in Türkiye, and weeds. The aim of this study is to fill this gap by conducting a survey in banana production areas, focusing on the root-knot nematodes causing problems and identifying weed species that could act as hosts. The study also aims to determine the family distribution of weed species in relation to root-knot nematodes. In addition, molecular methods are used to confirm the presence of root-knot nematodes on specific weed species and to elucidate their host status. The infection status of root-knot nematodes in weeds has been determined in banana plantations in the Mediterranean Region.

## Materials and Methods

Between 2021 and 2022, survey studies were conducted in the provinces of Adana, Antalya, Hatay, and Mersin in the Mediterranean Region to determine the relationship between root-knot nematodes and weeds in open and greenhouse banana plantations. In the Mediterranean Region, a total plantation area of 11,154.4 hectares was recorded in 2020 (TUIK, 2023). Employing the sampling method proposed by Bora & Karaca (1970), approximately 2% of the total production area, equivalent to 180.8 hectares of banana plantations, was investigated. Additionally, for species identification purposes, various laboratory chemicals and materials, an incubator, a freezer, an oven, a PCR machine, electrophoresis equipment, DNA isolation kits, a gel imaging system, and PCR materials were employed as consumables in the diagnostic process of root-knot nematodes.

**Root-knot nematodes (*Meloidogyne* spp.) identified on weed species**

In the sampled banana production area, transects were established along the diagonals of the plantation area. Ten frames of 1 $m^2$ each were randomly placed around the banana plants, and the dominant weed species within these frames were identified (Odum, 1971). Once the dominant species in the banana field had been identified, nematological sampling was carried out by collecting roots from the prominent weed species within a radius of approximately 2 m around randomly selected banana plants. At least one species of weed belonging to three different root-knot nematode orientations was tested in the banana sampling area. Weeds were pulled from the soil surface, and plant species with evidence of galls on roots were identified, thereby recording weed species capable of hosting root-knot nematodes (Ercan, 2009). Surveys in banana production areas were conducted throughout the year with periodic intervals (Nkoa et al., 2015).

**Molecular diagnosis through laboratory studies**

During the survey, weed species with nematode-infected and gall-forming roots were sampled, and subsequently transported to the laboratory. In the surveyed banana plantations, the weed species predominantly present at the sampling points were initially examined, and root samples were collected. Commonly recognized weed species from these samples were documented, while unidentified ones were identified using the Flora of Turkey (Davis, 1965-1989) guide. For molecular diagnosis of root-knot nematodes, DNA isolation was performed using Thermo DNA isolation kit from egg masses. Species identification of the isolated DNA samples was conducted using general and specific primers as specified in Table 1 (Blok et al., 1997; Courtright et al., 2000; Zijlstra et al., 2000; Tesarova et al., 2003).

Table 1. Primers and PCR programs to be used for the identification of root-knot nematodes

| Primer | Sequence | Length | Target Nematodes | Programs | References |
|---|---|---|---|---|---|
| 194<br>195 | TTAACTTGCCAGATCGGACG<br>TCTAATGAGCCGTACGC | 720 bp | 5S-18S Ribosome region | Preheat 95°C-5 min.<br>95°C for 1 min.<br>50°C for 30 sec.<br>72°C for 1 min.<br>35 cycles<br>72°C for 7 min. | Blok et al., 1997 |
| Fjav<br>Rjav | GGTGCGCGATTGAACTGAGC<br>CAGGCCCTTCAGTGGAACTATAC | 720 bp | *M. javanica* specific SCAR | Preheat 95°C-5 min.<br>95°C for 1 min.<br>64°C for 45 sec.<br>72°C for 2 min.<br>35 cycles<br>72°C for 10 min. | Zijlstra et al., 2000 |
| Far<br>Rar | TCGGCGATAGAGGTAAATGAC<br>TCGGCGATAGACACTACAAACT | 420 bp | *M. arenaria* specific SCAR | Preheat 95°C-5 min.<br>95°C for 1 min.<br>61°C for 45 sec.<br>72°C for 2 min.<br>35 cycles<br>72°C for 10 min. | Zijlstra et al., 2000 |
| SEC-F<br>SEC-R | GGGCAAGTAAGGATGCTCTG<br>GCACCTCTTTCATAGCCACG | 502 bp | *M. incognita* | Preheat 95°C-5 min.<br>95°C for 1 min.<br>56°C for 45 sec.<br>72°C for 2 min.<br>35 cycles<br>72°C for 10 min. | Tesarova et al., 2003 |
| D2<br>D3 | ACAAGTACCGTGAGGGAAAGTTG<br>TCCTCGGAAGGAACCAGCTACTA | 758-784 bp | General | Preheat 94°C-4 min.<br>94°C for 30 sec.<br>55°C for 1 min.<br>72°C for 1 min.<br>30 cycles<br>72°C for 10 min. | Courtright et al., 2000 |

Species identification from the DNA obtained after isolation was conducted using the classical PCR method with DreamTaq Green PCR Master mix. The PCR reaction was prepared using 1V PCR Master Mix (DreamTaq DNA Polymerase, 2X DreamTaq Green buffer, dNTPs, 4 mM MgCl2), 1V d2H2O, and 0.4 µM of each primer. The mixture was supplemented with 1 µl of DNA, and the reaction was carried out to a

final volume of 25 µl. Samples displaying a 720 bp band in PCR with general primers underwent specific primer PCR for *M. javanica*, *M. arenaria*, and *M. incognita* species. Samples producing bands of different lengths with primers specific to these species were subjected to PCR with the general D2/D3 primers for species diagnosis and sent for sequence analysis.

**Visualization of molecularly identified nematode species through agarose gel electrophoresis method**

For agarose gel electrophoresis of PCR, buffer was used to prepare the agarose gel. Six microlitres of loading buffer and 10 microlitres of PCR product mixture were pipetted into wells of the prepared agarose gel. The PCR products were electrophoresed and then ethidium bromide was applied for 15 minutes to visualise the bands. After washing the stained gel with distilled water, the bands were examined and photographed under ultraviolet light in a transilluminator (Sambrook et al., 1989).

## Results

### Infection status of root-knot nematodes (*Meloidogyne* spp.) on weed species in banana

When examining the banana plantations, both under cover and in open fields, in the Mediterranean Region, a total of 151 sampling fields were surveyed, covering 50.8 hectares in Mersin, 46.2 hectares in Antalya, 8.2 hectares in Hatay, and 75.6 hectares in Adana. Specifically, the districts of Akdeniz and Erdemli in Mersin, Alanya and Gazipaşa in Antalya, Arsuz and Erzin in Hatay, and Ceyhan and Yüreğir in Adana were investigated, revealing the highest nematode infections in weed populations. The proportion of nematode infections in weeds was found to be 44.54% in covered banana plantations and 34.79% in open fields (Table 2).

Table 2. Infection status of root-knot nematodes (*Meloidogyne* spp.) in weed populations examined in covered and open banana production in the Mediterranean Region for 2021-2022

| | | Covered banana | | | | Open field banana | | | |
|---|---|---|---|---|---|---|---|---|---|
| Provinces | Districts | Studied area (ha) | Studied area (number) | Infected area (number) | Infection (%) | Studied area (ha) | Studied area (number) | Infected area (number) | Infection (%) |
| Mersin | Akdeniz | 5.0 | 5 | 5 | 100.00 | | | | |
| | Anamur | 10.9 | 20 | 4 | 20.00 | 0.6 | 2 | 0 | - |
| | Aydıncık | 0.7 | 2 | 1 | 50.00 | | | | |
| | Bozyazı | 2.9 | 8 | 1 | 12.50 | 0.7 | 3 | 0 | - |
| | Erdemli | 9.8 | 16 | 13 | 81.25 | 1.0 | 2 | 0 | - |
| | Silifke | 7.5 | 10 | 6 | 60.00 | | | | |
| | Tarsus | 11.7 | 7 | 4 | 57.15 | | | | |
| Antalya | Alanya | 4.6 | 5 | 2 | 40.00 | 25.5 | 13 | 8 | 61.54 |
| | Gazipaşa | 2.0 | 2 | 1 | 50.00 | 3.6 | 3 | 0 | - |
| | Manavgat | 10.5 | 6 | 0 | - | | | | |
| Hatay | Arsuz | 4.2 | 10 | 7 | 70.00 | | | | |
| | Erzin | 4.0 | 4 | 3 | 75.00 | | | | |
| Adana | Ceyhan | 2.3 | 1 | 1 | 100.00 | | | | |
| | İmamoğlu | 2.1 | 2 | 0 | - | | | | |
| | Karataş | 38.4 | 14 | 3 | 21.43 | | | | |
| | Sarıçam | 1.6 | 2 | 0 | - | | | | |
| | Seyhan | 7.5 | 5 | 2 | 40.00 | | | | |
| | Yumurtalık | 9.7 | 5 | 1 | 20.00 | | | | |
| | Yüreğir | 14.0 | 4 | 3 | 75.00 | | | | |
| | TOTAL | 149.4 ha | 128 | 57 | 44.54 | 31.4 ha | 23 | 8 | 34.79 |

As a result of survey studies conducted in banana production areas, it was determined that out of a total of 1617 examined weed numbers, roots of 300 weeds (18.55%) were infected with root-knot nematodes. On plant family, the highest infection rates with root-knot nematodes were observed in Amaranthaceae (33.59%), Apiaceae (33.33%), Geraniaceae (33.33%), Malvaceae (32.41%), Portulacaceae (33.85%), and Solanaceae (22.45%). Among the 24 plant families surveyed, nematode infection was identified in 13 families (Table 3).

Table 3. Distribution of weeds examined in banana production areas of the Mediterranean Region according to plant families and infected status of plant families with root-knot nematodes (*Meloidogyne* spp.) for 2021-2022

| Family | Weed species (number) | Proportion (%) | Studied weeds (number) | Infected weeds (number) | Infected proportion (%) |
|---|---|---|---|---|---|
| Amaranthaceae | 5 | 9.62 | 393 | 132 | 33.59 |
| Apiaceae | 1 | 1.92 | 3 | 1 | 33.33 |
| Asteraceae | 3 | 5.77 | 171 | 8 | 4.68 |
| Boraginaceae | 2 | 3.85 | 24 | 1 | 4.17 |
| Brassicaceae | 3 | 5.77 | 54 | 0 | 0.00 |
| Caryophyllaceae | 1 | 1.92 | 12 | 1 | 8.33 |
| Convolvulaceae | 2 | 3.85 | 15 | 0 | 0.00 |
| Cucurbitaceae | 1 | 1.92 | 18 | 2 | 11.11 |
| Cyperaceae | 1 | 1.92 | 21 | 0 | 0.00 |
| Equisetaceae | 1 | 1.92 | 3 | 0 | 0.00 |
| Euphorbiaceae | 6 | 11.54 | 81 | 15 | 18.52 |
| Fabaceae | 1 | 1.92 | 21 | 0 | 0.00 |
| Geraniaceae | 1 | 1.92 | 3 | 1 | 33.33 |
| Malvaceae | 2 | 3.85 | 108 | 35 | 32.41 |
| Oxalidaceae | 1 | 1.92 | 63 | 0 | 0.00 |
| Papaveraceae | 1 | 1.92 | 6 | 0 | 0.00 |
| Plantaginaceae | 3 | 5.77 | 12 | 1 | 8.33 |
| Poaceae | 8 | 15.38 | 144 | 5 | 3.47 |
| Polygonaceae | 1 | 1.92 | 3 | 0 | 0.00 |
| Portulacaceae | 1 | 1.92 | 192 | 65 | 33.85 |
| Primulaceae | 1 | 1.92 | 3 | 0 | 0.00 |
| Ranunculaceae | 1 | 1.92 | 9 | 0 | 0.00 |
| Solanaceae | 2 | 3.85 | 147 | 33 | 22.45 |
| Urticaceae | 3 | 5.77 | 111 | 0 | 0.00 |
| TOTAL | 52 species | 100.00 | 1617 weeds | 300 weeds | 18.55 |

Surveys conducted in banana production areas examined 52 different weed species within a total of 151 areas for both open-field and covered plantations. Among these, the most extensively studied weed species are *A. retroflexus*, *P. oleracea*, *S. nigrum*, *Malva* spp. (Malvales: Malvaceae), and *Conyza* spp. (Asterales: Asteraceae). The number of weed species sampled in banana production areas is thought to be directly related to the root-knot nematode infections in the weed roots, resulting in more accurate results. In this context, when evaluating at the area-based infection of root-knot nematodes, *A. retroflexus* was determined to have an infection rate of 46.34%, *P. oleracea* 40.63%, and *S. nigrum* 37.84%, establishing them as the dominant species within the surveyed areas. Based on the formation of galls in the roots of weed species, *A. retroflexus*, *P. oleracea*, and *S. nigrum* were recorded with the highest infection rates, at 40.65%, 33.85%, and 22.52%, respectively. Additionally, the highest root-knot nematode infections were identified in *Malva* spp. (32.38%), *Mercurialis annua* L. (Malpighiales: Euphorbiaceae) (26.67%), *P. angulata* (22.22%), *A. viridis* (36.67%), *A. spinosus* (41.67%), *Abutilon theophrasti* Medik. (Malvales: Malvaceae) (33.33%), *Erodium cicutarium* (L.) L'Hér. ex Aiton (Geraniales: Geraniaceae) (33.33%), *E. heterophylla* (66.67%), *Kickxia commutata* (Bernh. ex Rchb.) Fritsch (Lamiales: Plantaginaceae) (33.33%), and *Visnaga daucoides* Gaertn. (Apiales: Apiaceae) (33.33%). A total of 22 weed species were found to be infected with root-knot nematodes (Table 4).

Table 4. Percentage of root-knot nematode (Meloidogyne spp.) infection in weed species examined in banana production areas in the Mediterranean Region during 2021-2022

| Weed species | Family | EPPO Codes | Field (number) | Infected field (number) | *Infected proportion (%) | Weeds (number) | Infected weeds (number) | **Infected proportion (%) |
|---|---|---|---|---|---|---|---|---|
| *Amaranthus retroflexus* L. | Amaranthaceae | AMARE | 82 | 38 | 46.34 | 246 | 100 | 40.65 |
| *Portulaca oleracea* L. | Portulacaceae | POROL | 64 | 26 | 40.63 | 192 | 65 | 33.85 |
| *Solanum nigrum* L. | Solanaceae | SOLNI | 37 | 14 | 37.84 | 111 | 25 | 22.52 |
| *Malva* spp. | Malvaceae | MALSS | 35 | 15 | 42.86 | 105 | 34 | 32.38 |
| *Conyza* spp. | Asteraceae | CNDSS | 33 | 0 | 0.00 | 99 | 0 | 0.00 |
| *Chenopodium album* L. | Amaranthaceae | CHEAL | 24 | 4 | 16.67 | 72 | 9 | 12.50 |
| *Oxalis corniculata* L. | Oxalidaceae | OXACO | 21 | 0 | 0.00 | 63 | 0 | 0.00 |
| *Sonchus oleraceus* L. | Asteraceae | SONOL | 19 | 4 | 21.05 | 57 | 8 | 14.04 |
| *Pilea microphylla* (L.) Liebm. | Urticaceae | PILMI | 18 | 0 | 0.00 | 54 | 0 | 0.00 |
| *Echinochloa crus-galli* (L.) P.Beauv. | Poaceae | ECHCG | 16 | 1 | 6.25 | 48 | 2 | 4.17 |
| *Cardamine occulta* Hornem. | Brassicaceae | 1CARG | 15 | 0 | 0.00 | 45 | 0 | 0.00 |
| *Mercurialis annua* L. | Euphorbiaceae | MERAN | 15 | 5 | 33.33 | 45 | 12 | 26.67 |
| *Parietaria judaica* L. | Urticaceae | PAIDI | 14 | 0 | 0.00 | 42 | 0 | 0.00 |
| *Setaria verticillata* (L.) P.Beauv. | Poaceae | SETVE | 13 | 1 | 7.69 | 39 | 3 | 7.69 |
| *Physalis angulata* L. | Solanaceae | PHYAN | 12 | 3 | 25.00 | 36 | 8 | 22.22 |
| *Amaranthus viridis* L. | Amaranthaceae | AMAVI | 10 | 5 | 50.00 | 30 | 11 | 36.67 |
| *Amaranthus spinosus* L. | Amaranthaceae | AMASP | 8 | 4 | 50.00 | 24 | 10 | 41.67 |
| *Amaranthus albus* L. | Amaranthaceae | AMAAL | 7 | 1 | 14.29 | 21 | 2 | 9.52 |
| *Heliotropium europaeum* L. | Boraginaceae | HEOEU | 7 | 1 | 14.29 | 21 | 1 | 4.76 |
| *Cyperus rotundus* L. | Cyperaceae | CYPRO | 7 | 0 | 0.00 | 21 | 0 | 0.00 |
| *Digitaria sanguinalis* (L.) Scop. | Poaceae | DIGSA | 7 | 0 | 0.00 | 21 | 0 | 0.00 |
| *Euphorbia nutans* Lag. | Euphorbiaceae | EPHNU | 7 | 0 | 0.00 | 21 | 0 | 0.00 |
| *Melilotus officinalis* (L.) Pall. | Fabaceae | MEUOF | 7 | 0 | 0.00 | 21 | 0 | 0.00 |
| *Cucumis melo* var. *agrestis* Naudin. | Cucurbitaceae | CUMMG | 6 | 2 | 33.33 | 18 | 2 | 11.11 |
| Senecio vernalis Waldst. & Kit. | Asteraceae | SENVE | 5 | 0 | 0.00 | 15 | 0 | 0.00 |
| *Urtica urens* L. | Urticaceae | URTUR | 5 | 0 | 0.00 | 15 | 0 | 0.00 |
| *Stellaria media* (L.) Vill. | Caryophyllaceae | STEME | 4 | 1 | 25.00 | 12 | 1 | 8.33 |
| *Convolvulus arvensis* L. | Convolvulaceae | CONAR | 4 | 0 | 0.00 | 12 | 0 | 0.00 |
| *Setaria viridis* (L.) P.Beauv. | Poaceae | SETVI | 4 | 0 | 0.00 | 12 | 0 | 0.00 |
| *Sorghum halepense* (L.) Pers. | Poaceae | SORHA | 4 | 0 | 0.00 | 12 | 0 | 0.00 |
| *Ranunculus muricatus* L. | Ranunculaceae | RANMU | 3 | 0 | 0.00 | 9 | 0 | 0.00 |
| *Chrozophora tinctoria (*L.) A.Juss. | Euphorbiaceae | CRZTI | 2 | 1 | 50.00 | 6 | 1 | 16.67 |
| *Capsella bursa-pastoris* (L.) Medik. | Brassicaceae | CAPBP | 2 | 0 | 0.00 | 6 | 0 | 0.00 |
| *Echinochloa colonum* (L.) Link | Poaceae | ECHCO | 2 | 0 | 0.00 | 6 | 0 | 0.00 |
| *Fumaria officinalis* L. | Papaveraceae | FUMOF | 2 | 0 | 0.00 | 6 | 0 | 0.00 |
| *Veronica arvensis* L. | Plantaginaceae | VERAR | 2 | 0 | 0.00 | 6 | 0 | 0.00 |
| *Abutilon theophrasti* Medik. | Malvaceae | ABUTH | 1 | 1 | 100.00 | 3 | 1 | 33.33 |
| *Erodium cicutarium* (L.) L'Hér. ex Aiton | Geraniaceae | EROCI | 1 | 1 | 100.00 | 3 | 1 | 33.33 |
| *Euphorbia heterophylla* L. | Euphorbiaceae | EPHHL | 1 | 1 | 100.00 | 3 | 2 | 66.67 |
| *Kickxia commutata* (Bernh. ex Rchb.) Fritsch | Plantaginaceae | KICCO | 1 | 1 | 100.00 | 3 | 1 | 33.33 |
| *Visnaga daucoides* Gaertn. | Apiaceae | AMIVI | 1 | 1 | 100.00 | 3 | 1 | 33.33 |
| *Anagallis arvensis* L. | Primulaceae | ANGAR | 1 | 0 | 0.00 | 3 | 0 | 0.00 |
| *Dactyloctenium aegyptium* (L.) Willd. | Poaceae | DTTAE | 1 | 0 | 0.00 | 3 | 0 | 0.00 |
| *Eleusine indica* (L.) Gaertn. | Poaceae | ELEIN | 1 | 0 | 0.00 | 3 | 0 | 0.00 |

Table 4. Continued

| Weed species | Family | EPPO Codes | Field (number) | Infected field (number) | *Infected proportion (%) | Weeds (number) | Infected weeds (number) | **Infected proportion (%) |
|---|---|---|---|---|---|---|---|---|
| *Equisetum arvense* L. | Equisetaceae | EQUAR | 1 | 0 | 0.00 | 3 | 0 | 0.00 |
| *Euphorbia helioscopia* L. | Euphorbiaceae | EPHHE | 1 | 0 | 0.00 | 3 | 0 | 0.00 |
| *Euphorbia prostrata* Aiton | Euphorbiaceae | EPHPT | 1 | 0 | 0.00 | 3 | 0 | 0.00 |
| *Ipomoea* spp. | Convolvulaceae | IPOSS | 1 | 0 | 0.00 | 3 | 0 | 0.00 |
| *Lithospermum arvense* L. | Boraginaceae | LITAR | 1 | 0 | 0.00 | 3 | 0 | 0.00 |
| *Polygonum aviculare* L. | Polygonaceae | POLAV | 1 | 0 | 0.00 | 3 | 0 | 0.00 |
| *Sinapis arvensis* L. | Brassicaceae | SINAR | 1 | 0 | 0.00 | 3 | 0 | 0.00 |
| *Veronica montana* L. | Plantaginaceae | VERMO | 1 | 0 | 0.00 | 3 | 0 | 0.00 |

* The higher number of samples from the surveyed of banana production area, the more accurate infection rate of root-knot nematodes (*Meloidogyne* spp.) shows in weed species.
** The greater the number of weed species sampled in the surveyed areas, the more accurately the infection rate of root-knot nematodes (*Meloidogyne* spp.) shows on weeds.

**The host status of root-knot nematodes (*Meloidogyne* spp.) assessed through molecular methods**

During the surveys, samples were taken from the roots of weeds growing within a 2 m radius of the banana plants. These samples, exhibiting galls on the roots, were taken to the laboratory. The roots of the weeds studied were subjected to molecular analysis to identify the species of root-knot nematodes. As a result, the host situation of *M. javanica*, *M. incognita* and *M. arenaria* nematodes on different weed species was revealed (Figure 1).

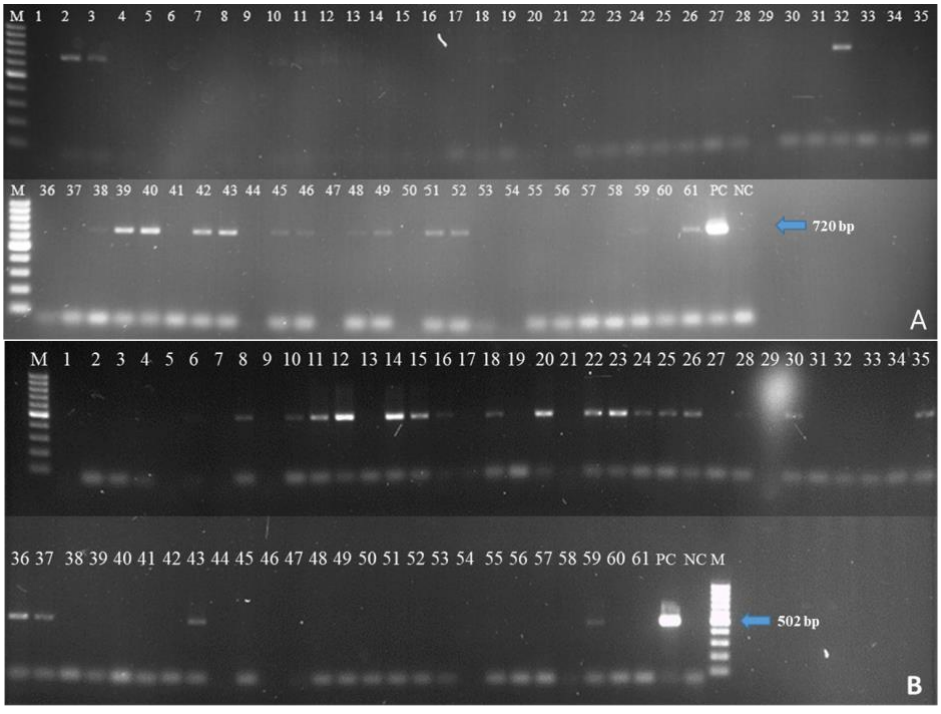


Figure 1. Molecular identification of *Meloidogyne* species in weed roots during surveys (*Meloidogyne javanica* (A); *Meloidogyne incognita* (B)) (M: Molecular marker, 1-61: DNAs obtained from weed roots, PC: Positive control, NC: Negative control).

After molecular studies, root-knot nematodes in the roots of weed samples evaluated in laboratory, such as *A. theophrasti*, *A. albus*, *A. retroflexus*, *A. spinosus*, *A. viridis*, *K. commutata*, *Malva neglecta* Wallr. (Malvales: Malvaceae), *M. sylvestris*, *M. annua*, *P. oleracea*, *S. nigrum*, and *Sonchus oleraceus* L., (Asterales: Asteraceae) were successfully diagnosed through molecular analyses. However, despite the formation of galls on the roots of other weed species collected from the field, molecular analysis did not show any results, as the diagnoses were based on the evaluation of nematode egg masses in weed roots.

## Discussion

In banana production, it has been reported that the growth of weeds prevents the initial development of banana seedlings, and some weeds also act as hosts for diseases and pests (Isaac et al., 2007; Fongod et al., 2010). Knowing the distribution, community status and floristic richness of the weed flora in open field and greenhouse banana plantations prevent yield losses. It is also important to understand which pests these weed species have as hosts. In countries with significant banana plantations, such as Colombia and Brazil, weed species have been reported to cause problems and damage crop production (Moura Filho et al., 2015; Quintero-Pertúz et al., 2020). In the banana fields of Türkiye, 68 weed species from 25 families have been identified, with Poaceae, Amaranthaceae and Euphorbiaceae being the top three. The weed species identified for banana fields were similar to those identified in surveys of weed roots for root-knot nematodes in banana fields. In fact, *Cardamine occulta* Hornem., *Amaranthus* spp., *P. oleracea*, *Conyza* spp., and *Oxalis corniculata* L. were among the most common weed species (Torun et al., 2023). Specifically, the interaction and host status of *Meloidogyne* spp., one of the main problems in banana production in the Mediterranean Region of Türkiye, with weeds, have been revealed by this study (Elekcioğlu et al., 2014; Özarslandan & Dinçer, 2015; Nacar & Özarslandan, 2021; Kalay Sarı et al., 2023).

The study found that *A. albus*, *A. retroflexus*, *A. spinosus*, *A. viridis*, *Cucumis melo* var. *agrestis* Naudin. (Cucurbitales: Cucurbitaceae), *P. oleracea* and *S. nigrum* are suitable hosts for the nematode species *M. javanica* and *M. incognita*. In fact, similar studies around the world have identified *M. javanica* and *M. incognita* as hosts for these weed species (Jain et al., 1983; Quénéhervé et al., 2006; Kaur et al., 2007; Brito et al., 2008; Rich et al., 2008; Singh et al., 2010; Kokalis-Burelle & Rosskopf, 2012; Faske, 2013; Ntidi et al., 2016). Similarly, in recent surveys, only *M. javanica* was found in the roots of *E. cicutarium*, *K. commutata*, and *S. oleraceus*, while only *M. incognita* was observed in the roots of *A. theophrasti*, *M. sylvestris*, *M. neglecta*, and *M. annua* (Goodey et al., 1965; Rich et al., 2008; Akyazı & Felek, 2022). The results of this study on host status are consistent with many other studies in the literature. Although root-knot nematodes are a known problem in banana fields (Sudha & Prabhoo, 1983; Saeed et al., 1988). The study of nematode infections in weed roots showed that *M. arenaria* did not act as a host in any weed species when analysed by molecular methods. However, this does not imply a lack of potential host interactions, as the presence of specific nematode species may vary depending on banana varieties, cultivars, and growing conditions. Because nematode populations always interact with plants (De Waele & Davide, 1999). Other studies have reported that nematode infected weed species do not act as hosts all the time or do not reproduce for other nematode species such as *R. similis*, *H. multicinctus* and *P. goodeyi* (Tedford & Fortnum, 1988; Quénéhervé et al., 2006). Despite some similarities observed in studies on weed species, it has been suggested that the major banana nematodes sometimes have a limited host range in these areas, infecting only a few plants depending on environmental conditions (Blake, 1972).

Consequently, weeds are potential reservoirs that can contribute to the rapid establishment of root-knot nematodes in bananas. A total of 151 sampling points were surveyed in the Mediterranean region, including indoor and outdoor production areas in Mersin, Antalya, Hatay and Adana. Surveys showed that the highest levels of root-knot nematode infection occurred in weeds of the Amaranthaceae, Apiaceae, Geraniaceae, Malvaceae, Portulacaceae and Solanaceae families. However, other studies have reported nematode development in prominent plant families such as Euphorbiaceae, Poaceae, and Solanaceae (Araya & De Waele, 2005; Quénéhervé et al., 2006; Duyck et al., 2009; Gebremichael, 2015). Regarding banana yield, it has been reported that if low population levels of *Meloidogyne* species observed on

*Amaranthus* sp., *S. nigrum*, *Crassocephalum crepidioides* (Benth.) S. Moore (Asterales: Asteraceae), *Commelina benghalensis* L. (Commelinales: Commelinaceae) and *E. indica* are not effectively managed, significant yield losses in bananas could occur in the future (Jonathan & Rajendran, 2000).

Weed control is a recommended management practice in banana plantations. Failure to control weeds can lead to an increase in nematode populations. Compared to open fields, daily irrigation, farm manures, and high humidity in greenhouses contribute to the population of weeds, thereby supporting the continued life cycle of nematode populations. In general, banana plantations have a rich exotic weed flora. It is therefore believed that integrated weed management (IWM), which involves the control of weed populations can reduce nematode densities. It is also considered that weed management indirectly plays an effective role in nematode management.

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*Original article (Orijinal araştırma)*

# The effectiveness of some rhizobacteria on *Meloidogyne incognita* (Kofoid & White, 1919) Chitwood, 1949 (Nematoda: Meloidogynidae) in cucumber plants[1]

Bazı rizobakterilerin hıyar bitkisinde *Meloidogyne incognita* (Kofoid & White, 1919) Chitwood, 1949 (Nematoda: Meloidogynidae)'ya karşı etkinliği

**Deniz YAŞAR[2]** **Galip KAŞKAVALCI[2*]**

## Abstract

In this study, the possibilities of using 3 specific rhizobacteria isolates for the control of *Meloidogyne incognita* (Kofoid & White, 1919) Chitwood, 1949 (Nematoda: Meloidogynidae) in cucumber plants of Beith alpha cultivar were investigated in 2023. The variables of the climate chamber experiment were seedling and seed treatments of the specific bacterial isolates and the QST713 (Serenade®) commercial isolate, nematode (1500 J2/pot) and non-nematode treatments, as well as positive and negative control treatments. As a result of the study, when the rate of root galling on cucumber roots was determined according to the Zeck scale, the most successful treatment in decreasing root galling was the seedling treatment of KD29 isolate (2.64), while the highest rate of root galling was observed in the positive control (6.27). When the bacterial treatments were compared with the positive control, it was observed that all bacterial treatments had a decreasing effect on the number of egg mass. When the effects of the treatments on the reproduction rate of the J2 population were analyzed, it was found that seedling treatments of isolate KD238 (0.69) and commercial isolate QST713 (0.86) had a decreasing effect on the J2 reproduction rate in the soil. As a result of the laboratory experiment, it was determined that KD157, KD238 and KD29 isolates had 42.25, 33.98 and 27.77% mortality effect on J2s after 96 hours, respectively. However, especially considering the decrease in the J2 population in the soil, the amount of root growth and the decrease in the number of egg mass, these bacteria stimulate the induced systemic resistance (ISR).

**Keywords:** *Bacillus thuringiensis*, *Pantoea* spp., PGPR, *Pseudomonas* spp., root-knot nematodes

## Öz

Bu çalışmada, 3 adet özgün rizobakteri izolatının, Beith alpha çeşidi hıyar bitkisinde *Meloidogyne incognita* (Kofoid & White, 1919) Chitwood, 1949 (Nematoda: Meloidogynidae) ile mücadelede kullanım olanakları 2023 yılında araştırılmıştır. Yapılan çalışmalarda bakteri uygulamalarının *M. incognita*'ya karşı etkinliği iklim odası ve laboratuvar denemesi yapılarak değerlendirilmiştir. İklim odası denemesinin karakterlerini özgün bakteri izolatlarının ve QST713 (Serenade®) ticari izolatının fide ve tohum kaplama uygulamaları, bu uygulamaların nematodlu (1500 J2/saksı) ve nematodsuz uygulamaları, pozitif ve negatif kontrol uygulamaları oluşturmuştur. Deneme sonunda, hıyar köklerindeki urlanma oranı Zeck skalasına göre değerlendirildiğinde, köklerdeki urlanma miktarını azaltma konusunda en başarılı uygulama KD29 izolatının (2.64) fide uygulaması olurken, en yüksek urlanma miktarı pozitif kontrolde (6.27) görülmüştür. Bakteri uygulamaları pozitif kontrol ile kıyaslandığında, yumurta kümesi oluşumu üzerinde tüm bakteri uygulamalarının azaltıcı etkiye sahip olduğu görülmüştür. Yapılan uygulamaların, J2 popülasyonunun üreme oranı üzerindeki etkileri araştırıldığında, KD238 (0.69) izolatı ve QST713 (0.86) ticari izolatının fide uygulamalarının, topraktaki J2 üreme oranı üzerinde azaltıcı etkiye sahip olduğu saptanmıştır. Yapılan laboratuvar denemesi sonucunda KD157, KD238 ve KD29 izolatlarının 96 saat sonunda J2'ler üzerinde sırasıyla %42.25, 33.98 ve 27.77 oranında öldürücü etkiye sahip olduğu saptanmıştır. Ancak özellikle topraktaki J2 popülasyonunun azalması, kökte oluşan ur miktarı ve yumurta kümesi sayılarındaki azalma göz önüne alındığında, bu bakterilerin uyarılmış sistemik dayanıklılığı (ISR) teşvik ettiği düşünülmektedir.

**Anahtar sözcükler:** *Bacillus thuringiensis*, *Pantoea* spp., PGPR, *Pseudomonas* spp., kök-ur nematodları

[1] This article has been drawn up from the first author's Master Science thesis.

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## Introduction

Cucumber is an annual plant species that grows in warm to subtropical climates. Cucumber, *Cucumis sativus* L. is a member of the Cucurbitaceae (Cucurbitales) family and is cultivated in most parts of the world as a rich source of vitamins and minerals. Cucumber is the most cultivated vegetable after tomato, watermelon, and onion, with a production of around 1.9 million tons in Türkiye (TÜİK, 2022). According to FAO statistics, cucumber production was 1.890.160 tons worldwide in 2021. China has the highest cucumber production in the world with 75.547.733 tons. Türkiye is the second cucumber growing country, followed by China (FAO, 2021). However, farmers need to manage important pests and diseases that prevent them from achieving the expected yields in cucumber production. Root-root nematodes, *Meloidogyne* spp. Göldi, 1892 (Nematoda: Meloidogynidae) have an important place among these pests. Root-knot nematodes spend part of their life in the soil as egg or J2 form. As a result of the feeding of J2s on the roots, root-knot formation is observed. The formed knots block the plant's absorbance of water and nutrients from the soil. Consequently, the plant becomes stunted, growth and development are impaired and fruit quality decreases (Echeverrigaray et al., 2010).

When necessary, precautions are not taken in agricultural areas where root-knot nematodes are contaminated with vegetables, crop losses depend on the intensity of the pest and the type and sensitivity of the plant cultivated. The crop losses can generally reach up to 15-85% in vegetables (Anonymous, 2008), and 16-47% in cucumber plants grown under greenhouse conditions (Netscher & Sikora, 1990). Different management methods are used to minimize the damage of plant parasitic nematodes that cause such crop losses in agricultural areas. Among these methods, nematicides has an important place in chemical control, which has a critical role in the global market with an annual share of 1.3 billion dollars (Oka, 2020). However, although chemical control is the first choice of farmers due to its ease of application and cheapness, it does not produce long-term and long-lasting results on plant parasitic nematodes.

On the other hand, biological management is one of the alternative control methods that have been intensively studied in recent years. In biological control against root-knot nematodes, bacteria living in the rhizosphere, called plant growth-promoting rhizobacteria (PGPR), have an important potential as biological control agent (Paul & Lade, 2014). The mechanism of action of this group, called plant growth-promoting rhizobacteria (PGPR), is quite broad. These mechanisms can be classified as direct antagonistic effect and indirect effect. Direct antagonistic effects include inhibition of nematode populations by producing toxins, enzymes, and other metabolic components, while indirect effects include activating mechanisms between the plant and nematode (promotion of systemic resistance), competition for nutrients, and reducing populations by regulating nematode behavior. The toxins produced by rhizobacteria inhibit nematode hatching, suppress their reproduction, or directly cause their death (Tian et al., 2007).

PGPR bacteria, which have an important place among biological control agents and are also used as biopreparations, are an alternative that does not cause residue problems compared to chemical control and supports plant growth. In this study, it was aimed to analyze the possibilities of using specific rhizobacteria preparations in the control of root-knot nematodes *in vivo* and *in vitro* trials.

## Materials and Methods

### Nematode culture

The root-knot nematode population used in the experiment was obtained by reproduction of *Meloidogyne incognita* (Kofoid & White, 1919) Chitwood, 1949 (Nematoda: Meloidogynidae) pure culture in Nematology climate chamber. Beith alpha cucumber variety, which is sensitive to root-knot nematode, was used for population multiplication. Seedlings were transplanted into 1:1 ratio of sand and clay sand and soil mixture in half-liter plastic pots at the 3-leaf stage. 1500 *M. incognita* J2 were introduced through holes drilled near the root collar of the plants. The pure culture was grown in a climate chamber at 16:8

photoperiod and 27±3ºC. Plants were harvested 8 weeks after nematode inoculation. Plant roots were gently cleaned from the soil and rinsed. Egg masses on the roots were collected and J2 were obtained by using the improved Baermann funnel technique. The J2 were kept at +4ºC to be used in the experiments.

**Preparation of bacterial suspensions**

Three specific bacterial isolates (KD29, KD157 and KD238), which were found to be the most successful as a result of *in vitro* PGPR tests, were used in the experiment and these isolates were obtained from the collection of Prof. Dr. Hatice Özaktan from Ege University Faculty of Agriculture, Department of Plant Protection, Bacteriology Laboratory. Serenade® (*Bacillus subtilis* QST713) was used as a control to compare the effectiveness of the bacteria. Bacteria grown on King B medium for 24-48 hours at 24ºC were suspended by adding sterile distilled water. The suspensions obtained for each specific bacterial isolate were adjusted to $OD_{600}$=0.1 (1x $10^8$ cfu/ml) using a spectrophotometer (Akbaba & Özaktan, 2018). Serenade® (*Bacillus subtilis* QST713) commercial isolate was used at a recommended dose of 1000 ml/100 l.

**Identification of bacteria**

Bacteria were cultured on King B medium, and pure colonies grown at 25ºC for 24 hours were suspended in sterile water in eppendorf tubes. Genomic DNA was then isolated by boiling the prepared suspensions at 95°C for 15 minutes. The DNA obtained was PCRed with 27F/1492F primers amplifying the 16S rRNA region. The PCR products obtained were sent for two-way sequence analysis through service procurement. The sequences of the Reverse primer of the incoming sequence files were translated into Forward primer by reverse translation. Both sequence files were aligned according to the ClusterW method with the help of the MEGAX program. Then, the alignment result was compared with the help of BioEdit program, and the Contig Sequence was created by removing unnecessary SNPs and completing the missing SNPs. The obtained Contig sequence was definitively diagnosed with the help of the BLAST program on the NCBI website (Akbaba & Özaktan, 2018).

**Effect of bacterial extracts on J2 immobility and mortality *in vitro* trials**

The experiment was achieved in the Nematology Laboratory of Ege University, Faculty of Agriculture, Department of Plant Protection between February 23 and May 8, 2023. This experiment was carried out to observe whether suspensions obtained from specific bacterial isolates have mortality effects on J2s.

The experiment was established with 6 variables, 4 replicates and repeated twice. In the experiment, 4 well plates with 12 wells each were used. In each well, 100 newly hatched J2 and 1 ml of bacterial suspension were added by micropipette. Since the suspensions contained sterile distilled water, in the experimental conditions sterile distilled water was added to see if this treatment influenced the J2 and 1 ml when applied to the wells. As a negative control, 1 ml of tap water was used. All plates were kept in a shaker (Biosan PSU-20i) at 105 rpm for 96 hours at room temperature of 25±3ºC to avoid bacteria settling to the bottom. Throughout the study, nematode mortality rates were recorded regularly at 24, 48, 72 and 96 hours. The mortality of nematodes was checked by touching the nematode needle and nematodes that did not move were recorded as dead.

**Effect of rhizobacteria on *Meloidogyne incognita* J2 *in vivo* trials**

This study was conducted between 2022 and 2023 in the Nematology Climate Room of Ege University, Faculty of Agriculture, Department of Plant Protection. Cucumber (*Cucumis sativus* L.) seeds of Beith Alpha cultivar and seedlings at 3-leaf stage were used in the experiment. Half-liter pots used in the experiment were filled with a 1:1 mixture of sand and clay soil. The plants used in the study were grown in controlled conditions at 16:8 photoperiod and 27±3ºC in the climate room. The pot experiment was setup with 2 replications with 6 replicates with 18 variables. The experimental variables were seed treatment and soil drenching application of each bacterial isolate (KD29, KD157 and KD238) and Serenade® (*Bacillus*

*subtilis* QST713) commercial isolate, nematode and non-nematode treatments of these treatments, as well as positive control (N+) and negative control (N-) treatments. In half of the variables treated with positive control and bacteria, J2 of *M. incognita* were given to each plant in the amount of 1500 J2 through 5 cm deep holes drilled around the root collar from two different directions. The other half were not given nematode treatment to compare the effectiveness of bacteria and plant growth with the negative control.

Bacterial treatments were applied as follows:

1-Seed treatment

Bacteria grown on King B medium for 24-48 hours at 24ºC were suspended by adding carboxymethyl cellulose (CMC, 1% v/v). The suspensions obtained for each specific bacterial isolate were adjusted to $OD_{600}$=0.1 (1x $10^8$ cfu/ml) using a spectrophotometer. Serenade® (*Bacillus subtilis* QST713) was applied at a recommended dose of 1000 ml/100 l. Cucumber seeds were sterilized with 1% sodium hypochlorite for 1 min and then rinsed three times with sterile distilled water. The sterilized seeds were added to the prepared suspensions and mixed in a shaker for 30 minutes. Bacteria-coated seeds were transferred on dryer sheets and left to dry in a sterile cabinet. At the end of 24 hours, cucumber seeds coated with bacteria were planted in sterile viols filled with sterile peat (Akbaba & Özaktan, 2018). When the plants reached the 3-leaf stage, they were transferred to pots.

2-Soil drenching

Prepared bacterial suspensions were applied to the roots of cucumber seedlings when the seedlings passed the one-leaf stage by injecting 5 ml of the suspension. When the plants reached the 3-leaf stage, they were transferred to pots.

The experiment was finalized 60 days after *M. incognita* application. Throughout the experiment, plant height was measured weekly, and the number of leaves was recorded. At the end of the experiment, to determine the effectiveness of the treatments on the nematode, the roots of J2 treated cucumber roots were analyzed according to the Zeck (1971) scale. Egg masses in the roots were counted to determine whether the bacterial treatments had a reducing effect on the reproduction of J2s in the roots. Also, J2s in the soil samples taken from the pots were counted and the final population of nematodes was recorded. The numbers of knots in the roots, egg masses and the number of J2 in the soil were analyzed. In addition, to determine the plant growth, at the end of the experiment, after the plants were harvested and the roots were cleaned from the soil, the wet weights of the roots were measured with a sensitive scale. The roots and green parts of the plants were dried in an oven at 80ºC for 48 hours. After drying, the dry weights of the roots and green parts were measured with a sensitive scale.

**Data analysis**

R statistical software program was used for the analysis of variance (ANOVA) of the values obtained after the experiment was completed, and the comparison of the averages was made according to LSD test at $p \leq 0.05$ level.

## Results

The identification of root-knot nematodes was made by using the Method of Preparation of Perineal Samples given by Taylor & Netsher (1974) and developed by Hartman & Sasser (1985). At the end of the experiment, the female root nematodes were obtained from the infected roots. When the preparations from the perineal patterns of the females were analyzed, it was identified that the individuals were belonging to *Meloidogyne incognita* (Kofoid & White, 1919) species.

At the end of the experiment, species identification of the specific rhizobacteria isolates used in the experiment was made. The results of the identification are given in Table 1.

Table 1. Specific rhizobacteria isolates identification

| Isolates | Species |
|---|---|
| KD29 | *Pantoea vagans* C (Enterobacterales: Enterobacteriaceae) |
| KD157 | *Bacillus thuringiensis* Berliner (Bacillales: Bacillaceae) |
| KD238 | *Pseudomonas* sp. Migula (Pseudomonadales: Pseudomonadaceae) |

**Effect of bacterial extracts on J2 immobility and mortality *in vitro* trials**

Abbott formula was used to calculate the effect of bacterial isolates on the mortality rate of *Meloidogyne incognita* J2. The results of the experiment are given in Table 2.

Table 2. Reducing effect rate (%) of *in vitro* experiment in the laboratory according to the counts at the end of 96 h

| Treatments | Numbers of active nematodes | | | | | | | | Percent effect (%) |
|---|---|---|---|---|---|---|---|---|---|
| | 24 h | | 48 h | | 72 h | | 96 h | | |
| **Control** | 106.50 | a* | 91.12 | a | 85.75 | a | 76.50 | a | - |
| **Sterile Water** | 105.25 | ab | 91.12 | a | 85.50 | a | 74.00 | a | 3.26 |
| **KD29** | 97.50 | abc | 80.87 | ab | 73.87 | ab | 55.25 | bc | 27.77 |
| **KD238** | 94.87 | bcd | 82.50 | ab | 63.12 | b | 50.50 | bc | 33.98 |
| **QST713** | 92.62 | cd | 82.75 | ab | 66.00 | b | 55.87 | b | 26.96 |
| **KD157** | 85.12 | d | 71.00 | b | 64.12 | b | 42.25 | c | 44.77 |
| **F** | 4,739 | | 2,552 | | 4,989 | | 8,552 | | |
| **p** | <0.0001 | | <0.0001 | | <0.0001 | | <0.0001 | | |
| **df** | 5, 42 | | 5, 42 | | 5, 42 | | 5, 42 | | |

* Means with the same letter are not statistically different according to LSD test (p≤0.05).

According to these results, control and sterile water treatments had the lowest mortality rate. The highest mortality rate was recorded in *Bacillus thuringiensis* KD157 (44.77%), followed by *Pseodumonas* sp. KD238 (33.98%), *Pantoea vagans* KD29 (27.77%) and *B. subtilis* QST713 (26.96%). These results showed that all bacterial treatments had mortality rates on nematodes significantly higher than the control group.

**Effect of rhizobacteria on *Meloidogyne incognita* J2 *in vivo* trials**

In order to determine the effectiveness of the bacterial treatments on the amount of root-knot, the roots of the nematode-treated plants were scored according to the Zeck (1971) scale (Table 3).

Compared to the positive control, it was observed that all treatments decreased the amount of root knots. S.D.29 (57.97%) was found to be the most effective treatment to decrease the amount of root knot. This treatment was closely followed by S.D.238 (56.52%) and S.T.QST713 (55.07%). The other treatments had a decreasing effect on the amount of root knot, respectively; S.T.238 (42.03%), S.T.157 (42.03%), S.D.QST713 (40.58%), S.D.157 (40.58%) and S.T.29 (34.78%). As a result of the experiment, it was observed that all bacterial isolates used in the experiment were found to be effective against the root knots caused by the feeding of *Meloidogyne incognita* in cucumber plants.

At the end of the study, the egg masses in the nematode treated plant roots were counted and the effect of bacterial treatments on egg mass production was determined. As a result of the statistical analysis, none of the treatments were in the same group with the positive control. The highest number of egg masses

was found in the positive control (31.09±12.10) and the lowest number of egg masses was found in the S.D.29 (12.18±5.38) treatment. When the bacterial treatments were compared with the positive control, it was observed that all bacterial treatments had a decreasing effect on egg mass production. S.D.29 (60.82%) treatment had the highest decreasing effect on egg mass production followed by S.D.238 (57.31%), S.T.157 (51.17%) and S.D.157 (50.00%) treatments.

Table 3. Effect of rhizobacteria applications against *Meloidogyne incognita* on cucumber

| Treatments** | Zeck scale index (X±SD) | | Percent effect on root galling | Zeck scale index F (df); p | Egg mass index (X±SD) | | Percent effect on egg masses | Egg mass index F (df); p |
|---|---|---|---|---|---|---|---|---|
| **Positive Control** | 6.27±1.01 | a* | - | | 31.09±12.10 | a* | - | |
| **S.T.29 (N+)** | 4.09±1.30 | b | 34.78 | | 18.91±5.56 | b | 39.18 | |
| **S.D.QST.713 (N+)** | 3.73±2.24 | b | 40.58 | | 15.91±8.42 | bcd | 48.83 | |
| **S.D.157 (N+)** | 3.73±1.19 | bc | 40.58 | | 15.55±7.55 | bcd | 50.00 | |
| **S.T.157 (N+)** | 3.64±1.43 | bc | 42.03 | F (8, 90)= 7,145; p <0.0001 | 15.18±4.85 | bcd | 51.17 | F (8, 90)= 6,694; p <0.0001 |
| **S.T.238 (N+)** | 3.64±1.43 | bcd | 42.03 | | 18.18±4.43 | b | 41.52 | |
| **S.T.QST713 (N+)** | 2.82±0.60 | cde | 55.07 | | 16.82±6.01 | bc | 45.91 | |
| **S.D.238 (N+)** | 2.73±1.49 | de | 56.52 | | 13.27±6.05 | cd | 57.31 | |
| **S.D.29 (N+)** | 2.64±0.92 | e | 57.97 | | 12.18±5.38 | d | 60.82 | |

* Means with the same letter are not statistically different according to LSD test (p≤0.05);

** Abbreviations: S.T.: Seed Treatment, S.D.: Soil Drenching.

For all the nematode treated variables, 1500 J2 was applied to the pots as a starting population. At the end of the experiment, 100 g of soil sample was taken from each pot and *M. incognita* J2 in the soil was sampled. Analysis of variance was applied to the J2 numbers determined and LSD test was performed to determine the effect of treatments on nematode population. The results obtained are given in Table 4.

Table 4. Effects of *Meloidogyne incognita* individuals in soil on J2 number (number/100 g) and reproduction rate

| Treatments** | Number of Nematodes (X±SD) | | F (df); p | Percent effect (%) | RF=Pf/Pi |
|---|---|---|---|---|---|
| **Positive Control** | 3967.27±2451.89 | ab* | | - | 2.64 |
| **S.T.238 (N+)** | 4574.55±4323.60 | a | | -15.33 | 3.05 |
| **S.T.157 (N+)** | 3359.09±2486.85 | abc | | 24.20 | 2.24 |
| **S.D.157 (N+)** | 3007.27±4132.67 | bc | | 41.34 | 2.00 |
| **S.T.QST713 (N+)** | 2327.27±1642.61 | cd | F (8, 90)= 2,260; p <0.05 | 43.17 | 1.55 |
| **S.T.29 (N+)** | 2254.55±1296.43 | cd | | 49.59 | 1.50 |
| **S.D.29 (N+)** | 2000.00±2467.39 | cd | | 67.46 | 1.33 |
| **S.D.QST713 (N+)** | 1290.91±1281.76 | de | | 73.88 | 0.86 |
| **S.D.238 (N+)** | 1036.36±1130.73 | de | | 77.34 | 0.69 |

* Means with the same letter are not statistically different according to LSD test (p≤0.05);

** Abbreviations: S.T.: Seed Treatment, S.D.: Soil Drenching.

When the treatments were compared with the positive control, it was found that all treatments except S.T.238 (-15.33%) influenced the number of *M. incognita* J2 in the soil. S.D.238 (77.34%) was found to be the most effective treatment in decreasing the number of *Meloidogyne incognita* J2 in the soil.

The effects of the treatments on the reproduction rate of the root-knot nematode population were studied. It was determined that S.D.238 (0.69) and S.D.QST713 (0.86) treatments had a reducing effect on the reproduction rate of *M. incognita* J2 in the soil.

Plant height and number of leaves were recorded weekly during the experiment. At the end of the experiment, root growth, root and green parts wet and dry weights were measured to analyze the effect of bacterial treatments on plant growth. However, as a result of the analyses, there was no statistically significant effect of bacterial treatments on the growth and development of cucumber plants.

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## Discussion

Soil drenching treatment of *Pantoea vagans* KD29 bacterial isolate was more effective in reducing the amount of root growth compared to seed coating treatment. Soil drenching and seed treatment of this bacterial isolate had 57.97% and 34.78% decreasing effect on the amount of knot on roots, respectively, when compared to the positive control. Mohamedova et al. (2016), in a similar study using *Pantoea agglomerans* isolate against *M. incognita* in eggplant plants, it was reported that it significantly reduced the number of J2 in plants subjected to root dipping and soil drenching treatments, and in the same study, it was reported that the amount of knots on the roots of plants showed a decreasing effect by 32.4% in the seed treatment and 44.6% in the soil drenching treatment compared to the positive control. As a result of the experiment, when the J2 population in the soil was compared with the positive control, *P. vagans* KD29 isolate had an effect of 67.46% in the soil drenching treatment, while this rate was 49.59% in the seed treatment.

However, when the effects of the treatments on the reproduction rate (RF) of the *M. incognita* population were analyzed, it was determined that *P. vagans* KD29 isolate had no effect on the nematode population in two different treatments. Soil drenching treatment of *P. vagans* KD29 isolate reduced the number of egg clusters by 60.82%. While this rate indicated the highest reduction effect among all treatments, the seed treatment of the same isolate had the lowest reduction effect with 39.18%. Although these results were obtained in the *in vivo* trial in the climate chamber, in the *in vitro* trial, when the J2s in the *P. vagans* KD29 solution were counted at the end of 96 hours, the mortality rate was 27.77% compared to the control group. On the other hand, Gowda et al. (2022) researched the activity of *Bacillus subtilis* DTBS 5, *Pantoea agglomerans* and *Bacillus amyloliquefaciens* DSBA 11 isolates against *M. incognita* in an *in vitro* study. In the *in vitro* study, it was observed that the isolates used at 100% concentration were 91.67% effective on J2 death after 96 hours.

When the treatments of *B. thuringiensis* KD157 bacterial isolate were compared with the positive control, seed coating treatment reduced the amount of root growth by 42.03% and soil drenching treatment decreased the amount of root knot by 40.58%. Elsharkawy et al. (2015), in a study conducted with *B. thuringiensis* CR-371 isolate against *M. incognita* in tomato plants, reported that while the root knotting rate of the plants in positive control group was 24.4%, this rate decreased to 5.5% in the roots of the plants treated with *B. thuringiensis* CR-371. In the present study, soil drenching treatment of *B. thuringiensis* KD157 bacterial isolate had a 41.34% reduction effect on the J2 population in the soil compared to the positive control, while this rate was 24.20% in the seed treatment. These results support the study of Khalil & El-Naby (2018), in which the use of *Bacillus thuringiensis* isolate against *M. incognita* in tomato decreased the number of knots in the root by 66.22% to 78.88% and the nematode population in the soil by 70.63% to 80.45%. In the present study, the seed coating treatment of *B. thuringiensis* KD157 isolate had a 51.17% reducing effect on the number of egg clusters, while this rate was 50.00% in the soil drenching treatment. In the greenhouse trial conducted by Khalil et al. (2012) against *M. incognita* on tomato plants, *B. thuringiensis* isolate reduced the J2 population in the soil by 80.5%, while *B. thuringiensis* prevented egg mass production by 74.9%.

In a similar study conducted by Dawar et al. (2008), *B. thuringiensis* (Bt-10) was tested against *M. javanica* by seed coating and soil drenching on mash bean and cowpea. It was reported that there was no significant difference between the application methods and both methods significantly reduced nematode damage in both plant varieties. Choi et al. (2020), in an *in vivo* trial to research the efficacy of *B. thuringiensis* KYC isolate against *M. incognita*, it was reported that the treatment significantly decreased the egg mass production in tomato plants with fertilizer alone. While these results were obtained in the *in vivo* trial in the climate chamber, when the J2s in the *B. thuringiensis* KD157 bacterial solution used in the *in vitro* trial in the laboratory were counted at the end of 96 hours, the mortality rate was 42.25% compared to the control group. This isolate was the bacterial isolate with the highest lethal effect against *M. incognita* in the *in vitro* trial compared to other treatments. Dawar et al. (2008) conducted an *in vitro* study with *B. thuringiensis* (Bt-10) isolate and found that the isolate eliminated 50% of *M. javanica* J2 survival and egg hatching. In a similar *in vitro* study conducted by Oliveira et al. (2007), it was reported that *B. thuringiensis* isolates reduced the number of J2 of *M. exigua*.

Soil drenching treatment of *Pseudomonas* sp. KD238 bacterial isolate reduced the amount of root growth by 56.52% compared to the positive control, while seed coating treatment reduced the amount of root growth by 42.03%. These results support the results of Kaşkavalcı et al. (2006), who found that seed treatment and seed treatment + soil drenching treatment of *Pseudomonas fluorescens* Pat1 strain reduced the root growth of *M. incognita* by 44% and 39%, respectively, in the climate chamber *in vivo* trial against *M. incognita* in cucumber plants. *Pseudomanas* sp. KD238 isolate had the highest effect on the decrease of *M. incognita* J2 number in the soil because of soil wetting application. As a result of the experiment, when the J2 population in the soil was compared with the positive control, the soil wetting treatment had a 77.34% reduction effect, while this rate was found to be -15.33% in the seed treatment.

When the effects of the treatments on the reproduction rate of the J2 population were examined, it was determined that the soil wetting treatment of *Pseudomanas* sp. KD238 isolate had the highest reducing effect on the J2 reproduction rate in the soil with a value of 0.69. This rate was lower than the reproduction rate of 0.86 of the commercial preparation QST713, which was the control group. These results support the results of Ashoub & Amara (2010), who reported that *P. fluorescens* RR isolate was highly effective in suppressing *M. incognita in vitro* and *in vivo* studies. In the soil drenching treatment of *Pseudomanas* sp. KD238 isolate, a 57.31% decreasing effect on the number of egg masses was seen, while this rate was 41.52% in the seed treatment. When J2s in *Pseudomanas* sp. KD238 bacterial solution were counted at the end of 96 hours in the *in vitro* experiment in the laboratory, it was determined that the mortality rate was 33.98% compared to the control group.

The results of the present study were similar to those of *in vitro* and *in vivo* trials established by Singh et al. (2021) to study the biocontrol potential of *P. fluorescens* against *M. incognita*. It was reported that *P. fluorescens* inhibited *M. incognita* egg hatching by 75% and caused 100% J2 mortality. In the same study, in the *in vivo* trial, *P. fluorecens* isolate was found to reduce egg mass, egg production, number and size of eggs when applied at a dose of $10^9$ (CFU/ml) against *M. incognita*. In a similar study reported by Abd-El-Khair et al. (2019), *P. fluorescens* (Pf1, Pf2) isolates were applied separately to cowpea plants in pots and inhibited the reproduction of *M. incognita* population by 69.8% and 62.3%, respectively. In a similar study by Singh et al. (2021), *P. fluorescens* isolate applied to tomato plants increased the weight of plant roots and shoots. However, in the present study, there was no change in the weight of root-green parts of cucumber plants treated with *Pseudomonas* sp. KD238 isolate compared to the negative control. In a similar study conducted by Almaghrabi et al. (2013) against *M. incognita* in tomato plants, it was reported that plant dry weight and plant height increased, while the amount of knot in the root, egg mass and the number of J2 in the soil decreased in the variables treated with *P. fluorescens* isolate.

When the data obtained were analyzed, it was shown that bacterial isolates did not have a significant effect on plant growth, but seed treatment of bacteria had a slight effect on root growth, plant height and leaf number compared to the negative control. It was found that all bacterial treatments had a decreasing effect on the amount of root-knot infections on the roots of cucumber plants. Soil drenching treatment (57.97%) of *P. vagans* KD29 isolate was the most successful treatment on the decrease in the amount of root knots. In addition, all the bacterial treatments significantly decreased the egg mass production on the roots compared to the positive control. Soil drenching treatments of *P. vagans* KD29 (60.82%) and *Pseudomonas* sp. KD238 isolates (57.31%) were found to have the highest decreasing effect on egg mass formation on roots.

At the end of the experiment, when the bacterial treatments were compared with the positive control, the soil wetting treatment of *Pseudomonas* sp. KD238 isolate (77.34%) was found to be the most effective treatment in reducing the number of *M. incognita* J2 in the soil. It was found that soil drenching treatments of bacterial isolates were more effective in decreasing the egg mass production on the roots and J2 population in the soil than seed treatment. Soil drench treatments of the specific bacterial isolates *P. vagans* KD29 and *Pseudomonas* sp. KD238 were found to be more successful in decreasing the amount of knots and the number of egg masses in the roots than the soil drenching treatment of *B. subtilis* QST713 commercial preparation used as a control. Soil drench treatment of *Pseudomonas* sp. KD238 isolate was more successful in decreasing the reproduction rate of *M. incognita* population in soil than the soil drench treatment of *B. subtilis* QST713 commercial preparation.

*Pseudomonas* sp. KD238 and *P. vagans* KD29 isolates were found to have 33.98% and 27.77% lethal effect on J2s, respectively, in the *in vitro* test. However, these bacteria are thought to promote induced systemic resistance (ISR), especially considering the decrease in the J2 population in the soil, the amount of root knot and the decrease in the number of egg masses. *Pseudomonas* sp. KD238 and *P. vagans* KD29 are important to be studied to understand the mechanism of action of bacterial isolates.

It is thought that the specific bacterial isolates used in this study may be an alternative to the use of nematicides in the control of root-knot nematode, which causes significant damage to cucumber plants. However, it is thought that further studies on the use of these bacterial isolates on cucumber plants in greenhouses and open fields will contribute more to this issue.

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*Original article (Orijinal araştırma)*

# The karyotype studies of some aphid species (Hemiptera: Aphidoidea) from Niğde province in Türkiye[1]

## Türkiye'nin Niğde ilinden bazı yaprakbiti türlerinin (Hemiptera: Aphidoidea) karyotip değerlendirilmesi

**Sümeyye Betül ŞAHİN[2]** **Özhan ŞENOL[2*]**

## Abstract

The biological features of aphids as holocentricity, parthenogenetic reproduction, and telescopic generation have fascinated researchers to conduct chromosomal studies. Because of their chromosomes' holocentricity, the fragmentation fusion leads to karyotypic variations in aphid species. In phytophagous insects like aphids, holocentrism can be related to production of compounds that induce chromosomal variations. In the current study, the evaluation of karyotypes of six aphid species belonging to six genera that infest different host plants at the Niğde Ömer Halisdemir University campus area was conducted between September and November 2022. Evaluated species were *Acyrthosiphon* (*Acyrthosiphon*) *ilka* Mordvilko, 1914, *Aphis* (*Aphis*) *spiraecola* Patch, 1914, *Brachycaudus* (*Brachycaudus*) *helichrysi* (Kaltenbach, 1843), *Cinara* (*Cinara*) *curvipes* (Patch, 1912), *Macrosiphum* (*Macrosiphum*) *rosae* (L., 1758), and *Pterochloroides persicae* (Cholodkovsky, 1898). The *C. curvipes* and *A. ilka* karyotypes were determined for the first time in this study.

**Keywords:** Aphid, chromosome, Hemiptera, karyotype, Türkiye

## Öz

Kromozomlarının holosentrik doğası, partenogenetik üreme, teleskopik jenerasyon gibi biyolojik karakteristik özellikleri, yaprakbitlerini kromozomal çalışmalar için çekici hale getirmektedir. Farklı konak bitkiler için önemli zararlı türler olan yaprakbitleri holosentrik kromozomlara sahiptir. Füzyon veya parçalanma, kromozomlarının holosentrik doğası nedeniyle karyotipik varyasyonlara yol açabilmektedir. Yaprakbitleri gibi fitofag böceklerde holosentrizm, kromozomal varyasyonlara neden olan bileşiklerin üretimi ile ilişkili olabilir. Bu çalışmada, Niğde Ömer Halisdemir Üniversitesi kampüs alanında farklı konak bitkileri istila eden altı cinse ait altı yaprakbiti türünün karyotiplerinin değerlendirilmesi Eylül ve Ekim 2022'de yapılmıştır. Bu türler *Acyrthosiphon* (*Acyrthosiphon*) *ilka* Mordvilko, 1914, *Aphis* (*Aphis*) *spiraecola* Patch, 1914, *Brachycaudus* (*Brachycaudus*) *helichrysi* (Kaltenbach, 1843), *Cinara* (*Cinara*) *curvipes* (Patch, 1912), *Macrosiphum* (*Macrosiphum*) *rosae* (L., 1758) ve *Pterochloroides persicae* (Cholodkovsky, 1898)'dir. *Cinara curvipes* ve *A. ilka'nın* karyotip verileri ilk kez bu çalışmada belirtilmiştir.

**Anahtar sözcükler:** Yaprakbiti, kromozom, Hemiptera, karyotip, Türkiye

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## Introduction

Nearly 6000 aphid species worldwide and 675 aphid species from Türkiye have been described up to date (Görür et al., 2012, 2023; Kök & Özdemir, 2021; Favret, 2024). The aphids have been recorded on almost 25% (nearly 94.000 plant species) of the known species of host plants, but nearly 100 aphid species were evaluated as economically significant. Currently, the chromosome numbers of 1.039 aphid species belonging to 14 families have been reported, comprising nearly 22% of all the described aphid species (Potan & Gautam, 2019; Sharma & Gautam, 2019; Kuznetsova et al., 2021).

The aphid cytological studies started at the beginning of the 20$^{th}$ century (Morgan, 1909). Blackman (1980) presented chromosome numbers of 180 aphid species, and pointed out that diploid chromosome numbers of them range from 6 [*Sarucallis kahawaluokalani* (Kirkaldy, 1907)] to 72 [*Amphorophora* (*Amphorophora*) *sensoriata* Mason, 1923)]. Gautam & Dutta (1994) provided information about the chromosomal compositions of 52 aphid species belonging to 34 genera and 21 of them were reported for the first time. The sex diagnosis and karyotype of *Cavariella aegopodii* (Scopoli, 1763) and *Tuberolachnus salignus* (Gmelin, 1790) were detected in different localities of India in a study by Dhatwalia & Gautam (2009). Although they determined the diploid chromosome number of *C. aegopodii* as 2n=8, 9 and 10, the male diploid chromosome number comprised eight autosomal and a single X chromosome. The common diploid chromosome number of *T. salignus* was 2n=20, but in the Solan region, the population showed variations in diploid chromosome number as 2n=18-20. In a study related to three clones of *Myzus persicae* (Sulzer, 1776) detailed karyotype analyses were conducted by using Hind200 satellite and subtelomeric repeat chromosomal markers. The results of the study showed that clone 1 diploid chromosome number was ten autosomal and double X (2n=12), clone 50 was 2n=13, and clone 70 was 2n=14 (Monti et al., 2012). Rivi et al. (2012) reported cytogenetic data of 66 *M. persicae* populations, infected aubergine, peaches, potato tobacco, and tomato host plants, distributed in different localities of Italy. The researchers indicated that the diploid chromosome number of *M. persicae* generally was 2n=12, but the diploid chromosome number of populations that were collected from tobacco host plants was 2n=11-14. In a study conducted in different regions of India, 27 aphid species belonging to 14 genera were evaluated karyomorphologically. It was determined that the chromosome number varied between 2n=6-18 in the aphid species evaluated (Sharma & Gautam, 2019). Kumari et al. (2022) aimed to give information about the karyotypes of four aphid species that damage to medically significant and common in host plants in India. It was shown that the chromosome number of *Macrosiphum euphorbiae* (Thomas, 1878) infecting *Malva parfiflora* L. (Malvales: Malvaceae) host plant was 2n=10, the chromosome number of *Myzus ornatus* Laing, 1932 infecting *Ajuga integrifolia* Buch.-Ham. ex D.Don (Lamiales: Lamiaceae) host plant was 2n=12, and the chromosome number of *Aphis odinae* (van der Goot, 1917) infecting *Duranta erecta* L. (Lamiales: Verbenaceae) host plant was 2n=8 respectively.

The holocentric structure of the aphid chromosomes results in centromeric activity that diffuses the full length of chromosomes. Thus, the holocentricity in their chromosomes has deep implications for chromosomal development (Normark, 1999; Blackman et al., 2000; Wilson et al., 2003). Holocentric chromosomes have several kinetochores along the length of the chromosome instead of the single centromere that is characteristic of other chromosomes. In 1935, the term holocentric was defined for the first time and currently stands for some features as follows;

i. The monocentric chromosomes show a lack of primary tightness, which corresponds to that of the centromere.

ii. There are several kinetochores at the chromosome axis.

iii. Microtubules move from the metaphase plate towards the poles and are attached to the chromosomes along their entire length. The term holokinetic chromosome stands for the chromatids that do not form the standard V-shaped during the cell division, characteristic of monocentric chromosomes; instead, they separate each other in parallel. Holocentric chromosomes have undergone many changes during the evolution of both animals and plants.

iv.Holocentric chromosomes can stabilize chromosomal fragments through extensive kinetochores, promoting karyotype rearrangements (Mandrioli & Manicardi, 2012; Manicardi et al., 2015).

However, holocentricity can also lead to restrictions for crossing over in homologous chromosomes that are adjacent to each other during meiosis due to the limitation of the number of chiasmas (Mandrioli & Manicardi, 2003, 2012; Melters et al., 2012; Manicardi et al., 2015; Lukhtanov et al., 2018). Both host plants and geographical conditions play important roles in chromosomal variation. Therefore, it is necessary to study the chromosomes of aphids from different host plants and geographical regions. Under these general approaches, this study aimed to determine the chromosome numbers of certain aphid species distributed in the campus area of Niğde Ömer Halisdemir University and to contribute to the karyological characteristics of various aphids.

## Materials and Methods

This study was conducted in the campus area of Niğde Ömer Halisdemir University in 2022. The parthenogenetic, viviparous female individuals were collected from different host plants (Table 1), and aphid species were identified according to the key provided by Blackman & Eastop (2024).

Table 1. The information about studied samples and ant attendance of aphid populations (+: presence of ant attendance; -: absence of ant attendance)

| Sample no. | Host plant | Species | Collection date |
|---|---|---|---|
| S1 | *Rosa* sp. L. (Rosaceae) | *Macrosiphum rosae* L., 1758 | 27. IX. 2022 |
| S2 | *Sonchus* sp. L. (Asteraceae) | *Acyrthosiphon ilka* Mordvilko, 1914 | 29. IX.2022 |
| S3 | *Prunus domestica* L. (Rosaceae) | *Pterochloroides persicae* (Cholodkovsky, 1898) | 30. IX. 2022 |
| S4 | *Cedrus* sp. Rich (Pinaceae) | *Cinara curvipes* (Patch, 1912) | 3. X. 2022 |
| S5 | *Hibiscus* sp. L. (Malvaceae) | *Aphis spiraecola* (Patch, 1914) | 6. X. 2022 |
| S6 | *Lepidium latifolium* L. (Brassicaceae) | *Brachycaudus helichrysi* (Kaltenbach, 1843) | 7. X. 2022 |
| S7 | *Sonchus* sp. Britton & Brown (Asteraceae) | *Acyrthosiphon ilka* Mordvilko, 1914 | 11. X. 2022 |
| S8 | *Acacia* sp. Miller (Fabaceae) | *Aphis spiraecola* Patch, 1914 | 18. X. 2022 |

The slide preparation for karyological studies was conducted as follows (amended from Manicardi et al., 1996);

1. Adult female individuals from each population were dissected primarily in Ringer's saline solution.
2. The embryos were taken into the mini tubes that included a 1% hypotonic solution of potassium chloride and kept for 10 minutes.
3. Embryos were transferred into new sterile mini tubes and centrifuged at 3000 Rpm for 15 minutes.
4. The fixative was added to the mini tubes that included pellets (3: 1 methanol: acetic acid) and then kept in deep freeze at -20°C for 15 minutes.
5. Then each mini tube was centrifuged at 3000 Rpm for 15 minutes.
6. The 4$^{th}$ step was repeated with fresh fixative.
7. The samples were kept in deep freeze at -20°C for 60 minutes.
8. 20 µL of the cell suspension was dropped onto clean slides by pipette at a distance of 30cm and air-dried.
9. Dried slides were kept in a chalet that includes 10% of Giemsa stain for 15 minutes.
10. After the samples were removed from the stain, they were washed and left to dry for 24 hours.

Detection of chromosomes was conducted under the bright field microscope using immersion oil at 100x ocular.

## Results and Discussions

In this study, six aphid samples collected from different host plants from the Niğde Ömer Halisdemir University campus area between September and October 2022 were used and chromosomal data was obtained from viviparous adult females of different species.

***Acyrthosiphon* (*Acyrthosiphon*) *ilka* Mordvilko, 1914**

The diploid chromosomal number of *A.ilka* that was collected from the host plant *Sonchus* sp. was 2n=8 and a single X chromosome (Figure 1 a-b). The idiogram of this species revealed a single X chromosome and two partners long, a partner medium-sized, and a partner of short chromosomes (Figure 1 c).

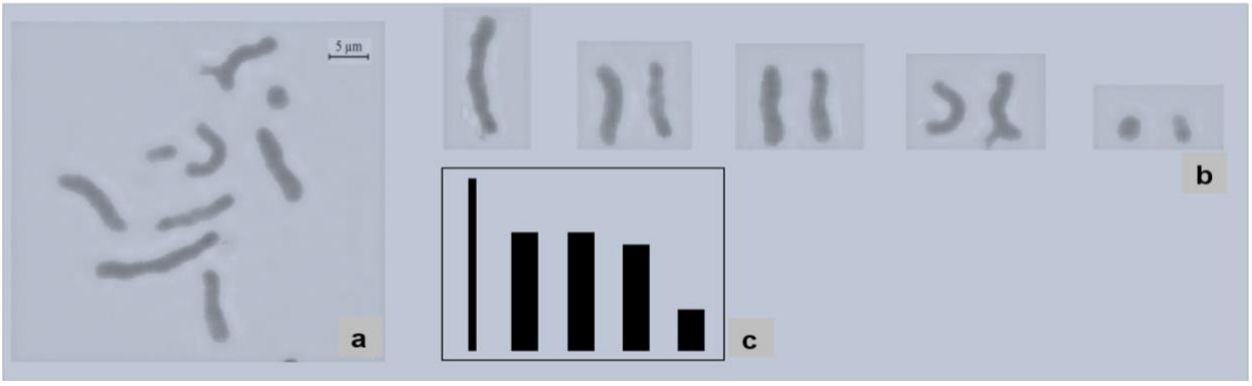


Figure 1. *Acyrthosiphon ilka*: a) Mitotic metaphase chromosomes; b) karyotype; c) idiogram.

***Aphis* (*Aphis*) *spiraecola* Patch, 1914**

The diploid chromosomal number of *A.spiraecola* that was collected from the host plant *Acacia* sp. and *Hibiscus* sp. was 2n=8 (Figure 2 a-b). The idiogram of this species revealed a partner of long, a partner of medium-sized, and two partners of gradually decreasing short chromosomes (Figure 2 c).

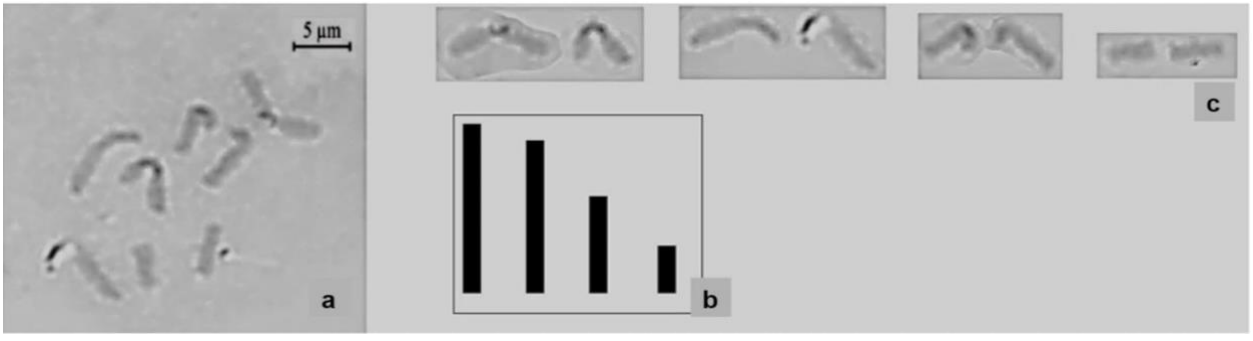


Figure 2. *Aphis spiraecola*: a) Mitotic metaphase chromosomes; b) karyotype; c) idiogram.

***Brachycaudus* (*Brachycaudus*) *helichrysi* (Kaltenbach, 1843)**

The diploid chromosomal number of *B.helichrysi* that was collected from the host plant *Lepidium latifolium* was 2n=12 (Figure 3 a-b). The idiogram of this species revealed two partners of long, two partners of medium size, and two partners of short chromosomes (Figure 3 c).

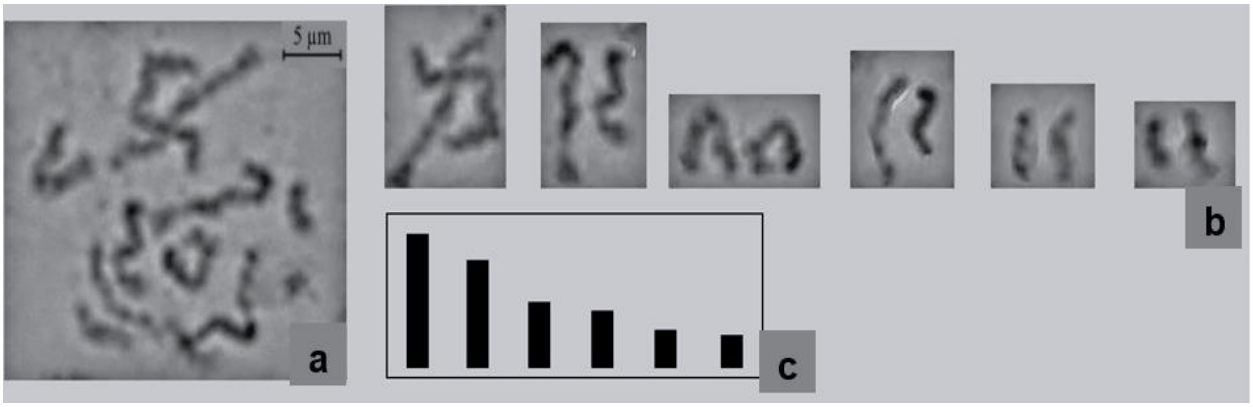


Figure 3. *Brachycaudus helichyrsi*: a) Mitotic metaphase chromosomes; b) karyotype; c) idiogram.

***Cinara* (*Cinara*) *curvipes* (Patch, 1912)**

The diploid chromosomal number of *C*. *curvipes* that was collected from the host plant *Cedrus* sp. was 2n=10 (Figure 4 a-b). The idiogram of this species revealed a partner of long, two partners of medium size, and two partners of short chromosomes (Figure 4 c).

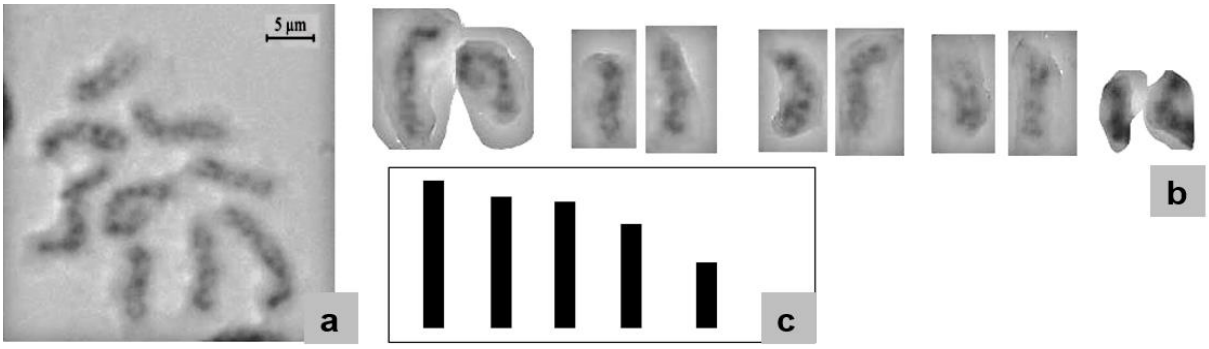


Figure 4. *Cinara curvipes*: a) Mitotic metaphase chromosomes; b) karyotype; c) idiogram.

***Macrosiphum* (*Macrosiphum*) *rosae* (L., 1758)**

The diploid chromosomal number of *M*. *rosae* that was collected from the host plant *Agropyron* sp. was 2n=10 (Figure 5 a-b). The idiogram of this species revealed a partner of long, a partner of medium-sized, and three partners of gradually decreasing short chromosomes (Figure 5 c).

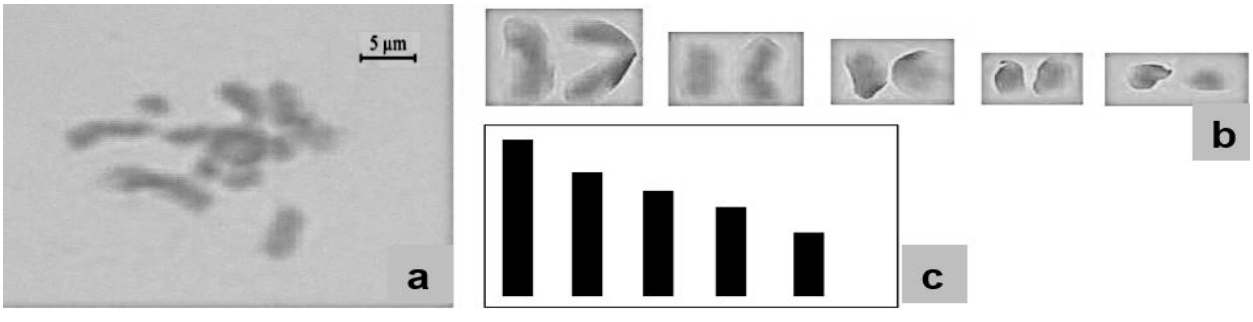


Figure 5. *Macrosiphum rosae*: a) Mitotic metaphase chromosomes; b) karyotype; c) idiogram.

***Pterochloroides persicae* (Cholodkovsky, 1898)**

The diploid chromosomal number of *P*. *persicae* that was collected from the host plant *Prunus cerasifera* was 2n=12 (Figure 6 a-b). The idiogram of this species revealed a partner of long, two partners of medium-sized, and three partners of short chromosomes (Figure 6 c).

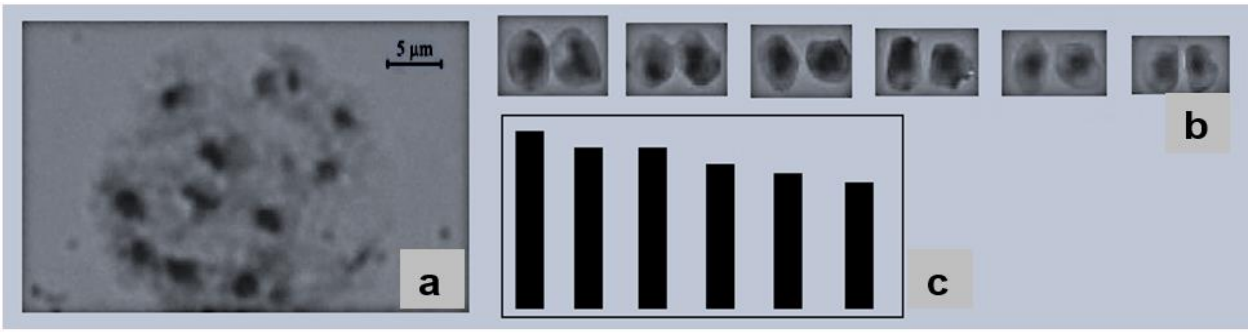


Figure 6. *Pterochloroides persicae*: a) Mitotic metaphase chromosomes; b) karyotype; c) idiogram.

Most of the data on holocentric chromosomes obtained so far have been derived from studies conducted on aphids and members of the Lepidopteran order. The phytophagous lifestyle of aphids can lead to the conservation of their chromosomal fragments. The tendency to favor the inheritance of chromosomal fragments causes repetitive substitutes in the karyotypes of some aphids like *Myzus persicae*. Furthermore, aphids have a repetitive expression of the gene encoding telomerase, therefore they can also start the resynthesis of telomeres at the inner cut-off points, leading to the stabilization of chromosomal fragments (Wilson et al., 2003; Dhatwalia & Gautam, 2009).

Currently, the standard chromosome number for all Aphidinea members can be considered as 2n=8, 10 and 12. Cytogenetically, 601 species in 119 genera belonging to Aphididae, the largest family with 3035 species in approximately 273 genera, were studied and the findings support this data. These chromosome numbers, or at least some of them, are also common in other relatively well-studied families such as Drepanosiphidae (2n=8, 4, and 18), Eriosomatidae (2n=6, 8, 10, 12 and 20) and Lachnidae (usually 2n=10, 12 and 14). In Hormaphididae, 2n=12 is the common chromosome number. However, all other families are too poorly studied to allow the determination of standard values (Manicardi et al., 2015; Kuznetsova et al., 2021). In previous studies, diploid chromosome numbers of *A. spiraecola* (Kapoor, 1994; Blackman & Eastop, 2024), *B.helichrysi* (Raychaudri & Das, 1987; Blackman & Eastop, 2024), *Macrosiphum rosae* (Samkaria et al., 2010; Blackman &Eastop, 2024) and *Pterochloroides persicae* (Blackman & Eastop, 2024) were determined as 8, 12, 10 and 20, respectively. The current study evaluated the karyotypes of six species that preferred different host plants and the chromosome numbers of them varied from 9 to 12. The karyotype data of *A. spiraecola* (2n=10), *B. helichyrsi* (2n=12), *M. rosae* (2n=10) showed similarity with previous studies (Dutta, 1993; Kapoor & Gautam, 1994; Samkari et al., 2010; Sharma & Gautam, 2019; Potan & Gautam, 2019; Blackman & Eastop, 2024). Although the chromosome number of *P. persicae* was indicated as 2n=20 by Blackman & Eastop (2024), as a result of this study it was 2n=12. This difference in the number of chromosomes in *Pterochloroides persicae* may be due to differences in the environmental conditions (geographical conditions, climate, host plant, etc.).

A range of unique cytogenetical processes are involved in the changeover between parthenogenetic and bisexual reproduction in the complex life time of the aphid. For example, in the case of cyclic parthenogenesis to happen, every descendants that develop from fertilised eggs must be XX females, while all of the sperm must have only one X chromosome. This occurs when one of the two X chromosomes is eliminated throughout the annual meiosis of the egg. However, the formation of parthenogenetic progeny consisting exclusively of females from bisexuals including the exclusion of male reproductive cells. Aphid

sex is controlled by endocrine factors responding to environmental cues, rather than to be reached by the random combination of male and female chromosomes during fertilisation. Such a complex and unique system emphasises a special "Aphidoid-type" sex determination system in parallel with such rare systems. The fact that some aphid species have multiple sex chromosomes most likely arose through X chromosome divisions, but other mechanisms can also be envisaged. The fact that some aphid species have multiple sex chromosomes probably results from X chromosome divisions, but other mechanisms are possible. Some species in the Adelgid and Greenid families have up to four pairs of X chromosomes, and some species in the Phylloxerid, Eriosomatid, Lachnid and Drepanosiphid families have two pairs of sex chromosomes. In some species, despite having multiple sex chromosomes, their sex determination system remains XnXn/Xn(0) (male/female) (Manicardi et al., 2015; Kuznetsova et al., 2021). The karyotype data of *C*. *curvipes* and *A*. *ilka* were given for the first time in the current study as 2n=10 and 2n=8+X, respectively. Identification of chromosomal landmarks is crucial in organisms with holocentric chromosomes, as the absence of a primary constriction and the difficulty in obtaining a clear banding pattern make cytogenetic studies in species with this unique chromatin organization challenging. The relevance of a cytogenetic approach to aphid chromosomes have shown that information on aphid genomes is not only scientifically important but also economically relevant. Manicardi et al. (2015) assessed *M. persicae* populations and suggested that, when their impact on economically important crops is considered, there is a need for chemical and/or biological control. Without a full understanding of its heredity, it may be hard to accurately assess the existence of infectious and adaptive variability that makes biological and chemically based controls less effective. The concept that populations of aphids are resistant over time and across geographical areas continues to be controversial since aphid colonies do not seem genetically uniform, as was previously thought. Aphid colonies can be aggregations of individuals of distinct karyotypes and thus respond differently to selective external factors. Therefore, a more detailed cytogenetic effort, expected to be supported by the identification of more chromosomal regions, would supply valuable data to assess the adaptive potential of aphids at short temporal and regional scales.Thereby, it could make a significant difference to our understanding of traits such as reproductive rate, host selection, resistance to pesticides, and the mechanisms of speciation (Wilson et al., 2003; Monti et al., 2011; Manicardi et al., 2016). Chromosomal variation occurs in aphids depending on the host plant and different geographical conditions (Sharma & Gautam, 2019). Chromosomal variation occurs in aphids depending on the host plant and different geographical conditions (Sharma & Gautam, 2019). Considering Turkey's geographical location and different climatic zones, it is assumed that chromosomal variations of aphids distributed in our country are quite diverse. In this regard, it is necessary to study the chromosomes of aphids from different host plants and geographical regions.

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*Original article (Orijinal araştırma)*

# Investigating insecticide resistance, *kdr* mutation, and morphology of the coastal mosquito *Aedes* (*Ochlerotatus*) *zammitii* (Theobald, 1903) (Diptera: Culicidae)

Bir kıyısal sivrisinek olan *Aedes* (*Ochlerotatus*) *zammitii* (Theobald, 1903) (Diptera: Culicidae)'nin insektisit direnci, *kdr* mutasyonu ve morfolojisinin araştırılması

**Fatma BURSALI[1*]**

## Abstract

This study aimed to assess the insecticide resistance levels and investigate the presence of the *kdr* mutation in *Aedes* (*Ochlerotatus*) *zammitii* (Theobald, 1903) (Diptera: Culicidae) mosquito populations collected from various locations within the Aegean region of Türkiye. Additionally, the study explored the morphological details of *Ae. zammitii* eggs and adults by using scanning electron microscopy (SEM). Mosquitoes were collected from their natural rocky breeding habitats from several provinces from April to October 2023 using larvae scoops at different aquatic stages. Emerged adult mosquitoes were identified using both taxonomic keys and molecular methods. The obtained images from SEM analysis revealed unique surface features that could potentially be used to identify the species. The susceptibility of adult *Ae. zammitii* to six insecticides, namely DDT (4%), fenitrothion (1%), bendiocarb (0.1%), deltamethrin (0.05%), permethrin (0.75%) and malathion (5%), was determined using the World Health Organization (WHO) susceptibility test. Results indicated that the mosquitoes exhibited varied possible resistance to the different pesticides tested. Mortality rates ranged between 72%-96%. This research confirmed the presence of *kdr* mutation associated with pyrethroid resistance in *Ae. zammitii*. The frequency of L1014F mutation ranged between 55 and 70% with the highest frequency determined in Antalya-Kaş population, followed by Karaburun and Çandarlı in İzmir. These findings significantly contribute to the understanding of insecticide resistance in *Ae. zammitii,* paving the way for developing effective mosquito control strategies in the Aegean region.

**Keywords:** *Aedes zammitii*, coastal mosquito, insecticide*, kdr*, SEM

## Öz

Bu çalışmada, Türkiye'nin Ege Bölgesi'nin çeşitli yerlerinden toplanan *Aedes* (*Ochlerotatus*) *zammitii* (Theobald, 1903) (Diptera: Culicidae) türünde insektisit direnç seviyelerinin değerlendirilmesi ve *kdr* mutasyonunun varlığının belirlenmesi amaçlanmıştır. Ek olarak, *Ae. zammitii* türünün yumurta ve ergin morfolojileri taramalı elektron mikroskobu (SEM) kullanılarak belirlemiştir. Sivrisinekler, Nisan-Ekim 2023 tarihleri arasında doğal kayalık üreme habitatlarından larva kepçeleri kullanılarak toplanmıştır. Ergin *Ae. zammitii* örnekleri hem taksonomik anahtarlar hem de moleküler yöntemler kullanılarak tanımlanmıştır. SEM analizinden elde edilen görüntüler, türün tanımlanmasında potansiyel olarak kullanılabilecek benzersiz yüzey özelliklerini ortaya çıkarmıştır. Ergin *Ae. zammitii* örneklerinin DDT (4%), fenitrothion (1%), bendiokarb (0.1%) deltametrin (0.05%), permetrin (0.75%) ve malathion'a (5%) karşı olan duyarlılıkları Dünya Sağlık Örgütü (DSÖ)'nün duyarlılık testi ile belirlenmiştir. DSÖ duyarlılık test sonuçlarına göre ölüm oranları %72-%96 arasında değişmiş, örneklerin farklı pestisitlere karşı direnç sergilediği ortaya konmuştur. *Ae. zammitii*'de piretroid direnciyle ilişkili *kdr* mutasyonunun varlığı doğrulanmıştır. L1014F mutasyonunun sıklığı %55-70 arasında değişmekte olup, en yüksek frekans Antalya-Kaş popülasyonunda belirlenirken, bunu Karaburun ve Çandarlı takip etmiştir.

**Anahtar sözcükler:** *Aedes zammitii*, kıyı sivrisineği, insektisit, *kdr*, SEM

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## Introduction

Mosquitoes, members of the family Culicidae, continue to pose a significant threat to global health. While certain mosquito species act as pathogen vectors of diseases like West Nile, dengue, Zika disease most of which lack effective vaccines or readily available treatments, others primarily cause annoyance and are categorized as nuisance mosquitoes. These nuisance species are capable of biting humans but lack the ability to transmit diseases (Petric et al., 2010; Heym et al., 2017; Becker et al., 2020). *Aedes* (*Ochlerotatus*) *zammitii* (Theobald, 1903) (Diptera: Culicidae) falls within the nuisance category. This species is a coastal mosquito species found in the Mediterranean region. It has a specific habitat preference, developing within the rock pool water, especially inhabiting the central and eastern regions of countries such as Italy, the Balkans, Sicily, Malta, Greece, and Türkiye (Becker et al., 2020).

*Aedes zammitii* is a closely related to *Aedes mariae* (Sergent & Sergent, 1903), a mosquito species that occupies the western Mediterranean coast (Mastrantonio et al., 2015; Yavasoglu et al., 2016; Robert et al., 2019). These coastal mosquitoes are morphologically similar in all stages, but *Ae. zammitti* has a more robust build and distinct coloration pattern. There is limited existing information about *Ae. zammitii's* morphology.

*Aedes zammitii* exhibits a highly zoo-anthropophilic blood-feeding behavior and venture beyond its typical habitats in search of blood meals, increasing the likelihood of dispersal to nearby residences. The intensity of daytime biting activity can become highly bothersome, rendering visits to these coastal areas particularly unpleasant during specific periods in late spring and summer (Mastrantonio et al., 2015; Yavasoglu et al., 2016). However, there are no reports of it transmitting diseases.

Insecticides have been a mainstay in conventional mosquito control programs, with four primary chemical classes historically employed: organochlorines (OCs), carbamates (CBs), organophosphates (OPs), and pyrethroids (PYs). The use of specific insecticides, such as malathion, deltamethrin, and permethrin, has been instrumental in managing mosquito populations. However, this approach faces a growing challenge: insecticide resistance (Liu, 2015; Naqqash et al., 2016; Touray et al., 2023). This resistance, driven by mechanisms like target site mutations (resulting in knockdown resistance) and increased insecticide metabolism, increasingly compromise the effectiveness of various insecticide classes and necessitates alternative strategies. The emergence of insecticide resistance among mosquito populations presents a significant and escalating challenge to global public health (Park et al., 2020; Clarkson et al., 2021; Yavaşoğlu et al., 2022). Pyrethroid insecticides disrupt insect nervous systems by targeting voltage-gated sodium channels (VGSCs) (Hołyńska-Iwan & Szewczyk-Golec, 2020), whereas mutations in the acetylcholinesterase-1 gene confer resistance to organophosphate and carbamate insecticides (Martinez-Torres et al., 1999; Weill et al., 2004). Mutations in the knockdown resistance (*kdr*) gene can lead to amino acid substitutions within the VGSC protein structure. These substitutions hinder pyrethroid binding, diminishing insecticide effectiveness. This necessitates increased insecticide concentrations to achieve the same lethal effect in insects (Davies et al., 2007; Bursalı, 2013; Dong et al., 2014; Uemura et al., 2024).

Mosquito control in Türkiye is a collaborative effort between the Ministry of Health and municipalities, employing both larval and adult control methods (Akiner et al., 2018; Touray et al., 2023). Monitoring insecticide resistance in various mosquito species is a global practice, and Türkiye is no exception. Extensive data exist regarding the resistance status of vector species like *Anopheles sacharovi* (Ramsdale et al., 1980; Hemingway et al., 1992; Kasap et al., 2000)*, An. superpictus* (Yavaşoğlu et al., 2019), *Culex pipiens* (Akiner et al., 2009; Akıner & Ekşi, 2015; Taşkın et al., 2015; Guntay et al., 2018; Ser & Çetin, 2019), *Cx. tritaeniorhynchus* (Yavaşoğlu et al., 2022), *Ae. albopictus* (Yavaşoğlu, 2021). However, there is no study about the insecticide resistance of *Ae. zammitii* Türkiye populations. Given the widespread use of insecticides and the emergence of resistance in other mosquito species within Türkiye, investigating the

insecticide resistance profile of *Ae. zammitii* populations is crucial to ensure effective mosquito control strategies. There is a critical gap in knowledge concerning this species.

This study investigated the insecticide resistance profiles of six *Ae. zammitii* populations collected from the Aegean and Mediterranean regions of Türkiye. The research evaluated the effectiveness of six insecticides commonly used in vector control programs: DDT, permethrin, fenitrothion, malathion, bendiocarb, and deltamethrin. Additionally, the study aimed to identify the presence of the *kdr* mutation, a genetic marker associated with insecticide resistance in mosquitoes. This research is expected to provide valuable insights into the current resistance status of *Ae. zammitii* populations in Türkiye. This information will be crucial for guiding the selection and implementation of effective vector control strategies, including the selection of appropriate insecticides for future control programs.

## Materials and Methods

### Sampling studies

Sampling was conducted across several Turkish provinces (İzmir, Antalya, Muğla, Aydın) during the summer months, from April to October 2023 (Figure 1). Samples containing different aquatic stages of *Ae. zammitii* were collected from designated locations, including rock pools, irrigation channels, and flooded farmlands using larvae scoops (Figure 2). Adult mosquitoes were sampled from barns around the coastal area using mouth aspirators. As *Ae. zammitii* exhibits exophilic behavior, they are found near their rocky breeding habitats and readily attack hosts. Larval samples were more abundant than adult samples.

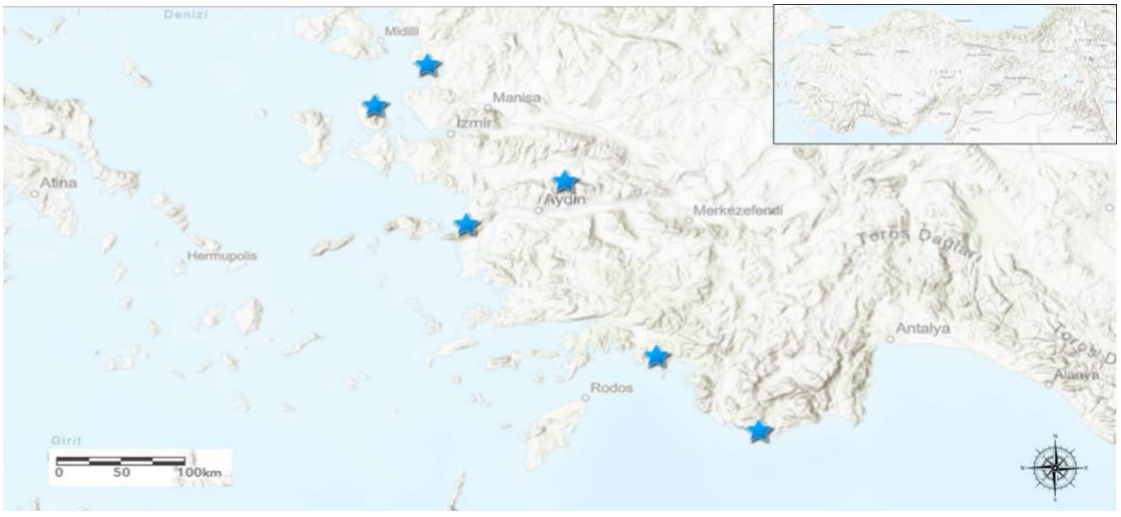


Figure 1. Sampling localities of *Ae. zammitii* populations. This map was generated with the aid of ArcGIS software (version 10.3).

The samples were placed in separate sampling tubes containing habitat water. Information about sampling time, date and coordinate information were recorded and samples were brought to the Vector Insects Research Laboratory, Aydın Adnan Menderes University, Türkiye. III.-IV. stage larvae and pupae samples collected from different localities were transferred to separate cages. Mosquitoes were reared under controlled conditions (28°C temperature, 12 h photoperiod, 60% humidity) and larval feeding with commercial fish food (Tetramin®) and allowed to develop into adults (Bursali & Simsek, 2024). Simultaneously, adult specimens obtained from animal enclosures using light traps and aspirators were maintained on a 10% sugar solution, with gravid females providing eggs for the establishment of F1 generations. Morphological identification of adult mosquitoes was performed using a stereomicroscope (Leica S8 Apo) and established taxonomic keys (Schaffner et al., 2001). F1 females, derived from both field-collected larvae and adults, were utilized in subsequent WHO insecticide susceptibility assays and molecular analyses.

Figure 2. Sampling sites showing breeding habitats of *Aedes zammitii.*

**Molecular identification**

Molecular methods were used to confirm morphological species identification. Four morphologically identified species from the 4 different provinces (Antalya, Muğla, Aydın,İzmir) were used (Z1-Z4). Genomic DNA was extracted from adult mosquitoes using the Invitrogen Pure Link Genomic DNA isolation kit, following the manufacturer's protocol for efficient and consistent DNA isolation. This method utilizes a multi-step process involving cell wall disruption, cellular content release, selective DNA binding, and purification. The quantity and quality of the extracted DNA will be assessed using a NanoDrop spectrophotometer (Thermo Scientific™ NanoDrop™ 2000/2000c) to ensure suitability for downstream applications. The extracted DNA was stored at -20°C. The cytochrome c oxidase subunit I (COI) genes were amplified using the primers LCO1490F (5'-GGTCAACAAATCATAAAGATATTGG-3') and HC02198R (5'-TAAACTTCAGGGTGACCAAAAAATCA-3') (Folmer et al., 1994). The PCR mix comprised 12.5 µl PCR mix, 0.25 µl 20 µM each of primers, 1 µl template DNA and 11µl ddH20. The thermal cycling protocol comprised of an initial denaturation step at 94°C for one minute. This was followed by five cycles with denaturation at 94°C for 40 seconds, annealing at 45°C for 40 seconds, and extension at 72°C for one minute. Subsequently, there were 35 cycles with denaturation at 94°C for 40 seconds, annealing at a higher temperature of 51°C for 40 seconds, and extension maintained at 72°C for one minute. Finally, a final extension step was performed at 72°C for 5 minutes. The amplified DNA fragments were loaded onto a 1% agarose gel and visualized under UV light after electrophoresis. Visualized products were documented before purification and submission for sequencing. Sequences were edited using BioEdit software and compared to other COI sequences available in GenBank using the BLAST tool.

To infer the evolutionary relationships between the investigated organisms, a phylogenetic tree was generated using the Neighbor-Joining algorithm within the MEGA software suite. The analysis was run on 1000 replicates for inferred bootstrap consensus and the reliability of the generated tree was assessed using the bootstrap test (Tamura et al., 2007). *Culex pipiens* mtCOI sequences obtained from the GenBank database was used as an outgroup on the topology.

**Insecticide susceptibility bioassays and detection of *kdr* mutation**

The susceptibility or resistance levels of mosquito populations to various insecticides was evaluated using standardized protocols established by the World Health Organization (WHO, 2016). Bioassays were conducted with commercially available diagnostic susceptibility bioassay tubes. Insecticide-treated papers, containing different insecticides at specific concentrations, were obtained from a WHO collaborating center (WHOPES) located at Universiti Sains Malaysia. The selected insecticides included

DDT 1,1′-(2,2,2-Trichloroethane-1,1-diyl) bis (4-chlorobenzene) (4%), fenitrothion (1%), malathion (5%), bendiocarb (0.1%), permethrin (0.75%), and deltamethrin (0.05%). These concentrations are those commonly reported in the literature (Kushwah et al., 2015; Liu, 2015; WHO, 2016). This study was done under insectarium conditions. Each test tube included 25 unfed, 3-5 days old, F1 generation *Ae. zammitii* females and each treatment had three replicates. These adult mosquitoes were exposed to insecticide-treated papers for a defined period (1 hour for most insecticides, 4 hours for DDT) following the WHO guidelines (WHO, 2016). Subsequently, they were transferred to holding tubes and provided with a 10% sugar solution for sustenance over a 24-hour period. A control group received identical treatment but with papers impregnated only with the carrier solvent used for the insecticides, as recommended by the World Health Organization (WHO, 2016). Mortality was assessed after 24 h incubation. Populations were considered as 'susceptible' if the mortality rates were ≥ 98%; 'possible resistant' if mortality rates between 90-97%; 'resistant' when the mortality rates were ≤ 90% (WHO, 2016).

Total DNA was isolated from female mosquitoes belonging to each population. Three to five-day-old, unfed females were selected to investigate *kdr* mutations. The DNA extraction protocol in the Invitrogen Pure Link genomic DNA isolation kit was employed to isolate DNA from a total of ten adult females per population. The eluted DNA was subsequently amplified to detect the presence of *kdr* mutations. For the detection of *kdr* mutation in the vgsc gene, the allele-specific primers primers (C1: 5′-CCT GCC ACG GTG GAA CTT-3′/C2: 5′-GGA CAA AAG CAA GGC TAA GAA-3′) used by Liu et al., (2013) were applied in this study to assay the polymorphisms from electrophoresis of the amplicons.

The PCR protocol involved denaturation at 94°C for 5 minutes, followed by 35 cycles with each cycle consisting of denaturation at 94°C for 30 seconds, annealing at 52°C for 30 seconds, and extension at 72°C for 40 seconds. A final extension step was performed at 72°C for 5 minutes to ensure complete amplification. The resulting DNA fragments were then visualized using gel electrophoresis on 1.5% agarose gels.

**Scanning electron microscope (SEM) analysis**

Morphological configurations of mosquito eggs and 1-day-old male body parts were determined using Scanning electron microscopy (SEM). Mosquitoes were dissected under stereoscope in 1 µl phosphate-buffered saline (PBS), pH 7.2. The antennae, the mouthparts, and the other body parts of the male were preserved in 70% ethanol. Then an ultrasonic cleaning machine was used to clean the samples twice for 10 min. Samples were serially dehydrated for 10 min in 70%, 80%, 85%, 90%, and 95% ethanol gradients, immersed in 100% ethanol for 30 min twice, and then treated with pure tert-butanol for 30 min. The samples were quickly dried and were fixed to aluminum stubs using double-sided carbon tape (Shi et al., 2021).

Samples were gold-coated in a layer of approximately 100 A° (8-10 nanometer), using a fine gold coating apparatus, with the method of magnetron sputter, ion sputtering device (Spi Supplies, SPI-MODULE Sputter Coater), and examinations of mosquito parts were carried out by a FEI-Quanta 250FEG source Scanning electron microscope (SEM) connected to an EDXS system at an acceleration voltage of 30 kV. The samples were viewed and photographed directly from the SEM video monitor. Eggs were left in-situ throughout this process.

**Statistical analysis**

Differences in the mosquito mortality rates after exposure to the different treatments (i.e. insecticides and control) were determined using two-way analysis of variance (ANOVA) and Tukey's HSD post-hoc test in SPSS version 23.0. Genotype frequencies, the allele frequency, and genetic conformity to Hardy-Weinberg Equilibrium (HWE) was assessed within each population using exact probability tests implemented in POPGENE software (Yeh, 1999). Statistical significance was $p < 0.05$.

## Results and Discussion

The Aegean and Mediterranean regions of Türkiye offer highly suitable climatic conditions for mosquito populations. This, coupled with factors like intensive agricultural practices, tourism, and industrial activity, contributes to the proliferation of mosquito species and the potential spread of mosquito-borne diseases. In this context, understanding the levels and underlying mechanisms of insecticide resistance within these mosquito populations becomes crucial. By investigating resistance patterns, this research aims to identify the most effective insecticides for mosquito control programs in Türkiye. This knowledge is essential for guiding vector control strategies and mitigating the risk of disease transmission. This study determined the susceptibility of *Ae. zammitii,* a nuisance mosquito species found in the Aegean region of Türkiye, to various insecticide classes and identified the presence of a *kdr* mutation, which is associated with pyrethroid resistance in other mosquito species.

*Aedes zammitii* mosquitoes were collected from coastal breeding sites and subsequently identified using both taxonomic keys and molecular methods. Sequence analysis of the cytochrome c oxidase subunit I (COI) gene revealed high nucleotide homology (>96%) with related species sequences deposited in GenBank, as determined by BLASTn searches. The resulting phylogenetic tree (Figure 3) generated using the neighbor-joining method positioned *Ae. zammitti* within the mariae group alongside *Ae. mariae* and *Ae. phoeniciae*. This finding aligns with previous research (Mastrantonio et al., 2015; Yavasoglu et al., 2016). Notably, these three species are considered sibling species, characterized by their development in rock pools located along Mediterranean coastal regions (Urbanelli et al., 2014).

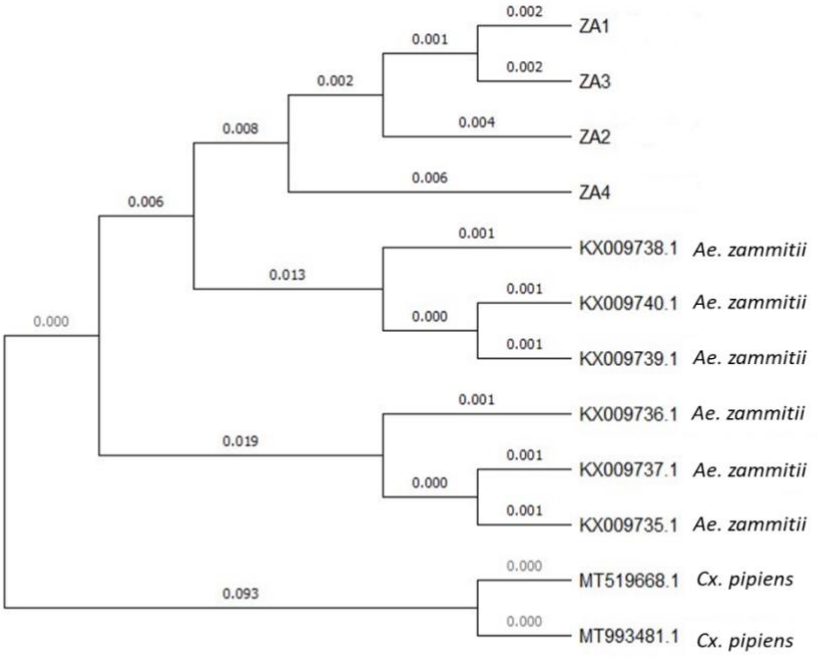


Figure 3. Phylogenetic tree created by NJ method for the COI gene region of *Aedes zammitii* (Z1-Antalya-Z2-Mugla, Z3-Aydin, Z4-İzmir).

Chemical insecticides remain the mainstay of mosquito control programs globally (WHO, 2018; Kumar et al., 2022; Duval et al., 2023). This reliance began with the discovery of DDT during World War II (Vezehegno, 2008). However, widespread use led to the development of resistance in mosquitoes and other insects, coupled with significant environmental and ecological damage (Coetzee vd., 1999). As a result, DDT has been banned in many countries. Consequently, newer chemical insecticides were

developed and integrated into mosquito control strategies. Pyrethroid insecticides, for instance, are utilized in both ultra-low-volume aerial sprays for adult mosquitoes and indoor residual spraying (IRS) programs (Duval et al., 2023). Additionally, biological control agents like *Bacillus thuringiensis israelensis*, and insect growth regulators (IGRs) are widely employed for effective larval control (Özbilgin et al., 2011; WHO, 2018). During the winter, targeted treatment of potential hibernation sites is conducted. Additionally, homeowners are encouraged to adopt indoor residual spraying or insecticide-impregnated nets (Guz et al., 2020; Touray et al., 2023). The financial burden, significant risks posed to both human health and ecosystems, and limited long-term efficacy of chemical insecticides, coupled with the widespread emergence of insecticide resistance among mosquito populations, underscore the need for alternative approaches (Liu, 2015; Pimentel et al., 1992).

WHO susceptibility bioassay results indicated that the mosquitoes exhibited varied possible resistance to the different pesticides tested. The different insecticides have varying degrees of effectiveness against mosquito. Mortality rates ranged between 72%-94. All populations were resistant to DDT even though it was banned in the 1980s (Akiner et al., 2009) and had the least effects against the mosquitoes. DDT resistance has been documented in various medically important mosquito species in Türkiye, including *An. sacharovi, An. maculipennis, An. superpictus* and *Cx. pipiens* (Akıner et al., 2013; Taskin et al., 2016; Yavaşoğlu et al., 2019). This study represents the first report of DDT resistance in *Ae. zammitii* populations from Türkiye. Following the widespread withdraw of DDT in the 1970s due to resistance concerns, mosquito control programs transitioned to carbamate (CB) and organophosphate (OP) insecticides, such as malathion, fenitrothion, bendiocarb, and propoxur (Ramsdale, 1980). The extensive use of malathion in agricultural pest control creates a strong selection pressure for resistance in mosquito populations inhabiting these areas (Kasap et al., 2000). Notably, *Cx. pipiens* populations in neighboring countries like Iran, Russia, and Greece have also shown high levels of resistance to various insecticides (Rahimi et al. 2020; Vereecken et al., 2022; ECDC, 2023).

Permethrin appears to be the most effective insecticide, achieving remarkably high mosquito mortality (over 92%) across all six testing sites. Fenitrothion, deltamethrin, and bendiocarb also displayed strong efficacy, eliminating 79% to 92% of mosquito adults collected from the various locations. Malathion and DDT were the least effective insecticide overall, with mosquito mortality rates ranging from 70% to 79% across the different sites. The two-way ANOVA analysis revealed that there were clear differences between the insecticide treatments and the control group ($F(6, 84) = 1573$; $p<0.0001$); between localities from which the populations were collected ($F(5, 84) = 103.5$; $p<0.0001$) and in the interaction between the treatments and localities ($F(30, 84) = 3.711$; $p<0.0001$).There were no statistical differences in the effects of the insecticides on mosquito mortality (Figure 4). Our bioassay results revealed resistance to all tested OPs (malathion and fenitrothion) and CBs (bendiocarb) across all *Ae. zammitii* populations. This constitutes the first record of OP and CB resistance in *Ae. zammitii* populations from Türkiye. The observed resistance likely stems from the intensive use of these insecticides, particularly malathion, in agricultural pest management programs (Kasap et al., 2000) (Table 1).

This research is significant because it contributes to the understanding of insecticide resistance in *Ae. zammitii,* which is crucial for developing effective mosquito control strategies in the region. Previous reports indicate resistance in other Turkish mosquito species, including *Anopheles superpictus* (Grassi, 1899) (Diptera: Culicidae), and *Anopheles sacharovi* (Favre, 1903) (Diptera: Culicidae) (Yavaşoglu et al., 2019), *Anopheles maculipennis* (Meigen, 1818) (Bursalı & Şimşek, 2016), *Culex pipiens* (L., 1758) (Diptera: Culicidae) (Akiner & Ekşi, 2015), *Culex tritaeniorhynchus* (Giles, 1901) (Diptera: Culicidae) (Yavaşoğlu et al., 2022), *Aedes albopictus* (Grassi, 1899) (Diptera: Culicidae (Yavaşoğlu, 2021), *Aedes caspius* (Pallas, 1771) (Diptera: Culicidae) (Yavaşoglu et al., 2024) in Türkiye. These studies revealed widespread resistance to various insecticides and elevated enzyme activity, suggesting multiple resistance mechanisms. We however note that our bioassays lacked a susceptible *Ae. zammittii* population for comparison. Because of its habitat preferences, we could not rear this species in the laboratory.

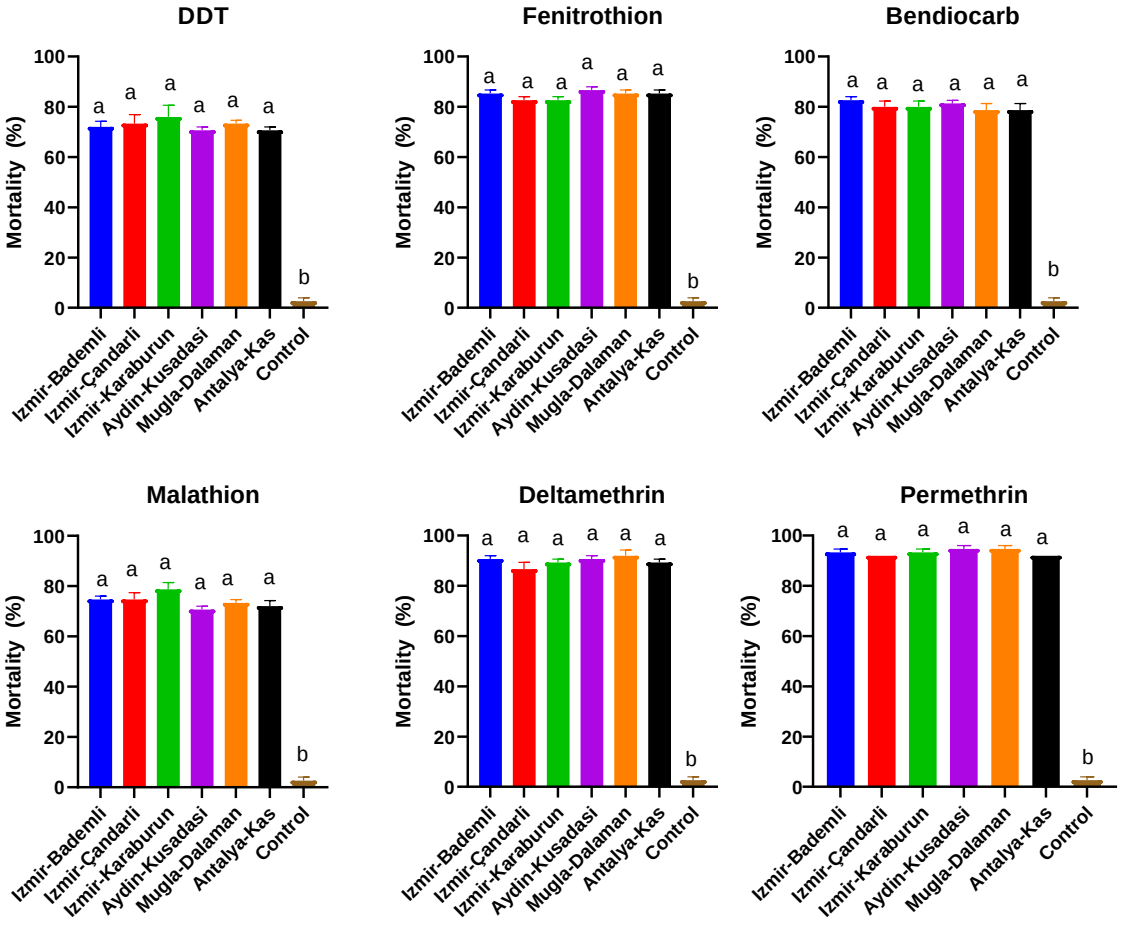


Figure 4. Insecticide resistance levels of *Aedes zammitii* collected from different localities in the Aegean and Mediterranean region of Türkiye. According to WHO susceptibility bioassay results, mortality rates ≥ 98% indicate susceptible; '90%-97% = possible resistant; mortality rates ≤ 90%resistant. The bars represent the mortality rates after exposure to treatments and error bars indicate standard deviation.

Table 1. Mortality rates (% ± standard deviation) and resistance levels of *Aedes zammitii* collected from different localities in the Aegean and Mediterranean regions of Türkiye. Mortality rates were ≥ 98% were considered as susceptible (S); '90%-97% possible resistant (PR); mortality rates ≤ 90%= resistant (R).

| Localities | Resistance levels to Insecticides (% mortality) | | | | | |
|---|---|---|---|---|---|---|
| | DDT (4%) | Malathion( 5%) | Bendiocarb (0.1%) | Fenitrothion (1%) | Deltamethrin (0.05%) | Permethrin (0.75%) |
| İzmir-Bademli | 72±3 (R) | 75±2 (R) | 83±3 (R) | 85±5 (R) | 91±4 (PR) | 93±2 (PR) |
| İzmir-Çandarlı | 73±3 (R) | 75±3 (R) | 80±3 (R) | 83±4 (R) | 87±7 (R) | 92±0 (PR) |
| İzmir-Karaburun | 76±2 (R) | 79±4 (R) | 80±3 (R) | 83±2 (R) | 89±6 (R) | 93±2 (PR) |
| Aydın-Kuşadası | 71±2 (R) | 71±3 (R) | 81±3 (R) | 87±3 (R) | 91±2 (PR) | 95±2 (PR) |
| Muğla-Dalaman | 73±2 (R) | 73±2 (R) | 79±2 (R) | 85±2 (R) | 92±2 (PR) | 95±2 (PR) |
| Antalya-Kaş | 71±2 (R) | 72±3 (R) | 79±4 (R) | 85±2 (R) | 89±3 (R) | 92±0 (PR) |

The *kdr* mutation has been implicated in DDT resistance in some mosquito species worldwide (Martinez-Torres et al., 1999; Ponce et al., 2016; Saha et al., 2019; Zhou et al., 2019). However, the absence of *kdr* mutation data in our study limits our ability to determine if this mechanism contributes to DDT resistance observed in these *Ae. zammitii* populations. DNA sequence from individual mosquitoes

was separated and amplified from 60 specimens of *Ae. zammitii*, using PCR. The *kdr* genotype and allele frequencies of the phenotypes, determined by the deltamethrin resistance bioassay in *Ae. zammitii,* populations of various regions, are shown in Table 2. In *Ae. zammitii*, three genotypes were identified homozygous resistance (TTT/TTT-L1014F/L1014F), heterozygous resistance (TTT/TTA-L1014F/L1014), and homozygous susceptibility (TTA/TTA-L1014/L1014). No TCA (L1014S) mutation was found *Ae. zammitii*. The frequency of L1014F mutation in *Ae. zammitii* ranged between 55 and 70% with the highest frequency determined in Antalya-Kaş population (70%), followed by Karaburun (65%) and Çandarlı (60%) in İzmir. Heterozygous genotypes were observed in all assessed populations (Table 2).

Table 2. *Kdr* genotypes and allele frequencies in *Aedes zammitii* at the study sites

| Sites | Coordinates | Sample Size | *kdr* genotype | | | Allele frequency (%) | | $X^2$ | p |
|---|---|---|---|---|---|---|---|---|---|
| | | | TTT/TTT | TTT/TTA | TTA/TTA | TTT | TTA | | |
| Antalya-Kaş | 36°11'32.9"N 29°38'54.7"E | 10 | 4 | 6 | 0 | 70 | 30 | 0.400 | 0.527 |
| Muğla-Dalaman | 36°42'20.3"N 28°43'24.0"E | 10 | 3 | 5 | 2 | 55 | 45 | 1.400 | 0.497 |
| Aydın-Kuşadası | 37°54'20.3"N 27°16'04.8"E | 10 | 3 | 5 | 2 | 55 | 45 | 1.400 | 0.497 |
| İzmir-Bademli | 39°02'27.5"N 26°48'45.7"E | 10 | 4 | 3 | 3 | 55 | 45 | 0.200 | 0.905 |
| İzmir-Çandarlı | 38°56'05.1"N 26°57'02.8"E | 10 | 5 | 2 | 3 | 60 | 40 | 1.400 | 0.497 |
| İzmir-Karaburun | 38°37'46.4"N 26°31'28.7"E | 10 | 5 | 3 | 2 | 65 | 35 | 1.400 | 0.497 |

Pyrethroids are commonly used for *Aedes* control, but their widespread and sustained use has selected for resistance globally (Bursalı, 2013; Amelia-Yap et al., 2018; Melo Costa et al., 2020; Mashlawi et al., 2022; WHO, 2023). For example, *Aedes aegypti* (Linnaeus in Hasselquist, 1762) (Diptera: Culicidae), populations in Thailand displayed incipient or full resistance to various insecticides including deltamethrin and permethrin, with only a few susceptible populations found in specific areas of Songkhla and Chiang Rai provinces (Jirakanjanakit et al., 2007). These *Ae. aegypti* mosquitoes had mutations linked to pyrethroid resistance. Kushwah et al. (2015)'s study indicated resistance to DDT in both *Ae. aegypti* and *Ae. albopictus* (Grassi, 1899) (Diptera: Culicidae), with variable resistance to other insecticides. They did not detect mutations associated with pyrethroid resistance and these did not consistently correlate with phenotypic resistance. Konkon et al. (2023) investigated the susceptibility of *Ae. aegypti* and *Ae. albopictus* mosquitoes exposed to commonly used insecticides (deltamethrin, permethrin, alpha-cypermethrin, pirimiphos-methyl, and bendiocarb) in southern Benin. They observed that *Ae. albopictus* showed varying levels of resistance to alpha-cypermethrin, while *Ae. aegypti* presented widespread resistance to nearly all tested pyrethroids. Notably, resistance persisted even after pyrethroid withdrawal in specific regions Despite the cessation of their public use in 2000, resistant *Ae. aegypti* adults were detected in São Paulo during a robust monitoring a decade later (Macoris et al., 2018). Two known *kdr* mutations (Val1016Ile and Phe1534Cys) were determined with a significant decrease in the susceptible allele over time (Macoris et al., 2018). The L1014F and L1014C mutations in the *kdr* gene have been implicated in DDT and pyrethroid resistance in *Cx. pipiens* populations worldwide (Taskin et al., 2016; Fotakis et al., 2017; Tmimi et al., 2018). For instance, both mutations were identified in *Cx. pipiens* from the Aegean region of Türkiye (Taskin et al., 2016) and Greece (Fotakis et al., 2017), while *Cx. pipiens* populations in Morocco (Tmimi et al., 2018) displayed a high frequency of the L1014F allele. Variations exist in the specific *kdr* mutation responsible for resistance geographically. For example, the L1014S mutation confers resistance in *Cx. quinquefasciatus* from China (Xu et al., 2006), whereas L1014F is responsible in New Jersey (Johnson et al., 2016) and L1014C is observed in some Chinese populations (Wang et al., 2012). This study contributes significantly to understanding insecticide resistance in *Ae. zammitii*, a critical step towards effective mosquito control in the Aegean region of Türkiye.

We identified resistance or possible resistance to various insecticide classes and confirmed the presence of a *kdr* mutation associated with pyrethroid resistance. Our findings reinforce the widespread emergence of insecticide resistance among mosquito species in Türkiye, including *Ae. zammitii, Ae. caspius, Ae. albopictus,* and others. Such reports highlight the significant problem of resistance development in populations from different countries and underscore the need for diverse strategies such as utilizing standardized WHO methods to map resistance trends and identify mechanisms, investing in developing insecticides with new modes of action to counter existing resistance and exploring and implementing non-chemical control methods like source reduction, development of bio-agents, and insect growth regulators (Hancock et al., 2020; Touray et al., 2023; WHO, 2023). Bioagents, such as *Bacillus thuringiensis* and its derivatives, alongside entomopathogenic fungi, holds promise for mosquito control (Mampalil etal., 2017; Accoti et al., 2021).

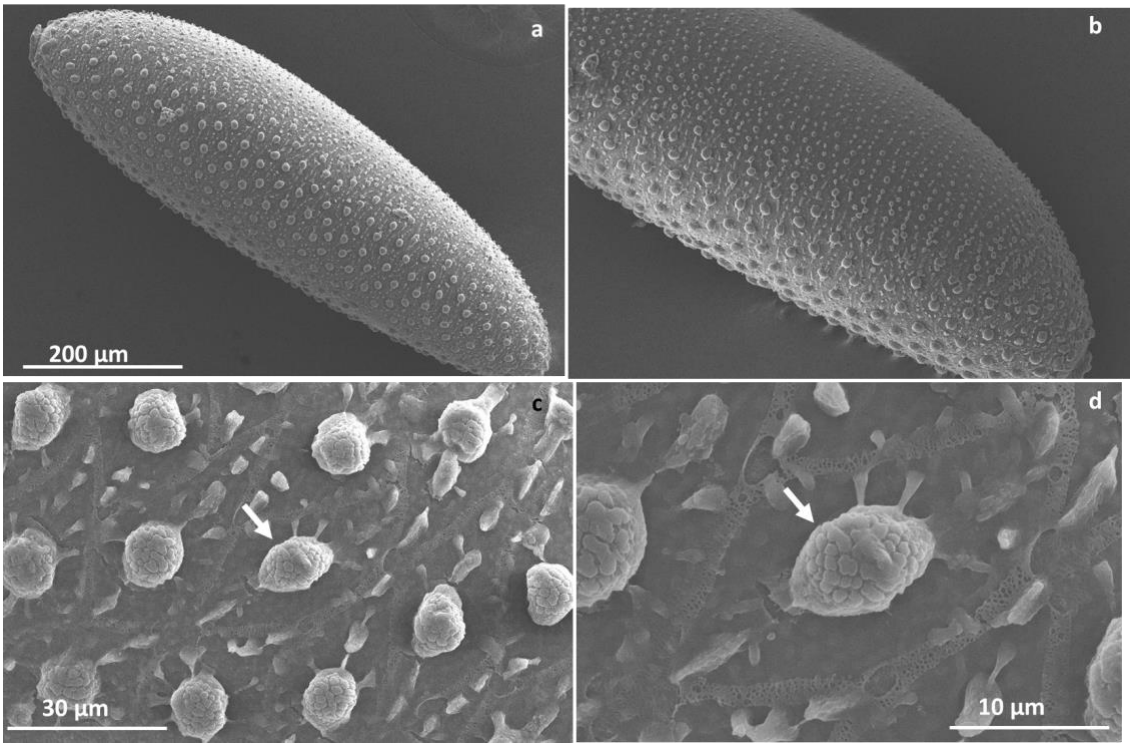


Figure 5. Scanning electron microscopy (SEM) images of *Aedes zammitii* eggs.

Identifying mosquitoes traditionally relies on microscopic analysis of morphology and molecular analysis of genes. Scanning electron microscopy (SEM) offers detailed descriptions of species such as the surface topography of adults and developmental stages (Mello et al., 2017; Kim et al., 2020). There is limited existing information about *Ae. zammitii'* morphology. While not directly connected to the genotyping work, our SEM observations can serve as valuable baseline for future research, including morphology-based identification. This study captured the SEM images of various adult *Ae. zammitii* body parts, including the head, maxillary palps, antennae, wings, scales, and abdomen. The images revealed that the adult mosquito's body is covered in numerous triangular-shaped setae and scales. These scales displayed pointed apices (emergence points) and blunt, broad distal ends. Additionally, the setae were observed to be long and backwardly bent. The abdominal scales displayed 17 longitudinal ridges interconnected by numerous small cross-ribs. The head of the male mosquito exhibited plumose antennae and long, hairy maxillary palps (Figure 6). These observations share similarities with the reported characteristics of *Ae. albopictus* and *Ae. aegypti* adults (Supriyono et al., 2023). *Ae. zammiti* eggs are characterized by their black color and cigar-shaped morphology. Females lay these eggs individually on the surface of saline

water (Figure 5). The eggs measured 200 ± 11.33 µm in length and 96.23 ± 3.0 µm in width, tapering towards both ends. SEM analysis revealed a unique chorionic surface featuring an air-covering plastron network and clusters of globular tubercles of varying sizes. Notably, large oval tubercles were uniformly distributed around the eggs, while smaller, irregularly shaped tubercles filled the spaces between. These structures are believed to contribute to egg buoyancy. Previous SEM studies have explored the surface topography of numerous *Aedes* species, including *Ae. aegypti, Ae. albopictus, Ae. cinereus* (Hinton & Service, 1969; Linley, 1989a, b; Linley & Clark, 1989; Choochote et al., 2001; Alencar et al., 2003, 2008). While generally cigar/boat shaped, *Aedes* eggs exhibit variations in size, morphology, exochorionic tubercle patterns, and micropylar structures. Notably, *Ae. zammitii* eggs share some similarities with *Ae. aegypti* and *Ae. albopictus*; however, (Linley, 1989b) and (Supriyono et al., 2023) described the eggs of these latter species as having a shinier jet-black appearance and more regular, smoothly rounded large tubercles surrounded by nearly tubercle-free cell fields. In contrast, *Ae. lineatopennis* eggs, measuring 510 ± 40 µm in length and 182 ± 18.90 µm in width, possess a fragmented micropylar collar and an irregular exochorionic sculpture characterized by membrane-like walls and a mix of large and small irregular tubercles (Choochote et al., 2001) (Figure 7).

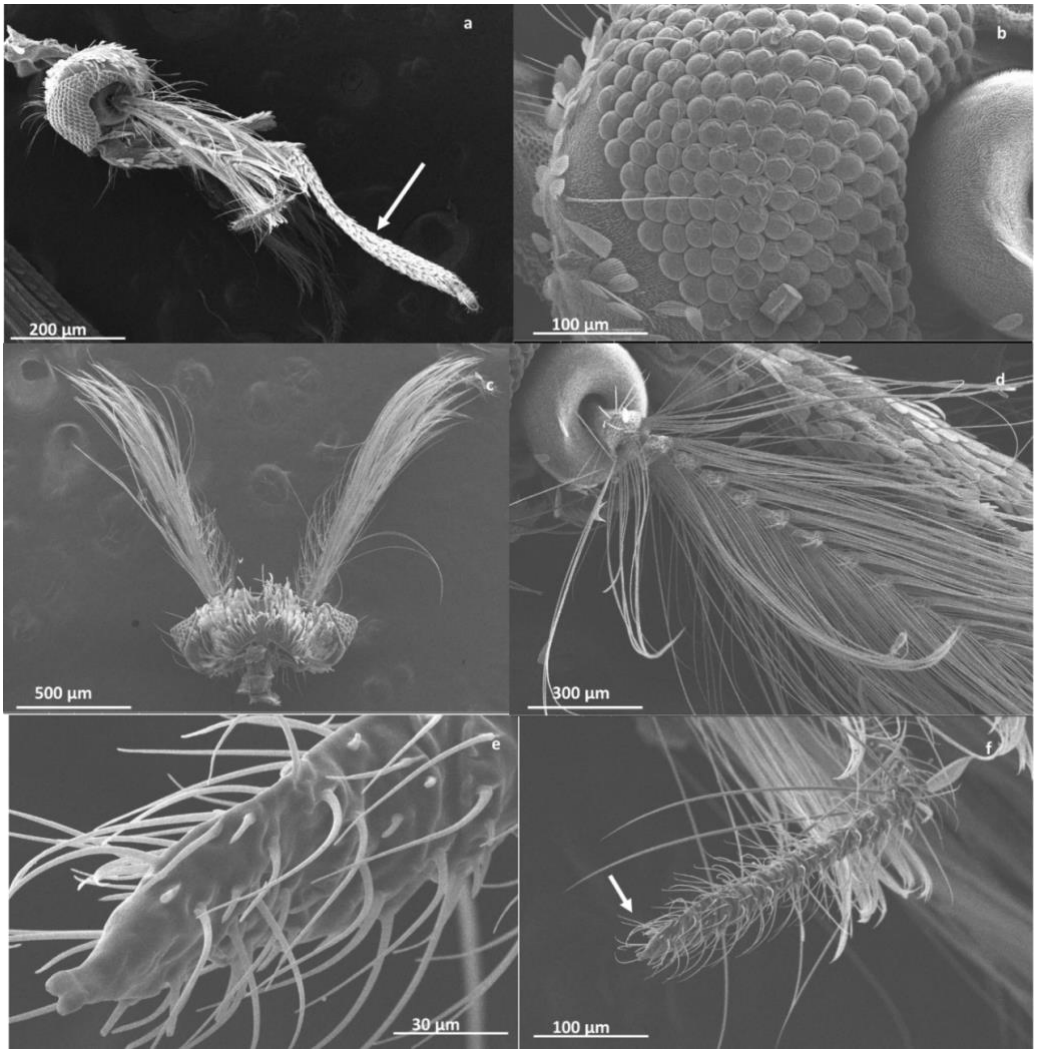


Figure 6. Scanning electron microscopy (SEM) images of scale from different head parts of the mosquito, *Aedes zammitii*. (a) Head of male showing antenna and proboscis (arrow) (b) Detail of compound eye with tiny ommatidia (c-d) Plumose antenna of male (e-f) details of the labium and labellum.

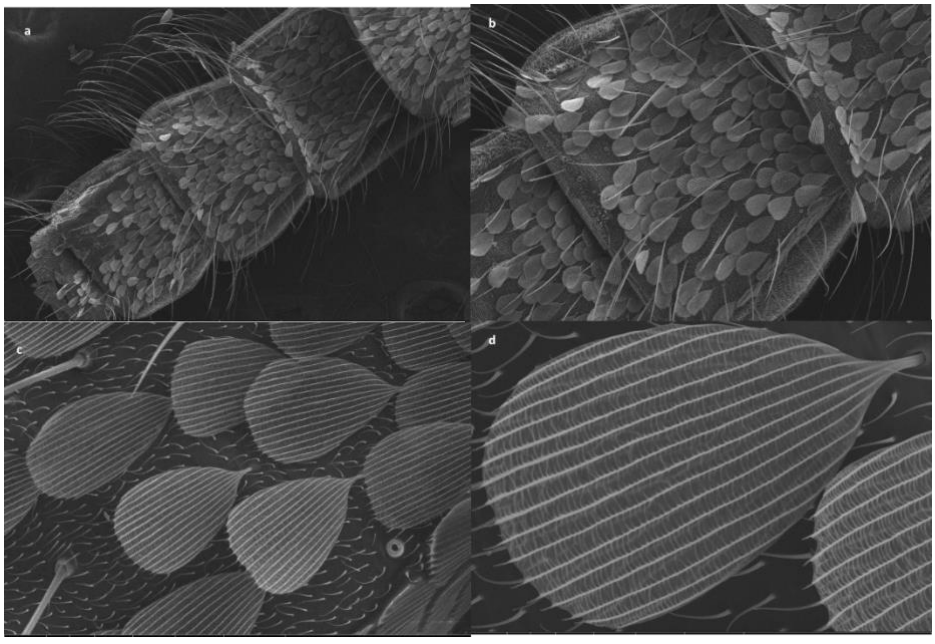


Figure 7. Scanning electron microscopy (SEM) images of scale from different abdomen parts of the mosquito, *Aedes zammitii*.

## Conclusion

This study contributes to the understanding of insecticide resistance in *Ae. zammitii,* which is crucial for developing effective mosquito control strategies in the Aegean region of Türkiye. The findings highlight the need for diverse strategies to manage mosquito populations, including utilizing standardized methods to track resistance trends, developing new insecticides, and exploring non-chemical control methods. SEM analysis provided detailed descriptions of the morphology of *Ae. zammitii* eggs and adults, including their unique surface features.

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*Original article (Orijinal araştırma)*

# Genetic diversity of *Steinernema feltiae* Filipjev, 1934 (Rhabditida: Steinernematidae) and *Heterorhabditis bacteriophora* Poinar, 1976 (Rhabditida: Heterorhabditidae) in potato production areas of Türkiye[1]

Türkiye'de patates üretim alanlarında, *Steinernema feltiae* Filipjev, 1934 (Rhabditida: Steinernematidae) ve *Heterorhabditis bacteriophora* Poinar, 1976 (Rhabditida: Heterorhabditidae) genetik çeşitliliği

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## Abstract

Entomopathogenic nematodes (EPNs) are one of the most important biological control agents and have proved their biocontrol success against a variety of insect pests. However, limited knowledge exists regarding the genetic structure of various species and populations of EPNs. Thus, this study was conducted to isolate and elucidate the EPN's phylogenetic diversity sourced from potato (*Solanum tuberosum* L.) (Solanales: Solanaceae) crops in 2020 at Bolu Abant Izzet Baysal University. Through ribosomal DNA sequencing, we investigated genetic variability within and among isolates of *Steinernema* and *Heterorhabditis* species. Widespread sampling across Afyonkarahisar, Bolu, İzmir, Sivas, Niğde, Kayseri, and Konya provinces, covering a total area of 795 hectares, led to the recovery of two EPN isolates, constituting 10% of the samples. Molecular characterization involved ribosomal DNA sequencing, which, upon integration with sequences from 41 populations, confirmed the identification of *Steinernema feltiae* Filipjev, 1934 (Rhabditida: Steinernematidae) and *Heterorhabditis bacteriophora* Poinar, 1976 (Rhabditida: Heterorhabditidae), displaying monophyly in most *Steinernema* and *Heterorhabditis* clades, respectively. This survey emphasizes the common occurrence of these EPNs in key potato-growing areas in Türkiye, highlighting their biocontrol potential against arthropod pests of agricultural importance.

**Keywords:** Beneficial nematodes, genetic variability, ribosomal DNA sequencing

## Öz

Entomopatojen nematodlar (EPN) en önemli biyolojik mücadele ajanlarından biridir ve çeşitli böcek zararlılarına karşı biyolojik savaşta başarılarını kanıtlamıştır. Ancak çeşitli türlerin ve EPN popülasyonlarının genetik yapısına ilişkin bilgiler sınırlıdır. Bu nedenle, bu çalışma, patates alanlarından elde edilen EPN'lerin filogenetik çeşitliliğini ortaya koymak ve tel kurtlarının, özellikle *Agriotes* spp. (Coleoptera: Elateridae) türlerinin mücadelesi için alternatif mücadele yöntemlerini belirlemek amacıyla 2020 yılında Bolu Abant İzzet Baysal Üniversitesi'nde yapılmıştır. Ribozomal DNA dizileme yoluyla, *Steinernema* ve *Heterorhabditis* türlerinin izolatları arasındaki genetik farklılıklar araştırılmıştır. Afyonkarahisar, Bolu, İzmir, Sivas, Niğde, Kayseri ve Konya illerini kapsayan geniş bir örnekleme ile, toplamda 795 hektarlık bir alanı kapsayarak, örneklerin %10'unu oluşturan iki EPN izolatının elde edilmiştir. Moleküler karakterizasyon, ribozomal DNA dizileme içermekte ve 41 popülasyonun dizileriyle incelendiğinde, *Steinernema feltiae* Filipjev, 1934 (Rhabditida: Steinernematidae) ve *Heterorhabditis bacteriophora* Poinar, 1976 (Rhabditida: Heterorhabditidae)'nın varlığını doğrulayarak, çoğu *Steinernema* ve *Heterorhabditis* kladlerinde yer almıştır. Bu araştırma, bu EPN'lerin Türkiye'deki önemli patates yetiştirme alanlarında yaygın olarak bulunduğunu göstermekte ve bunların tarımsal öneme sahip arthropod zararlılara karşı biyolojik savaşta potansiyellerini vurgulamaktadır.

**Anahtar sözcükler:** Faydalı nematodlar, genetik çeşitlilik, ribosomal DNA sekans

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## Introduction

The potato (*Solanum tuberosum* L.) (Solanales: Solanaceae) is a vital food and a significant industrial commodity globally. It holds promise in reducing hunger and poverty worldwide. With an output of 388 million tons across 162 nations, potatoes rank as the fourth most crucial main food following maize, rice, and wheat (FAOSTAT, 2021). However, potato cultivation faces numerous challenges from pathogens and pests, including nematodes. Among these pests, wireworms, specifically *Agriotes* spp. Eschscholtz, 1829 (Coleoptera: Elateridae), pose a substantial threat, causing substantial economic losses in various crops, notably potatoes (Furlan & Tóth, 2017). Wireworms predominantly reside underground during their larval stage, feeding on subterranean plant parts of potato, causing a significant reduction in tuber yield and tuber quality (Furlan et al., 2021). Moreover, the feeding punctures on tubers by wireworm larvae make plants susceptible to other soil-borne pathogens (Keiser et al., 2012). As a result, damaged potato tubers lose their commercial value, and the profitability of potato production drops dramatically (Keiser et al., 2012). Traditionally, chemical insecticides with broad-spectrum compounds like carbamates, organophosphates, and organo-chlorine have been primary tools for wireworm control among most growers. Yet, due to environmental and health concerns, numerous synthetic chemicals face regulatory restrictions and bans. Consequently, researchers have been prompted to investigate eco-friendly alternatives for controlling wireworm populations (Reddy & Tangtrakulwanich, 2014).

In recent years, entomopathogenic nematodes (EPNs) from the families Heterorhabditidae and Steinernematidae have garnered significant attention for their potential in biological pest control, targeting various economically significant insect pests (Bhat et al., 2020; Peçen & Kepenekci, 2022). Taxonomists have described over 100 species of *Steinernema* and 21 of *Heterorhabditis* (Bhat et al., 2020). These nematodes have shown remarkable efficacy in controlling agricultural pests, particularly those belonging to the orders Lepidoptera, Diptera, and Coleoptera, across a wide range of crops (Garriga et al., 2020; Özdemir et al., 2021; Yüksel, 2022; Wakil et al., 2023). Notably, EPNs have formed symbiotic relationships with insect pathogenic bacteria of the genera *Photorhabdus* and *Xenorhabdus* (Boemare, 2002). During the infective juvenile (IJ) stage, these nematodes, residing in the soil, actively seek out insect hosts, penetrating their bodies through natural openings or by breaching the cuticle directly. Upon locating a suitable host, they release their bacterial symbionts upon detecting chemical cues in the insect's hemolymph. These bacteria then proliferate, generating virulence factors and toxins that incapacitate the host (Boemare, 2002). Additionally, the bacteria release exoenzymes that break down insect tissues and generate metabolites essential for the growth, development, and reproduction of nematodes (Forst et al., 1997). Moreover, they produce potent secondary metabolites with antibiotic properties, deterring scavenging arthropods. Upon exhausting resources, the succeeding generation of nematodes disperses to seek out new hosts (Dreyer et al., 2018). The soil-dwelling nature of EPNs, coupled with their effective host-searching strategies, makes them ideal candidates to suppress pest populations that live in the soil environments (Hazir et al., 2003a).

Accurate identification of EPN species is essential for devising effective control strategies. However, relying solely on morphological characteristics for nematode diagnosis is challenging and time-consuming, requiring substantial expertise. Consequently, molecular techniques are increasingly favored for disease diagnosis, offering precise and swift identification, along with insights into population origins and introduction pathways. Consequently, genomic and ribosomal DNA analysis has emerged as the preferred method for nematode identification (Hashmi et al., 1996). In a prior investigation, a comprehensive field survey was conducted in key potato cultivation regions of Türkiye to assess the genetic diversity of EPN species for controlling significant potato pests. Here, the current study aims to (i) employ molecular data, specifically sequencing of the ITS-rDNA expansion segments, to identify various isolates of the genera *Steinernema* and *Heterorhabditis*, and (ii) explore the genetic relationships among EPN species.

## Materials and Methods

### Sampling area and EPN Isolation

The sampling area encompassed potato fields from 407 locations spread across 7 provinces (Afyonkarahisar, İzmir, Bolu, Sivas, Konya, Niğde and Kayseri) situated in various regions of Türkiye, which are significant centers for potato production (Figure 1).


Figure 1. The survey of entomopathogenic nematodes conducted in potato areas of Türkiye

A grand total of 407 soil samples were gathered, with each farm contributing nine samples, covering a combined area of 325.7 hectares (as indicated in Table 1). Using a hand shovel, the rhizosphere of potato plants was sampled. The samples were taken at a depth of 0-20 cm, labeled, and put in plastic boxes in bags. All samples were transferred to laboratory for isolation process of EPNs.

Table 1. The sampled location for entomopathogenic nematodes in potato growing area in Türkiye

| No | Province | Production areas (decare) | Samples |
|---|---|---|---|
| 1 | Bolu | 30 | 22 |
| 2 | İzmir | 112 | 55 |
| 3 | Afyonkarahisar | 129 | 65 |
| 4 | Konya | 139 | 70 |
| 5 | Sivas | 60 | 30 |
| 6 | Kayseri | 90 | 45 |
| 7 | Niğde | 235 | 120 |
| Total | | 795 | 407 |

After eliminating plant debris and stones in the soil, samples were subjected to the Galleria trap technique (Akhurst & Bedding, 1986). Samples were poured into clean plastic containers (8×8×10 cm) containing eight last-instar larvae of *Galleria mellonella* (L., 1758) (Lepidoptera: Pyralidae). These containers were covered and inverted every 24 hours to facilitate interaction between the wax moth larvae and the infective juveniles (IJs) of nematodes. After a week of incubation in darkness at 25°C, the containers were examined every three days to check for dead larvae. Any deceased larvae found were individually transferred to modified White traps to collect the emerging infective-stage juveniles. During the initial week, the emerging infective juveniles (IJs) were washed with distilled water, and each nematode isolate underwent pathogenicity assessments on 10 *G. mellonella* larvae to validate Koch's postulates (Kaya & Stock, 1997). Subsequently, the juveniles coming out of the cadavers were reproduced on *G. mellonella* larvae by re-inoculating the IJs to larvae. All these processes were conducted at the Plant Protection Department of Bolu Abant Izzet Baysal University.

**Molecular studies**

The collected isolates of *Steinernema* and *Heterorhabditids* were cultured *in vivo* using the last instar larvae of *Galleria mellonella*. Subsequently, DNA extraction was performed from a single F1 female of each isolate using the Quiagen® DNeasy blood and tissue kit, according to the manufacturer's guidelines. The DNA samples containing a concentration of approximately 20 ng/µL DNA were used for further molecular analyses. Following this, DNA pattern and phylogenetic analyses were run using two standard barcoding loci from the nuclear genome, namely the ITS and LSU rDNA regions.

For each locus, PCR amplifications were conducted by combining 2 µL of DNA (20 ng/µL), 2.5 µL of 10× PCR Buffer (NH4)2SO4, 2 µL of 25 mM MgCl2, 1 µL of 20 mM dNTPs, 0.4 µL of each forward and reverse primer (10 mM), 0.5 µL of 5× BSA, 1 µL of 10% Trehalose, 0.16 µL of Taq DNA polymerase (5 U/µL), and 15 µL of ultrapure water, resulting in a total volume of 25 µL. The primer sets utilized are listed in Table 2. The PCR protocol began with an initial denaturation step at 95°C for 600 s and this step was followed by 32 cycles consisting of 45 s denaturation at 55°C, annealing at 55°C for 45 s, and extension at 72°C for 60 s, with a final extension step at 72°C for 60 s. To determine the length of the PCR products, amplified DNA of isolates was subjected to electrophoresis in 1.5% agarose gels and run at 120 V for 45 minutes. All PCR products were purified, and their concentrations were verified by electrophoresis using 1 µL of the purified product.

Table 2. The primer sets used for both PCR amplification and sequencing reactions targeting entomopathogenic nematodes (EPNs)

| Primer | Region | Primer Sequence | Orientation | |
|---|---|---|---|---|
| AB28 | ITS rDNA | ATATGCTTAAGTTCAGCGGGT | Forward | Joyce et al. (1994) |
| TW81 | ITS rDNA | GTTTCCGTAGGTGAACCTGC | Reverse | |

Genetic diversity quantification among EPN species was conducted using Mega 7 (Kumar et al., 2018). Moreover, the calculation of segregating sites was conducted by assessing the average number of polymorphic nucleotides between sequences and the G + C content, employing DnaSP 5.1 (Librado & Rozas, 2009). Notably, all nucleotide sequences of EPN isolates have been archived in the GenBank NCBI database (refer to Table 3). Maximum Likelihood (ML) analyses were executed with 1000 replicates of bootstrap support, utilizing the General Time Reversible model (Tavaré, 1984), incorporating invariable sites (I) (Shoemaker & Fitch, 1989), and accounting for variations across sites (G) (Yang, 1994). A concatenated analysis was also conducted, incorporating sequences from 41 species reported for ITS (Tavaré, 1984). The Maximum Likelihood (ML) analysis comprised 1000 bootstrapped sequence alignments, which underwent global rearrangement with random replications. The phylogenetic relationship between *S. feltiae* and *H. bacteriophora* populations was compared to international isolates, with *Caenorhabditis elegans* (Maupas, 1900) (Rhabditida: Rhabditidae) serving as an outgroup to root the phylogeny. Reference sequence *Steinernema carpocapsae* (Weiser, 1955) (Rhabditida: Steinernematidae) *(*AY944007) (Nadler et al., 2006) was employed to identify all nucleotide-level substitutions.

Table 3. Sequenced entomopathogenic nematode samples that were collected from surveyed areas

| No | Code | Species | Accession Number |
|---|---|---|---|
| 1 | Bolu-1 | *Steinernema feltiae* | OR979106 |
| 2 | Bolu-2 | *Steinernema feltiae* | OR979107 |
| 3 | Bolu-7 | *Steinernema feltiae* | OR979108 |
| 4 | Bolu-8 | *Steinernema feltiae* | OR979109 |
| 5 | Bolu-9 | *Steinernema feltiae* | OR979110 |
| 6 | Bolu-10 | *Steinernema feltiae* | OR979111 |
| 7 | Bolu-14 | *Steinernema feltiae* | OR979112 |
| 8 | Bolu-22 | *Steinernema feltiae* | OR979113 |
| 9 | Bolu-24 | *Steinernema feltiae* | OR979114 |
| 10 | Bolu-26 | *Steinernema feltiae* | OR979115 |
| 11 | Konya-3 | *Steinernema feltiae* | OR979123 |
| 12 | Konya-4 | *Steinernema feltiae* | OR979124 |
| 13 | Konya-6 | *Steinernema feltiae* | OR979125 |
| 14 | Konya-7 | *Steinernema feltiae* | OR979126 |
| 15 | Konya-8 | *Steinernema feltiae* | OR979127 |
| 16 | Konya-10 | *Steinernema feltiae* | OR979128 |
| 17 | Konya-12 | *Steinernema feltiae* | OR979129 |
| 18 | Konya-14 | *Steinernema feltiae* | OR979130 |
| 19 | Konya-15 | *Steinernema feltiae* | OR979131 |
| 20 | Konya-16 | *Steinernema feltiae* | OR979132 |
| 21 | Konya-18 | *Steinernema feltiae* | OR979133 |
| 22 | Konya-19 | *Steinernema feltiae* | OR979134 |
| 23 | Konya-20 | *Steinernema feltiae* | OR979135 |
| 24 | Konya-22 | *Steinernema feltiae* | OR979136 |
| 25 | Kayseri-4 | *Steinernema feltiae* | OR979116 |
| 26 | Kayseri-5 | *Steinernema feltiae* | OR979117 |
| 27 | Kayseri-10 | *Heterorhabtidis bacteriophora* | OR979118 |
| 28 | Kayseri-14 | *Steinernema feltiae* | OR979119 |
| 29 | Kayseri-16 | *Heterorhabtidis bacteriophora* | OR979120 |
| 30 | Kayseri-18 | *Steinernema feltiae* | OR979121 |
| 31 | Kayseri-20 | *Heterorhabtidis bacteriophora* | OR979122 |
| 32 | Afyonkarahisar-1 | *Heterorhabtidis bacteriophora* | OR979098 |
| 33 | Afyonkarahisar-2 | *Steinernema feltiae* | OR979099 |
| 34 | Afyonkarahisar-4 | *Heterorhabtidis bacteriophora* | OR979100 |
| 35 | Afyonkarahisar-8 | *Steinernema feltiae* | OR979101 |
| 36 | Afyonkarahisar-12 | *Heterorhabtidis bacteriophora* | OR979102 |
| 37 | Afyonkarahisar-14 | *Heterorhabtidis bacteriophora* | OR979103 |
| 38 | Afyonkarahisar-35 | *Steinernema feltiae* | OR979104 |
| 39 | Afyonkarahisar-54 | *Steinernema feltiae* | OR979105 |
| 40 | Sivas-6 | *Steinernema feltiae* | OR979137 |
| 41 | Sivas-8 | *Steinernema feltiae* | OR979138 |

## RESULTS and DISCUSSION

### Sampling area and EPN isolation

Through an extensive sampling effort in potato cultivation areas across seven provinces (Niğde, Bolu İzmir, Konya, Kayseri, Sivas and Afyonkarahisar) in Türkiye (Figure 1), forty-one out of 407 soil samples (10%) tested positive for EPNs. These isolates were predominantly recovered from Konya, Afyonkarahisar, Bolu, Kayseri, and Sivas provinces. Among the positive samples, the majority of nematode isolates were from the *Steinernema* genus, with 34 (82.4%) out of the 41 samples testing positive. *Steinernema feltiae* was the most frequently encountered species, found in 7 soil samples (16.6%), followed *by Heterorhabditis bacteriophora*.

This study represents the first comprehensive assessment demonstrating the widespread presence of entomopathogenic nematodes in potato fields across seven provinces in Türkiye, a key region for potato production. Among the 407 soil samples collected from various districts within the provinces, 41 entomopathogenic nematode isolates were obtained, indicating a recovery rate of 10%. This recovery rate (17.9%) aligns closely with findings from Karaman province (19.2%) (Yavuzaslanoglu et al., 2016), and it notably surpasses rates reported in other surveys, such as 4.71% in Rize (Keskin et al., 1995), 9% in Adana and Kahramanmaras provinces (Canhilal et al., 2016), and 2.03% across Türkiye (Hazır et al., 2003b). Similarly, recovery rates in subtropical regions of other European countries were 13.8% in Southern Italy (Tarasco & Triggiani, 2016), 4.6% in Spain (Del Pino & Palomo, 1996), and 9.5% in Egypt (Shamseldean & Abd-Elgawad, 1994).The relatively high recovery rate in this study may be attributed to meticulous sampling from a confined land area at optimal times, particularly after rainfall, as soil moisture and temperature are crucial factors influencing the survival of entomopathogenic nematodes in the soil environment (Wright, 1992; Ehlers, 1996). Among the isolates obtained, *S. feltiae* was the most prevalent species, accounting for 84.6% of the isolates, while the occurrence of *H. bacteriophora* was less common, at 16.4%. This observation aligns with previous studies in Turkey where *S. feltiae* was consistently identified as the most common entomopathogenic nematode species, followed by *H. bacteriophora* (Laznik et al., 2009; Canhilal et al., 2016; Yuksel & Canhilal, 2019).

### Molecular Identification

The rDNA ITS regions from all 41 nematode populations were effectively amplified using specific primers. This region, which includes the ITS1-5.8S-ITS2 segments covering flanking regions of the 18S and 28S genes, yielded a consistent fragment approximately 859 base pairs in length across all populations. Importantly, no PCR products were observed in the negative control lacking DNA template, confirming the specificity of the amplification. Subsequently, sequencing efforts produced 42 sequences from the sampled nematode populations, identifying them as belonging to *Steinernema feltiae* and *Heterorhabditis bacteriophora* species. For each species, a single consensus sequence was generated from the obtained sequences. Alignment of these sequences with corresponding 18S rRNA gene sequences from nematode isolates revealed matches with 41 nematode species cataloged in the GenBank database (Table 1).

The rRNA sequence was discovered to be less efficient in resolving taxonomic conflicts at the species level, mainly because it represents fewer taxa, which is linked to its slower evolutionary pace (Stock, 2009). However, this trait has been leveraged to distinguish the monophyletic origins of nematode groups (Peat et al., 2009). Additionally, the 5.8S rRNA region within the ITS is comparatively shorter and more conserved than the ITS-1 and ITS-2 regions, yet it evolves more rapidly than the 18S and 28S genes, rendering it suitable for taxonomic and population genetic studies of entomopathogenic nematodes (EPNs) at the species (population) level (Stock, 2009). Specifically, the ITS-1 region has been demonstrated to be adequate for species differentiation and assessment of *Heterorhabditis* spp. evolutionary relationships (Peat et al., 2009; Stock, 2009). On the contrary, the 28S rRNA gene displays a quicker rate of variation

compared to the 18S rRNA gene and presents fewer uncertainties in alignment than the ITS region (Stock, 2009). Despite this, it's considered more informative and appropriate for evaluating phylogenetic relationships, defining terminal taxa, and fulfilling diagnostic roles within *Steinernema* spp. (Stock & Hunt, 2005; Stock, 2009). The identification approach employed in this study aligns with previous findings (Liu et al., 1999).

A phylogenetic tree was constructed based on genetic distance, clustering populations at various levels using ITS sequence alignment (Figure 2).

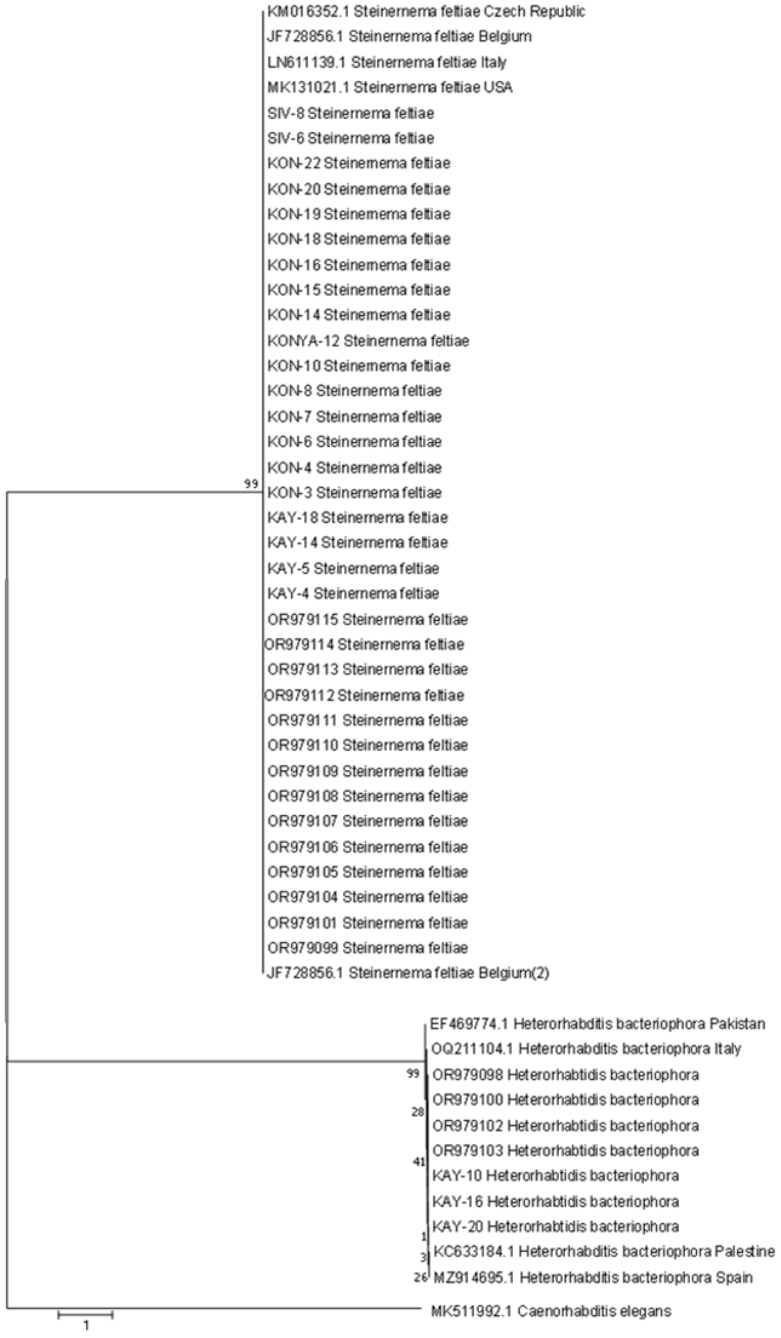


Figure 2. A phylogenetic tree (Neighbour-joining) was generated using the ITS sequence alignment derived from 41 populations of *Steinernema feltiae* and *Heterorhabtidis bacteriophora*. Bootstrap values are provided for the relevant clades to indicate their statistical support. Accession numbers for the population codes are listed in Table 1.

This analysis evaluated samples from five geographically distant sites, including 34 populations of *Steinernema feltia*e, 7 populations of *Heterorhabditis bacteriophora,* and one outgroup, *Caenorhabditis elegans* (MK511992.1). The resulting tree exhibited a distinct separation between the outgroup and *S. feltiae* and *H. bacteriophora* populations. Species with bootstrap values exceeding 99% were considered well-supported. The analysis revealed differences in ribosomal DNA sequences among the 41 isolates of *H. bacteriophora and S. feltiae*, indicating the presence of intraspecific polymorphism among the nematode populations. For the ITS region, a 859 bp fragment was obtained for the isolate of *Steinernema feltiae*. A BLAST analysis conducted against sequences archived in GenBank revealed a striking similarity ranging from approximately 96% to 99% with sequences belonging to the same species. The consensus tree derived from Bayesian inference prominently displayed a well-supported cluster (100% bootstrap) comprising sequences of *S. feltiae* originating from diverse geographical locations, including Belgium (JF28856.1), Czech Republic (KM016352.1), Italy (LN611139.1), and the USA (MK131021.1), which were sequenced in this study (Figure 4). Similarly, a 859 bp fragment was obtained for the *Heterorhabditis bacteriophora* isolate, which exhibited approximately 98–99% similarity with sequences of the same species in GenBank. The Bayesian inference consensus tree also displayed a well-supported group (100% bootstrap) consisting of sequences of H. bacteriophora from different countries, including Pakistan (EF469774.1), Italy (OQ211104.1), Palestine (KC633184.1), and Spain (MZ914695.1), which were sequenced in this study (Figure 2). Overall, the topology of our phylogenetic tree closely resembles that of previous studies (Liu et al., 1999; Emelianoff et al., 2008).

Historically, species determination within the Steinernematid nematode group has relied on crossbreeding experiments, morphometrics, and morphological characterization (Hominick et al., 1997). Morphometric differences in nematodes could be attributed to intra-specific variability (Poinar, 1992; Stock & Hunt, 2005). However, molecular tools may offer a solution to this issue and provide a novel approach for evaluating species boundaries in this group. Adams (1998) established several criteria for species description in nematology, emphasizing the importance of identifying autapomorphies (unique derived characters) as a primary requirement for establishing a new species.

Entomopathogenic nematodes, particularly *H. bacteriophora* and *S. feltiae*, have demonstrated significant potential for biological control of insects (Bhat al., 2020). Field tests have shown that *H. bacteriophora* effectively controls various target pests such as white grubs, cucumber beetles, black vine weevil, potato beetle, strawberry root weevil, among others (Grewal, 2012). While efforts have been made to assess the efficacy of these nematodes against foliar pests, challenges including desiccation, sunlight exposure, and high temperatures, which can be fatal to exposed nematodes, limit such applications (Grewal, 2012). The current survey indicates that *S. feltiae* and *H. bacteriophora* are frequently found in key potato-growing regions in Türkiye, suggesting they may hold promise for insect pests' biological control.

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*Original article (Orijinal araştırma)*

# Comparison of effectiveness of molecular markers linked to *Me1* and *N* genes in pepper (*Capsicum annuum* L.) (Solanales: Solanaceae)

Biber (*Capsicum annuum* L.) (Solanales: Solanaceae)' de *Me1* ve *N* genlerine bağlı moleküler markörlerin etkinliğinin karşılaştırılması

**Gülsüm UYSAL[1]** **Zübeyir DEVRAN[2*]**

## Abstract

Pepper (*Capsicum annuum* L.) (Solanales: Solanaceae) is one of the most important agricultural products consumed in the world. Root-knot nematodes (RKNs (*Meloidogyne* spp.)) are major pests that occur dramatically damage on pepper. However, the management of RKNs has some difficulties and one of the most effective methods is using resistant cultivars in infested areas. In this study, the efficiency of molecular markers linked to *Me1* and *N* genes was investigated. The study was conducted in laboratory and under controlled conditions at Akdeniz University Faculty of Agriculture, Department of Plant Protection Nematology Laboratory in 2022. Pepper genotypes belonging to two main varieties (Charleston pepper and Bell pepper) were tested against S6 isolate of *Meloidogyne incognita* (Kofoid & White, 1919) Chitwood 1949 (Tylenchida: Heteroderidae), and screened with molecular markers. As a result, molecular markers linked to two genes gave compatible results with pathologic tests. These markers can be successfully used for marker assisted selection in pepper genotypes.

**Keywords:** *Meloidogyne incognita*, pathologic tests, PCR primers, resistance

## Öz

Biber (*Capsicum annuum* L.) (Solanales: Solanaceae) dünyada tüketilen en önemli tarımsal ürünlerinden biridir. Kök-ur nematodları (*Meloidogyne* spp.) biberde ciddi hasara neden olan başlıca zararlılardır. Ancak Kök-ur nematodlarının mücadelesinde bazı zorluklar vardır ve bulaşık bölgelerde en etkili mücadele yöntemlerden biri dayanıklı çeşitlerin kullanılmasıdır. Bu çalışmada *Me1* ve *N* genlerine bağlı moleküler belirteçlerin etkinliği araştırılmıştır. Çalışma, 2022 yılında Akdeniz Üniversitesi Ziraat Fakültesi Bitki Koruma Bölümü Nematoloji laboratuvarında, laboratuvar ve kontrollü iklim odası koşullarında yürütülmüştür. İki ana çeşide (Charleston biberi ve Dolma biberi) ait biber genotipleri *Meloidogyne incognita* (Kofoid & White, 1919) Chitwood 1949 (Tylenchida: Heteroderidae) S6 izolatına karşı test edilmiş ve moleküler belirteçlerle taranmıştır. Sonuç olarak iki gene bağlı moleküler belirteçler patolojik testlerle uyumlu sonuçlar vermiştir. Bu belirteçler biber genotiplerinde markör destekli seleksiyonda başarılı bir şekilde kullanılabilir.

**Anahtar sözcükler:** *Meloidogyne incognita*, patolojik test, PCR primerler, dayanıklılık

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## Introduction

Solanaceae family has significant importance in agricultural productivity due to economically produced crops. Pepper (*Capsicum annuum* L.) (Solanales: Solanaceae) belonging to Solanaceae family is one of the most important agricultural vegetable yields consumed in the world (Pinto et al., 2016; Barka & Lee, 2020). There are approximately 20-30 *Capsicum* species cultivated in the different parts of the agricultural areas. Among these species, there are five main cultivated species; *Capsicum annuum*, *Capsicum chinense* Jacq., *Capsicum frutescens* L. Kuntze., *Capsicum baccatum* L., and *Capsicum pubesces* Ruiz & Pav. (Solanales: Solanaceae) (Bosland & Votava, 2005).

Pepper cultivations have an important role in economy and pharmacy. Pepper is known as a high-value crop including carotenoid, provitamin A and vitamin C (Bosland et al., 2012). It is also preferred as spice, while has been consumed for nutraceutical and nutritional properties, and industrial use (Lu et al., 2020).

Many pathogens and pests can attack pepper during cultivation. Root-knot nematodes (RKNs) (*Meloidogyne* spp.) (Tylenchida: Heteroderidae) are major pests that cause dramatically damage on pepper (Lizardo et al., 2022). RKNs are well-adapted obligate endoparasites which have more than one hundred species all over the world (Rehak Biondić et al., 2023). Major RKN species are *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: Heteroderidae). It was reported that *M. incognita* is the most common and significant species of RKNs all around the world (Jones et al., 2013). They cause serious damage on plants due to forming galls which prevent the nutrient uptake and absorption of water from soil. It was reported that 52% yield losses were caused by RKNs in pepper-growing areas in the Mediterranean area (Talavera-Rubia et al., 2022).

In the management of RKNs, cultural practices including crop rotation, use of resistant plant etc., solarization, biological control agents, and chemical nematicides were employed (Liu et al., 2023). Using resistant cultivars is one of the most effective methods in infested areas (Devran & Söğüt, 2014; Devran et al., 2015; Bucki et al., 2017). Breeding of nematode-resistant cultivars offers an economically and environmentally friendly sustainable strategy to controlling RKNs (Devran et al., 2013). The resistance against RKNs in pepper confers with several genes which are *N, Me1, Me2, Me3, Me4, Me5, Me6, Me7, Mech1, Mech2* and *CaRKNR* (Wang & Bosland, 2006; Djian-Caporalino et al., 2007; Wang et al., 2009; Mao et al., 2015). Resistance response of the genes occurs as the hypersensitive response (HR), which is gene to gene reaction in the plant tissue (Wang et al., 2009, 2018; Özalp & Devran, 2018). *Me* and *N* resistance genes in pepper have been reported to have resistance against RKNs including *M. incognita, M. javanica*, and *M. arenaria* (Djian-Caporalino et al., 2007; Thies & Ariss, 2009; Barbary et al., 2014).

In the resistance screening programs mostly pathogenicity tests are used, and it depends on several factors such as seedling stage, temperature and nematode inoculation levels (Djian-Caporalino et al., 2001; Devran et al., 2013; Barbary et al., 2016). In addition, classical pathogenicity tests have some difficulties with respect to labor and time required and involve a long process as well as serving limited materials. To overcome these difficulties, molecular markers, which are a powerful tool in plant breeding, can be preferred for providing an accurate and fast screening of large populations (Francia et al., 2005; Özkaynak et al., 2014; Nadeem et al., 2018). Molecular markers are also more advantageous as they are cost effective, quick and help checking more plant material. Molecular markers linked to resistance genes against RKNs have been developed from pepper lines/cultivars (Djian-Caporalino et al., 2007; Wang et al., 2009; Fazari et al., 2012; Uncu et al., 2015; Çelik et al., 2016). However, there is limited information on the efficacy of markers in different pepper types carrying resistant genes. This study aimed to describe the efficiency of the previously published molecular markers closely linked to *N* and *Me1* genes in pepper genotypes.

## Materials and Method

### Nematode isolate

*Meloidogyne incognita* S6 isolate was used in this study. This isolate was identified in previous studies (Devran & Söğüt, 2009, 2011). The isolate has been cultured as a pure nematode population since 2008 in Devran's Laboratory (Devran et al., 2023). The study was conducted in laboratory and under controlled conditions at Akdeniz University, Faculty of Agriculture, Department of Plant Protection, Nematology Laboratory in 2022.

### Nematode culturing

Reproduction of *M. incognita* S6 isolate was performed on susceptible pepper cultivar Safran F1 (Yüksel Seed, Türkiye). Pepper seedlings which had three or four true stages of leaves were planted into 250 cc plastic pot containing sterile soil. Approximately one week after the transplanting, egg masses of nematode were inoculated into plant roots 2-3 cm deep. Eight weeks after nematode inoculation, the plants were harvested, and the roots were washed carefully under tap water. Egg masses were counted under the light microscope and put into 1.5 ml centrifuge tubes to use inoculation.

### Nematode inoculation

Egg masses of nematodes were put into petri and incubated at 27±2°C in an incubator to the hatching of second stage juveniles (J2s) (Hooper, 1986). Then, every day hatched J2s were taken to 15 ml tube and J2s were counted under the stereo microscope. Approximately one week after transplanting, plants were inoculated with 1000 J2s of *M. incognita* S6 isolate into the 2 cm depth (Devran & Söğüt, 2009; Mıstanoğlu, et al., 2016).

### Plant material

Total 9 pepper genotypes belonging to Charleston and Bell pepper were used in this study. Both homozygous and heterozygous pepper cultivars for *Me1* and *N* genes and susceptible Safran F1 were used as control plants in the experiment (Table 1).

Table 1. Pepper plants used in this study

| Plant Code | Pepper type/ cultivar |
|---|---|
| P1 | Charleston pepper |
| P2 | |
| P3 | |
| P4 | |
| P5 | |
| P6 | Bell pepper |
| P7 | |
| P8 | |
| P9 | |
| P10 | Resistant Control 1 (*N/n*) |
| P11 | Resistant Control 2 (*Me1/me1)* |
| P12 | Resistant Control 3 *(Me1/Me1)* |
| P13 | Susceptible Control (Safran F1) |

Safran F1: No *Me1* and *N* genes

### Testing of pepper genotypes

The pepper seeds were germinated under 25±2°C controlled climate chamber in perlite and turf (1:1) mixture. Four true leaves seedlings were potted into 250 cc plastic pots filled with autoclaved (at 120°C, 2 h) sandy soil (75% sand, 15% silt, and 10% clay) after germination of seeds. When peppers have four true leaves, nematode inoculations were done. The study was conducted with 8 replicates in total plant (as 2 repeats with 4 replicates) according to the completely randomized design. Plants were grown in climate chamber at 25±1°C for 16:8 (light: dark) photoperiod with 60-65% relative humidity. Then plants were uprooted approximately sixty days after the inoculation, and plant roots were evaluated for the number of galls and egg masses and their indices (Hartman & Sasser, 1985).

### Data analyses

Plant roots were evaluated according to parameters including the number of egg masses, number of galls, and root galling and egg masses index (Hartman & Sasser, 1985). Based on the index, it was represented as resistant between 0-2 while 3-5 as susceptible.

In order to clear observation of roots, each plant's roots were stained with 0.15 g/L Phloxine B solution (Hussey & Barker, 1973). Following this application, the egg masses were counted under the light microscope.

The statistical analysis of these parameters was done using the statistical package program SAS (v. 9.0). Significant differences in pepper genotypes were analyzed using ANOVA with multiple comparison test Tukey HSD at $p<0.05$.

### DNA extraction

Total genomic DNA of pepper genotypes was isolated from young pepper leaves using Wizard Magnetic DNA Kit (Promega, Madison, WI, USA).

### Molecular marker analyses

For *Me1* gene and *N* genes, molecular markers linked to these genes were used in this study (Table 2). PCR was performed according to references mentioned in Table 2 and previous study (Nas et al., 2023). PCR yields were run a agarose gel in buffer TAE, stained with Xpert Green DNA Stain (Grisp, Portugal), and visualized in a Gel iX Imager (Intas Science, Germany).

Table 2. Molecular markers linked to *Me1* and *N* genes used in this study

| Gene | Marker name | Sequence (5′-3′) | Reference |
|---|---|---|---|
| *Me1* | SCAR_CD | GAAGCTTATGTGGTAMCC<br>GCAAAGTAATTATATGCAAGAGT | Djian-Caporalino et al. (2007) |
| | SCAR_HM60 | TATCCGTGGTCATCCTAGCC<br>TGTGGTTCATCGGGACTGTA | Fazari et al. (2012) |
| | SCAR_PM54 | CTGCAGGGTAGCAAAGTAATTATAT<br>CCAAAATTAGTCATGTTCTTATGTTCTTAC | |
| | 16880-1-V2 | TGACCCCTCAGACTGAACAG<br>CTCCTTCGCTGCTACCTTCT | Wang et al. (2018) |
| *N* | N-SCAR-315 | AATTCAGAAAAAGACTTGGAAGG<br>TAAAGGGATTCATTTTATGCATAC | Wang et al. (2009) |
| | CASSR37 | ACATACCCAAAAACTCTCTCAC<br>GATTGACCATGTTTCCGTAT | Çelik et al. (2016) |

## Results

### Response of peppers to *Meloidogyne incognita*

In the pathogenicity test, two pepper types which are Charleston and Bell were used. Pathogenicity test data were evaluated based on quantified parameters, the number of gall and egg masses and gall and egg masses index in 1000 J2 inoculation, pepper genotypes showed different levels of resistance to *M. incognita* S6 isolate. As expected, resistant control genotypes which carried *Me1* and *N* genes showed the highest level of resistance to *M. incognita*. (Table 3).

All Charleston genotypes exhibited the highest resistance to *M. incognita* and were classified as resistant according to scale (Hartman & Sasser, 1985). The highest and lowest gall number of Charleston genotypes which are P5 and P1 was 3.87 and 1.75, respectively. The number of egg masses ranged between 0.75 and 2.50 on P1 and P4, respectively. Gall index values were found between 1.12 and 1.87 while egg masses index was 0.62 and 1.50 on P1 and P5, respectively (Table 3). The response of Charleston pepper genotypes to *M. incognita* S6 isolate resulted in significant differences in the variables; the number of galls, the number of egg masses, gall index and egg masses index ($p < 0.05$) (Table 3).

Table 3. The number of galls, egg masses and indices of Charleston and Bell pepper genotypes and their marker reactions

| Plant Code | Gall | Egg masses | Gall index | Egg masses index | Pathological Reaction | Claimed phenotype[1] | SCAR_CD | SCAR_HM60 | SCAR_PM54 | 166880-1-V2 | NSCAR-315 | CASSR37 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| P1 | 1.75±1.03 b* | 0.75±1.03 b | 1.12±0.64 bc | 0.62±0.74 b | R | *Me1* | R | R | R | RR | S | S |
| P2 | 2.12±1.72 b | 1.37±1.50 b | 1.25±0.70 bc | 0.87±0.99 b | R | *Me1* | R | R | R | RR | S | S |
| P3 | 2.00±1.06 b | 1.75±1.28 b | 1.25±0.46 bc | 1.12±0.64 b | R | *Me1* | R | R | R | RR | S | S |
| P4 | 3.25±2.71 b | 2.50±2.72 b | 1.37±0.51 bc | 1.12±0.83 b | R | *Me1* | R | R | R | RR | S | S |
| P5 | 3.87±1.45 b | 2.50±1.30 b | 1.87±0.35 b | 1.50±0.53 b | R | *Me1* | R | R | R | RR | S | S |
| P6 | 3.37±3.11b | 1.25±1.16 b | 1.25±0.70 bc | 0.75±0.70 b | R | *N* | S | S | S | S | RR | RR |
| P7 | 2.75±3.05 b | 1.62±2.77 b | 1.37±0.51 bc | 0.75±0.88 b | R | *N* | S | S | S | S | RR | RR |
| P8 | 3.87±2.79 b | 1.50±2.32 b | 1.62±0.51 bc | 0.75±0.70 b | R | *N* | S | S | S | S | Rr | Rr |
| P9 | 4.00±2.30 b | 2.14±1.77 b | 1.71±0.48 bc | 1.28±0.75 b | R | *N* | S | S | S | S | RR | RR |
| P13 | 58.25± 3.15 a | 61.75±3.10 a | 4.00±0.0 a | 4.00±0.0 a | S | *S* | S | S | S | S | S | S |
| P10 | 3.12±2.10 b | 0.12±0.35 b | 1.50±0.53 bc | 0.12±0.35 a | R | *N* | S | S | S | S | Rr | Rr |
| P11 | 1.57±1.81 b | 0.42±0.78 b | 0.85±0.89 c | 0.28±0.48 b | R | *Me1* | R | R | R | Rr | S | S |
| P12 | 1.87±1.55 b | 1.25±1.66 b | 1.12±0.64 bc | 0.62±0.70 b | R | *Me1* | R | R | R | RR | S | S |

*Means ± SD Different letters within a column show significant differences ($p<0.05$ by ANOVA) between genotypes analyzed by Tukey multiple comparison tests.

[1]Phenotype information was obtained from the company. R: Resistant, S: Susceptible, RR: Homozygote resistant, Rr: Heterozygote resistant.

Gall and Egg masses index were evaluated according to Hartman & Sasser (1985).

Bell pepper genotypes represented the highest resistance to *M. incognita* S6 isolate. All genotypes were found resistant according to gall and egg masses indices (0-5). The highest gall number was found on P9 with 4.0, while the lowest gall number was 2.75 on P7 bell pepper genotypes (Table 3). The number of egg masses ranged between 1.25 and 2.14 on P6 and P9, respectively. Gall index values were found between 1.25 and 1.71 while egg masses index was 0.75 and 1.28 on P6 and P9, respectively (Table 3). Bell pepper genotypes showed significant differences for *M. incognita* S6 isolate according to the number of galls, the number of egg masses, gall index and egg masses index ($p < 0.05$) (Table 3).

**Molecular marker amplification**

For *Me1* gene, SCAR_CD, SCAR_PM54, SCAR_HM60, and 16880-1-V2 molecular markers were used for screening of plants. Results showed that marker 16880-1-V2 was codominant, and the others were dominant. The presence of *Me1* gene in all Charleston pepper genotypes was determined successfully. Molecular markers were compatible with pathological tests (Table 3).

For *N* gene, NSCAR-315 and CASSR37FR markers which are codominant were used in bell pepper genotypes. Bell pepper genotypes were homozygous and heterozygous allele for *N* gene (Table 3). The presence of *N* gene in Bell pepper genotypes was determined successfully by molecular markers, NSCAR-315 and CASSR37FR. These markers were correctly determined resistant genotypes. Molecular markers were compatible with pathological tests of bell pepper genotypes (Table 3).

## Discussion

Among the root-knot nematodes, *Meloidogyne incognita* is one of the most common species (Jones et al., 2013). Due to widespread geographical distribution of *M. incognita*, a significant reduction of yield and damage on pepper are observed in different locations of the world (Fullana et al., 2023). One of the main management strategies of RKN is validated as using resistant plant varieties especially in Solanaceous plants (Djian-Caporalino et al., 2007; Abdel-Mageed et al., 2023; Pradhan et al., 2023). Using resistance gene in plants helps growers to decrease economic losses in pepper production areas. In order to find resistant genes, plants need to screen both molecular and pathogenicity tests. In this study, the efficiency of the previously published molecular markers closely linked to *N* and *Me1* genes in pepper genotypes was described to test the response of peppers to *M. incognita*.

Charleston pepper genotypes bearing *Me1* gene were tested with *M. incognita*. Gall and egg masses indexes showed that all Charleston pepper genotypes used in this study were highly resistant. Similarly, pepper genotypes carrying *Me1* and *Me3* genes were tested with *M. incognita, M. javanica* and *M. arenaria* isolates and were found as resistant (Castagnone-Sereno et al., 1996, 2001). In another study conducted by Bucki et al. (2017), pathological response of accessions of pepper carrying the *Me1, Me3*, and *N* genes was found highly variable against *M. incognita* populations. Göze Özdemir & Uysal (2018) reported that the number of egg mass and gall reduced in pepper cultivars carrying *Me1* and *N* genes tested with *M. incognita*. Gürkan et al. (2018) reported the reactions of *M. incognita* race 1 against some pepper lines and varieties and all lines and varieties of pepper were found as susceptible. In this study, molecular markers SCAR_CD, SCAR_PM54, SCAR_HM60, and 16880-1-V2 were used for screening of *Me1* gene in Charleston peppers and the results of them were in accordance with pathology tests. SCAR_CD and SCAR_PM54 markers are dominant and SCAR_HM60 and 16880-1-V2 markers are codominant (Djian-Caporalino et al., 2007; Fazari et al., 2012; Wang et al., 2018). These markers help to find homozygous and heterozygous positions of gene in pepper genotypes. Pinar et al. (2016) used some molecular markers which are SCAR_CD and SCAR_PM54 to determine nematode resistance in diverse peppers genotypes and SCAR_PM54 were found fully successful in confirming both pathogenicity test and resistant genotypes. In the study dominant CAPS markers were used to screen the pepper to predict resistant and susceptible genotypes.

Bell pepper genotypes carrying *N* gene were tested with *M. incognita*. Gall and egg masses indexes showed that all Bell pepper genotypes were highly resistance. Thies et al. (1997) reported that pepper genotype of Carolina Ceyenne bearing *N* gene was resistance. In the other study De Souza- Sobrinho et al. (2002), it was determined the high resistance to *M. incognita* in backcrossed as seed parents of hot pepper cv. Carolina Cayenne and the sweet pepper cv. Agronômico-8. Wang et al. (2009) developed the molecular marker called as N-SCAR-315 linked *N* gene in pepper genotype using 320 F2 individuals obtained crossing of sweet pepper line (Carolina Wonder, *N* gene), and an inbred line '20080-5-29' (*C. annuum*). In this study, N-SCAR-315 marker was successful in the detection of *N* gene. In another study, the *N* gene linked to marker CASSR37FR were developed from F2 populations of crossed with resistant Carolina Wonder and susceptible pepper cultivar (Çelik et al., 2016). In the present study, molecular markers NSCAR-315 and CASSR37FR were in accordance with pathology tests in Bell peppers. These markers successfully predicted the homozygous and heterozygous in bell pepper genotypes.

In conclusion, pepper genetical variation was crucial in pepper breeding to nematode resistance. Our study emphasizes the molecular markers of *Me1* and *N* genes successfully determined the resistance of Charleston and Bell pepper genotypes. During molecular screening of pepper cultivars, we found all *Me1* gene markers produced positive results on all Charleston pepper genotypes, and *N* gene markers successfully gave products as expected size on Bell pepper genotypes. Both results pathogenicity and molecular markers were well matched. These marker sets can be used to determine the *Me1* and *N* genes in pepper breeding programs. However, further research is needed to better determine the efficacy of these markers. The markers should be screened in more pepper genotypes with different genetic backgrounds and types.

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