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Original article

Interactions of predatory coccinellids (Coleoptera: Coccinellidae) and aphids (Hemiptera: Aphididae) in pome and stone fruit orchards of Çanakkale Province

Çanakkale ili yumuşak ve sert çekirdekli meyve bahçelerindeki predatör coccinellidler (Coleoptera: Coccinellidae) ve afitlerin (Hemiptera: Aphididae) etkileşimleri

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ABSTRACT

This study revealed the tritrophic interactions of predatory coccinellids-host aphids-host plants on pome and stone fruit trees and herbaceous plants in fruit orchards of Çanakkale Province, Turkey. Field sampling was done during the spring and summer seasons in 2020 and 2021. Twelve predatory species belonging to eight genera from the family Coccinellidae (Coleoptera) were found interacting with eleven host aphids from the family Aphididae (Hemiptera) on eight host plants. A total of 33 tritrophic interactions of predatory coccinellids-host aphids-host plants were revealed on the pome and stone fruit orchards in the Çanakkale Province. From the predators, *Harmonia axyridis* (Pallas) associated with seven aphids was the most common coccinellid, followed by *Oenopia conglobata* (L.) which was associated with six aphids. From the aphids, *Brachycaudus helichrysi* (Kaltenbach) was the most common species; it was associated with ten different predatory coccinellids. Also, from the host plants, the highest number of the interactions of predatory coccinellids-host aphids were revealed on *Cydonia oblonga* Mill. (Rosaceae) and *Prunus domestica* L. (Rosaceae) in the pome and stone fruit orchards of Çanakkale Province. As a result, it is considered that the tritrophic interactions of predatory coccinellids-host aphids-host plants should be better understood to increase the success of biological control of pest aphids on the fruit orchards.

INTRODUCTION

Aphids (Hemiptera: Aphididae) are one of the most important agricultural pests that cause severe economic losses from damage done to a large number of crop and forest plants as a result of sap-sucking, and honeydew secretion. They also vector more than 270 plant phytopathogenic viruses that cause serious economic damage to agricultural crops (Katis et al. 2007). These insects are mostly distributed in terrestrial ecosystems worldwide (Alford 2011, Diehl et al. 2013, Jouraeva et al. 2006, van Emden and Harrington 2007).

The family Coccinellidae, known as ladybird beetles or lady beetles, is the largest in the superfamily Cucujoidea (Coleoptera) with nearly 6000 species belonging to 360 genera in two subfamilies and 30 tribes. Most coccinellid species are predators (Slipinski 2007); however, certain species can feed on plant tissues or fungal material, as well as various facultative food sources such as nectar, pollen and honeydew (Chinery 1993, Slipinski and Tomaszewska 2010). The vast majority of predatory coccinellids prefer insect species of Sternorrhnycha (Hemiptera) suborder, as well as mites, nymphs of Thysanoptera, and early instar larvae of some orders such as Diptera, Lepidoptera, Hymenoptera (Pervez 2004); hence such predatory coccinellids have been successfully used in biological control of many pest species such as aphids, scale insects, whiteflies, thrips, mealybugs, leaf hoppers and other soft bodied pests worldwide (Magro et al. 2010). Some coccinellid species are known as major predators of aphid pests (Volkl et al. 2007) and their predation on aphids contributes to the suppression of aphid pests in several agroecosystems (Deguine et al. 2007, Lee et al. 2005, Michels and Matis 2008).

Although predatory coccinellids of aphids cannot effectively impact the long-term population dynamics of aphid species in agroecosystems, they are quite efficient predators capable of reducing seasonal densities of selected aphid pests (Obrycki et al. 2009). It is known that the prey preferences of predatory coccinellids are quite variable. Giorgi et al. (2009) presented important data on the evolution of food preferences in Coccinellidae. One of the most important factors affecting the host aphid prey preferences of predatory coccinellids is the aphid-host plant interactions. Pervez and Chandra (2018) revealed that the prey diet of predatory coccinellids depends largely on the host aphid-host plant combination, and that host plant allelochemicals had a direct effect on the palatability of prey consumed by coccinellids. Also, Pervez and Kumar (2017) emphasized that most plant toxic constituents can alter the biochemical composition of the most preferred aphid prey of predatory coccinellids and make them the least preferred.

More tritrophic interaction studies in different agroecosystems are needed to better understand the interactions of predatory coccinellids-aphids-host plants in terms of biology, ecology and evolution, and to increase the success of biological control studies of aphids using predatory coccinellids. In this study, we revealed that the tritrophic interactions of predatory coccinellids-host aphids-host plant on pome and stone fruit trees and herbaceous plants in fruit orchards of Çanakkale Province, Turkey.

MATERIALS AND METHODS

This study aimed to determine the predatory coccinellid species (Coleoptera: Coccinellidae), which is one of the important natural enemies of aphids, on pome and stone fruit trees such as quince, almond, apple, plum, cherry, peach, and herbaceous host plants on the edges of fruit orchards in Çanakkale province. The sampling of predatory coccinellids and their host aphids were collected from Bayramiç, Biga, Ezine, Lâpseki and Merkez districts of Çanakkale province where fruit production is common between spring and summer seasons in 2020 and 2021.

Collection and identification of predatory coccinellids

Adult coccinellid individuals found on fruit trees and herbaceous host plants infested with aphids were collected by hand searching and suction tube, and later brought to the laboratory in glass jars covered with a net. Also, the adult coccinellid specimens were dropped into the Japanese umbrella and Steiner funnel by using the knock method on the branches in different directions, heights and inside-outside parts of fruit trees infested with aphids. For the collection of larvae, the larval stages of coccinellid individuals feeding on aphid colonies on fruit trees and herbaceous host plants were brought to the laboratory with parts of host plants infested with aphids in glass jars or plastic boxes covered with a net. Later, these larval stages were allowed to develop into adult individuals in the climate chamber with 22.5 °C temperature, 65% relative humidity and 16:8 lighting. For the preparation of coccinellid specimens, coccinellids individuals from fruit trees and herbaceous host plants in the orchards were killed in the glass jars using ethyl acetate and pinned from the appropriate parts of the body for identification. The predatory coccinellids species in this study were identified by Assist. Prof. Dr. Derya ŞENAL (Bilecik Şeyh Edebali University, Faculty of Agriculture and Natural Sciences, Department of Plant Protection, Bilecik, Turkey)

Collection and identification of host aphids

The sampling of host aphids of predator coccinellids was also collected from the fruit trees and herbaceous host plants infested with aphids in fruit orchards. Aphid colonies that do not contain sufficient number of adults were brought to the laboratory together with the infested parts of host plants such as stem, branch, shoot and leaf in order to obtain adult aphid individuals. A sufficient number of apterous, alate and nymph of aphid individuals were put in the Eppendorf tubes containing 70% ethyl alcohol using a (00) soft brush. For the preparation of the host aphid specimens, Hille Ris Lambers (1950) method was followed. The specimens of host aphids were identified by using a LEICA DM 2500 microscope with a mounted HD camera and 4.1 version of LAS software based on Blackman and Eastop (2006, 2021). For the current taxonomic status and species names of the identified aphids in this study were followed Favret (2021).

Predatory coccinellids-host aphids-host plants interactions

To visualize the structural patterns of the predatory coccinellids-host aphids-host plants tritrophic network in the fruit orchard in the Çanakkale province, the graphs of tripartite interactions were constructed based on the data of coccinellids, aphids and host plants relative abundances using the function of "plotweb2" in the bipartite package in the R software version 3.6.1 (Anonymous 2021).

RESULTS AND DISCUSSION

TThis study was conducted to determine the predatory coccinellid species which are important natural enemies of aphids feeding on pome and stone fruit trees and herbaceous host plants on the edges of fruit orchards of Çanakkale province. A total of twelve predatory species belonging to 8 genera of the family Coccinellidae (Coleoptera) were found on eleven host aphids from the family Aphididae (Hemiptera) on eight different host plants. The species names of predatory coccinellids and their sampling location, sampling date, number of individuals, host aphid species and host plant species are given below in the taxonomic order.

Order Coleoptera

Family Coccinellidae

Adalia bipunctata (Linnaeus, 1758)

Material examined: Çanakkale, Lâpseki, Subaşı, (1), *Brachycaudus* (*Brachycaudus*) *helichrysi* (Kaltenbach, 1843) on *Prunus domestica* L. (Rosaceae), 27.IV.2020; Çanakkale, Ezine, Akköy, (3), *Ovatus* (*Ovatus*) *insitus* (Walker, 1849), *Aulacorthum* (*Aulacorthum*) *solani* (Kaltenbach, 1843) and *Aphis* (*Aphis*) *spiraecola* Patch, 1914 on *Cydonia oblonga* Mill. (Rosaceae), 19.V.2020; Çanakkale, Biga, (1), *Brachycaudus* (*Brachycaudus*) *helichrysi* (Kaltenbach, 1843) on *Prunus domestica* L. (Rosaceae), 07.V.2021.

***Adalia decempunctata* (Linnaeus, 1758)**

Material examined: Çanakkale, Biga, (8), Brachycaudus (Brachycaudus) helichrysi (Kaltenbach, 1843) on Prunus domestica L. (Rosaceae), 07.V.2021.

***Adalia fasciatopunctata revelieri* Mulsant, 1866**

Material examined: Çanakkale, Lâpseki, (1), *Brachycaudus* (*Brachycaudus*) *helichrysi* (Kaltenbach, 1843) on *Prunus domestica* L. (Rosaceae), 21.V.2020; Çanakkale, Ezine, Akköy, (6), *Ovatus* (*Ovatus*) *insitus* (Walker, 1849), *Aulacorthum* (*Aulacorthum*) *solani* (Kaltenbach, 1843) and *Aphis* (*Aphis*) *spiraecola* Patch, 1914 on *Cydonia oblonga* Mill. (Rosaceae), 19.V.2020; Çanakkale, Biga, (4), *Brachycaudus* (*Brachycaudus*) *helichrysi* (Kaltenbach, 1843) on *Prunus domestica* L. (Rosaceae), 07.V.2021;

Coccinella septempunctata Linnaeus, 1758

Material examined: Çanakkale, Lâpseki, Subaşı, (1), *Brachycaudus* (*Brachycaudus*) *helichrysi* (Kaltenbach, 1843) on *Prunus domestica* L. (Rosaceae), 27.IV.2020; Çanakkale, Lâpseki, (1), *Brachycaudus* (*Brachycaudus*) *helichrysi* (Kaltenbach, 1843), *Prunus domestica* L. (Rosaceae), 21.V.2020; Çanakkale, Bayramiç, Evciler, (1), *Myzus* (*Nectarosiphon*) *persicae* (Sulzer, 1776) on *Prunus persica* (L.) Batsch (Rosaceae), 23.VI.2021.

***Coccinula quatuordecimpustulata* (Linnaeus, 1758)**

Material examined: Çanakkale, Biga, (1), *Brachycaudus* (*Brachycaudus*) *helichrysi* (Kaltenbach, 1843) on *Prunus domestica* L. (Rosaceae), 07.V.2021.

***Harmonia axyridis* Pallas, 1773**

Material examined: Çanakkale, Ezine, Akköy, (6), *Ovatus* (*Ovatus*) *insitus* (Walker, 1849), *Aulacorthum* (*Aulacorthum*) *solani* (Kaltenbach, 1843) and *Aphis* (*Aphis*) *spiraecola* Patch, 1914 on *Cydonia oblonga* Mill. (Rosaceae), 19.V.2020; Çanakkale, Çan, (1), *Phorodon* (*Phorodon*) *humuli* (Schrank, 1801) on *Prunus* sp. (Rosaceae), 11.VI.2020; Çanakkale, Çan, (2), *Myzus* (*Myzus*) *varians* Davidson, 1912 on *Prunus persica* (L.) Batsch (Rosaceae), 01.V.2021; Çanakkale, Biga, (1), *Brachycaudus* (*Brachycaudus*) *helichrysi* (Kaltenbach, 1843) on *Prunus domestica* L. (Rosaceae), 07.V.2021; Çanakkale, Bayramiç, Evciler, (1), Myzus (*Nectarosiphon*) *persicae* (Sulzer, 1776) on *Prunus persica* (L.) Batsch (Rosaceae), 23.VI.2021.

***Oenopia conglobata* (Linnaeus, 1758)**

Material examined: Çanakkale, Ezine, Akköy, (9), *Ovatus* (*Ovatus*) *insitus* (Walker, 1849), *Aulacorthum* (*Aulacorthum*) *solani* (Kaltenbach, 1843) and *Aphis* (*Aphis*) *spiraecola* Patch, 1914 on *Cydonia oblonga* Mill. (Rosaceae), 19.V.2020; Çanakkale, Ezine, Akköy, (2), *Phorodon* (*Phorodon*) *humuli* (Schrank, 1801) and *Brachycaudus* (*Brachycaudus*) *helichrysi* (Kaltenbach, 1843) on *Prunus domestica* L. (Rosaceae), 29.V.2020; Çanakkale, Çan, (1), *Dysaphis* (*Pomaphis*) *plantaginea* (Passerini, 1860) on *Malus domestica* Borkh. (Rosaceae), 11.VI.2020; Çanakkale, Biga, (3), *Brachycaudus* (*Brachycaudus*) *helichrysi* (Kaltenbach, 1843) on *Prunus domestica* L. (Rosaceae), 07.V.2021.

***Propylea quatuordecimpunctata* (Linnaeus, 1758)**

Material examined: Çanakkale, Lâpseki, Çardak, (2), *Acyrthosiphon* (*Acyrthosiphon*) *pisum* (Harris, 1776) and *Aphis* (*Aphis*) *craccae* Linnaeus, 1758 on Vicia sp. (Leguminosae), 15.VII.2021.

***Psyllobora vigintiduopunctata* (Linnaeus, 1758)**

Material examined: Çanakkale, Lâpseki, Subaşı, (1), *Brachycaudus* (*Brachycaudus*) *helichrysi* (Kaltenbach, 1843) on *Prunus domestica* L. (Rosaceae), 27.IV.2020.

***Scymnus apetzi* Mulsant, 1846**

Material examined: Çanakkale, Ezine, Akköy, (1), *Myzus* (*Myzus*) *lythri* (Schrank, 1801) on *Prunus armeniaca* L. (Rosaceae), 16.V.2020; Çanakkale, Biga, (2), *Brachycaudus* (*Brachycaudus*) *helichrysi* (Kaltenbach, 1843) on *Prunus domestica* L. (Rosaceae), 07.V.2021.

***Scymnus pallipediformis* Gunther, 1958**

Material examined: Çanakkale, Lâpseki, Çardak, (2), *Acyrthosiphon* (*Acyrthosiphon*) *pisum* (Harris, 1776) and *Aphis* (*Aphis*) *craccae* Linnaeus, 1758 on *Vicia* sp. (Leguminosae), 15.VII.2021.

***Scymnus rubromaculatus* (Goeze, 1778)**

Material examined: Çanakkale, Ezine, Akköy, (1), *Brachycaudus* (*Brachycaudus*) *helichrysi* (Kaltenbach, 1843) on *Cynoglossum creticum* Mill. (Boraginaceae), 13.VI.2020.

Thirty-three different tritrophic interactions of predatory coccinellids-host aphids-host plants were revealed on the pome and stone fruit orchards in the Çanakkale province. From the identified species, *H. axyridis* associated with seven aphid species was the most common predatory coccinellids, followed by *O. conglobata* associated with six aphid species. On the other hand, it was determined that *A. decempunctata*, *C. quatuordecimpustulata*, *P. vigintiduopunctata* and *S. rubromaculatus* were associated with only one aphid species. From the aphids, *B. helichrysi*, which is known as the leaf-curling plum aphid, was the most common species associated with ten different predatory coccinellids on the pome and stone fruit orchards. On the other hand, *D. plantaginea*, *M. lythri* and *M. varians* were the least common aphid species, all associated with only one predatory coccinellids. As for the host plants, the highest number of the interactions of predatory coccinellids-host aphids were revealed on *C. oblonga* and *P. domestica* in the fruit orchards (Figure 1).

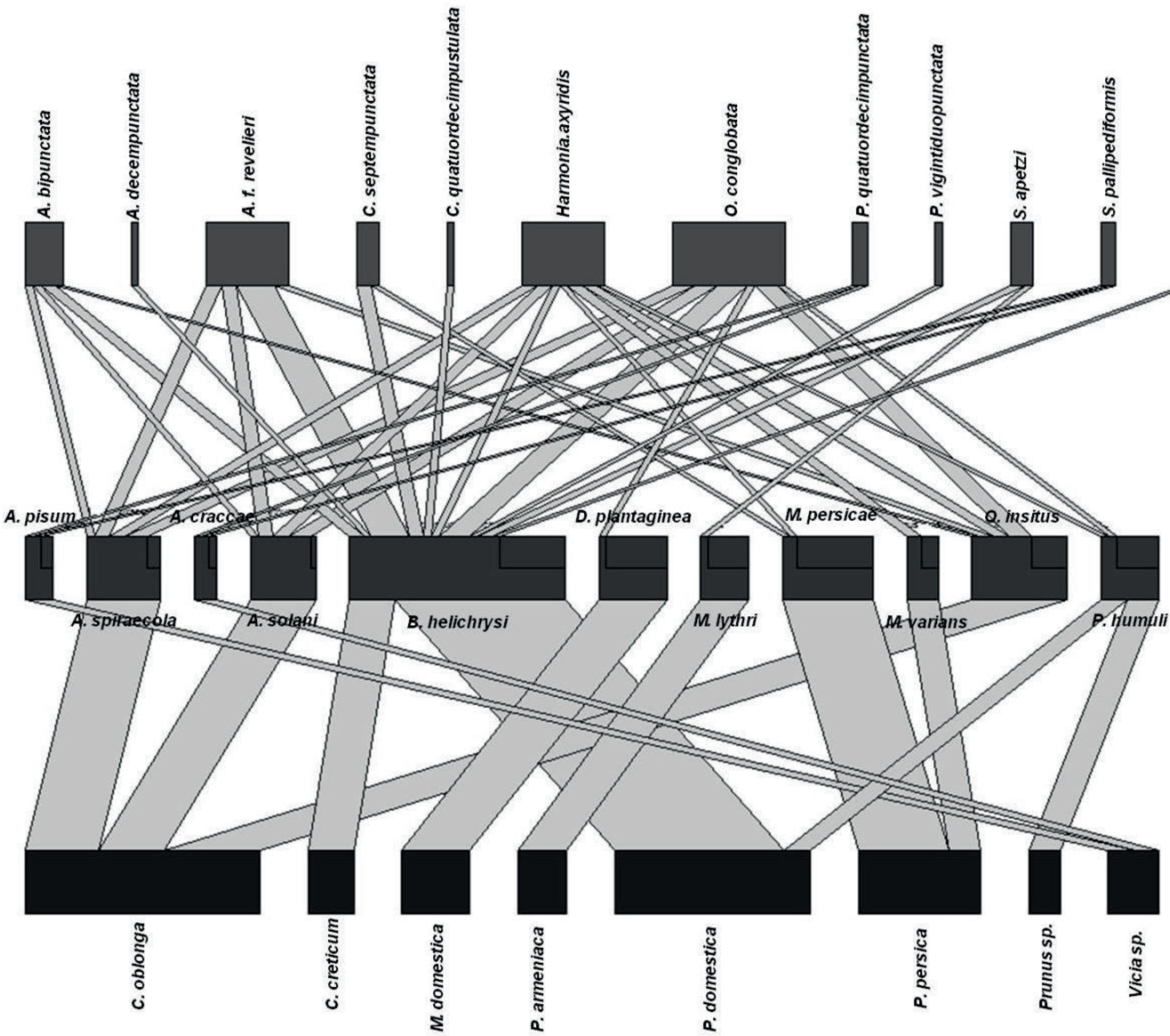

Figure 1. The graph of tripartite network interactions between predatory coccinellids (top), host aphids (mid) and host plants (bottom) species in the pome and stone fruit orchards in the Çanakkale province. Black bars represent the abundance of the species and gray bars represent interactions

Many studies have determined the coccinellid predators of aphids on different host plant in Turkey (Bayram 2009, Bolu et al. 2007, Daşcı and Güçlü 2008, Kaçar and Koca 2020, Kaplan and Turanlı 2016, Kök et al. 2017, Kök and Kasap 2019, Kütük and Güçlü 2016, Öztürk and Muştu 2018). The interactions between predatory insect and pest species such as aphids are quite complex in ecosystems with different host plant diversity. Natural enemies such as coccinellids have specialized sensory nervous systems that allow them to find and identify prey. Of these, chemical cues emitted by plants and used by aphids to find plants location and physical cues are very important for predators to find their preys' locations. For example, *C. septempunctata*, *Coleomegilla maculata* De Geer, *H. axyridis* and *Hippodamia convergens* Guerin specifically attack red and green individuals of *Acyrthosiphon pisum* (Harris, 1776) (Harmon et al. 1998). Similarly, predatory coccinellids did not respond to chemicals emitted by host plants that were not infested by aphids, while they did respond to chemicals emitted from aphid-damaged plants. For example, *C. septempunctata* was attracted to odours from host plants damaged or previously damaged by the aphids (Han and Chen 2002, Ninkovic et al. 2001). For these reasons, it is very important not only to determine the predatory coccinellid species of aphids in agricultural or non-agricultural areas but also to consider the predatory coccinellids-host aphids-host *plants* interactions as a whole. The results of our study support the idea that these tritrophic interactions should be examined in more detail as clearly seen that *B. helichrysi* was determined on both *C. creticim* and *P. domestica*, but the colonies of this aphid on *P. domestica* were much more preferred by predator coccinellids. Differently, *M. persicae* and *M. lytri*, feed on *P. persica,* which is commonly found on fruit orchards in the study region and on which densely aphid colonies are determined, were less preferred by predatory coccinellids. Also, the results of our study showed that *H. axyridis*, Asian lady beetle, preferred as prey seven aphid species on host plants such as *C. oblonga*, *P. domestica*, *P. persica* and *Prunus* sp. in the fruit orchards. Similarly, Jovičić et al (2020) reported that *Harmonia axyridis* fed on 43 aphid species on 58 cultivated plant species such as ten fruit, seven field crops, five vegetables and 16 ornamental species, as well as 20 non-cultivated plants. In this context, it has been announced that plant volatiles originating from aphid damaged have an important role in guiding prey foraging and in increasing aphid predation rates of *H. axyridis*, whose host prey aphid number is quite high (Francis et al. 2004, Xiu et al. 2019). We think this indicates that the host plants play an important role in the fact that *H. axyridis* has a wide aphid preys.

In conclusion, this study revealed the tritrophic interactions of the predatory coccinellids-host aphids-host plants on fruit trees and herbaceous plants in fruit orchards of Çanakkale province. Data obtained is a starting point for future more detailed studies. Better understanding tritrophic interactions from different perspectives may increase the success of biological control studies in agricultural areas.

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ÖZET

Bu çalışma ile Türkiye'nin Çanakkale ilinde meyve bahçelerindeki yumuşak ve sert çekirdekli meyve ağaçları ve yabancı otlar üzerindeki predatör coccinellid-konukçu afit-konukçu bitki tritrophic etkileşimlerinin ortaya çıkarılması amaçlanmıştır. 2020 ve 2021 yılları bahar ve yaz ayları boyunca yapılan arazi örneklemeleri sonucunda, sekiz farklı konukçu bitki üzerindeki Aphididae (Hemiptera) familyasından 11 konukçu afit ile ilişkili Coccinellidae (Coleoptera) familyasına ait sekiz cins içerisinde 12 predatör tür tespit edilmiştir. Çanakkale ili yumuşak ve sert çekirdekli meyve bahçelerinde toplam 33 predatör coccinellid-konukçu afit-konukçu bitki etkileşimi ortaya çıkarılmıştır. Predatör türlerden, yedi afit türü ile ilişkili *Harmonia axyridis* (Pallas) en yaygın coccinellid türü olurken, onu altı afit türü ile ilişkili *Oenopia conglobata* (L.) izlemiştir. Afitlerden, on farklı predatör coccinellid ile ilişkili olan *Brachycaudus helichrysi* (Kaltenbach) en yaygın afit türü olarak belirlenmiştir. Ayrıca, Çanakkale ili yumuşak ve sert çekirdekli meyve bahçelerindeki en yüksek predatör coccinellid-konukçu afit etkileşimi *Cydonia oblonga* Mill. (Rosaceae) ve *Prunus domestica* L. (Rosaceae) konukçu bitkileri üzerinde tespit edilmiştir. Sonuç olarak, meyve bahçelerinde zararlı afitlerin biyolojik mücadelesindeki başarının arttırılabilmesi için predatör coccinelli-konukçu afit-konukçu bitki etkileşimlerinin daha iyi anlaşılması gerektiği düşünülmektedir.

Anahtar kelimeler: predatör coccinellid, afit, meyve bahçesi, tritrofik etkileşim, Çanakkale

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Original article

Effects of harmful *Bangasternus planifrons* (Brulle, 1832) (Coleoptera: Curculionidae) control with different insecticides on yield and quality in safflower

Bangasternus planifrons (Brulle, 1832) (Coleoptera: Curculionidae)'un farklı insektisitler ile mücadelesinin aspirde verim ve kaliteye etkisi

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ABSTRACT

The production of safflower (*Carthamus tinctorius* L.) (Asterales: Asteraceae), which is cultivated worldwide, is significantly affected by different abiotic and biotic factors such as insects. Different insecticides have been applied during safflower production to control pests. The application of insecticides can affect plants as well as pests. This study was carried out in Ankara and Eskisehir provinces in 2016 using four different insecticides against the *Bangasternus planifrons* (Brulle, 1882) (Coleoptera: Curculionidae) pest in Balcı safflower variety. The pest population was 73-91% effectively suppressed as of the 14th day after the application of insecticides, and the yield increased by 60.43-123.18% when compared to the control group. Deterioration of seed quality, loss of oil ratio, and loss of 1000 grain weight occurred as a result of damage occurred by pest feeding. Through control of pests and reduction of pest population, an increase in quality and productivity was achieved. Regression analysis made on the data obtained from the application areas determined that there were 83.73%, 75.83%, and 75.44% negative relationship between the number of adults of the pest and the yield, oil rate, and 1000-grain weight, respectively. In conclusion, *B. planifrons* is an important factor causing a loss in yield, oil rate, and loss of 1000-grain weight in safflower plant. The damage caused by the pest can be prevented by the application of a suitable insecticide.

INTRODUCTION

Safflower (*Carthamus tinctorius* L.) (Asterales: Asteraceae) is an annual oil plant with a 30-50% oil content in its seeds. It is a broad-leaved and highly branched industrial plant grown especially for its seeds and petals that are used in the production of biodiesel or as pulp used to feed animals. Safflower leaves and oil are used in fabric dyes, food coloring and treatment of some diseases, human nutrition, and biodiesel production (Köse 2019a). Approximately 60% of consumed oil in Turkey is imported.

A result of limited production of existing oil crops and the gradual decrease in irrigable agricultural lands have increased the importance of the cultivation of droughtresistant plants such as safflower in recent years. Safflower cultivation area reached 43.107 ha, and the production amount reached 70.000 tons in 2015 with the support given to encourage agricultural production. However, safflower is affected by several harmful organisms that cause economic losses. Among these organisms, insects are the most important factors causing a decrease in production (Köse 2019b); for example, those in the *Bangasternus* genus cause significant economic damage.

Eight species in the genus *Bangasternus* are known to be in the Palearctic region (Hoffmann 1954), and three are in Turkey (Lodos et al. 1978, 2003). *Bangasternus* spp. (Coleoptera: Curculionidae) within the Asteraceae family, is especially specific to Centaurea species and is widely used in biological control studies of Centaurea spp. (Asterales: Asteraceae) conducted in the world. It has been determined that *Bangasternus orientalis* (Capiomont 1873) (Coleoptera: Curculionidae) feeds within the flower capsule of yellow pyrethrum [*Centaurea solstitialis* L. (Asterales: Asteraceae)] and causes damage to its seeds (Maddox et al. 1991). Sobhian et al. (1992a) stated that *B. orientalis* was fed in the laboratory and field trials were carried out for the control of the yellow pyrethrum. It was found that the white pyrethrum [Centaurea *diffusa* Lam. (Asterales: Asteraceae)] plant is infested with *Bangasternus fausti* (Reitter 1890) (Coleoptera: Curculionidae) at a rate of 72-100% (Sobhian et al. 1992b). Although it was stated that *B. planifrons* (Brulle) can be used as a biological control agent in studies carried out abroad, Damkacı (2013) first noted that it is a pest in safflower fields in Turkey. For instance, Yücel et al. (2019) stated that *B. planifrons* is an important pest in safflower areas, and it causes economic loss by feeding in the green parts and flower capsules of the safflower plant.

There has been no study, so far, on the biology and natural enemies of *B. planifrons*, the main pest of the safflower plant, both in Turkey and abroad. But since this pest has become an important limiting factor in safflower production, it must be controlled. There are a limited number of studies conducted in our country on this pest, and little is known of its natural enemies and their activities, so control is dependent on chemical means. Therefore, we investigated the effectiveness of different insecticides in order to prevent the economic loss caused by the pest. The effects of the capsule proboscis beetle, which causes a decrease in the seed quality and quantity of the safflower plant, on the yield and the effectiveness of the insecticides that can be used in the chemical control of the pest were determined. The studies were carried out in 2016 in two different experimental areas in Ankara and Eskisehir provinces where safflower is cultivated.

MATERIALS AND METHODS

Plant materials and insecticides

The experiments were conducted on the Balcı variety of safflower plants. Balcı safflower variety has a plant height of 55-70 cm, a yield of 120-240 kg/da in dry conditions, and 300-350 kg/da in watery conditions. Insecticides and active ingredients used in the study were; Insect.A (100 g/l Chlorantraniliprole + 50 g/l Lambda-cyhalothrin), Insect.B (5% Emamectin benzoate), Insect.C (227 g/l Chlorpyrifos-methyl) and Insect.D (141 g/l Thiamethoxam + 106 g/l Lambda-cyhalothrin).

Growing conditions and insecticide application

Safflower seeds were sown in 45 cm row spacing with 45 kg/ha seed sowing norm in the field on March 2, 2016, in the Transitional Zone Agricultural Research Institute Directorate's trial field of Eskisehir Province Tepebasi district, (39°45''57'N, 30°24''5'E 868 m, 10 da) and in Gülhüyük neighborhood, Sereflikochisar district, Ankara Province on 3 March 2016. (39° 06'54 N 33° 34'39 E 970 m, 40 da). Eighty kg/ha of nitrogen (33 ammonium nitrate) and 60 kg/ha of phosphorus (diammonium phosphate) fertilizer were used in planting. Safflower in the experimental fields was grown under rain-fed conditions and weed control was done manually during the growth period. In order to determine the effects of insecticides against *B. planifrons*, the experiment was conducted against the overwintered adults of the pest on May 24, 2016. The doses of insecticides used in the experiment were determined based on the recommended doses in our country (Insect.A: 50 ml/da, Insect.B: 50 g/da, Insect.C: 200 ml/da, Insect.D: 30 ml/da). A knapsack sprayer was used in spraying. The insecticides doses were calibrated according to the 30 liters/da water norm, and the amount of effective substance per parcel and the water to be disposed of the norm were calculated and the application was made.

The applications were set randomized block design in four replications, together with the control plot (4 insecticides + 1 control; 5x4 = 20 plots) in a 100 m^2 plot (distance between each block is one meter). In counts, the average number of pests per plant was calculated by counting the adults of the pests on 25 plants randomly selected from each plot four times (pre-counting, third, 7^{th}, and 14^{th} days). The efficiency of the insecticides was assessed based on 3 factors (insecticide*time*location) and factor levels were assessed according to the experimental design.

Measurement of growth and yield parameters

Safflower plants were hand-harvested in duplicates from 1 m^2 area of each parcel on August 12, 2016. The harvested capsules were cleaned in the laboratory. For the productivity and parcel yields, 5 sets random of 100 seeds were weighed and the average was taken to determine the 1000 grain weights. To determine the oil content, 10 g of ground safflower seeds were weighed and placed in cones. The samples were extracted for 6 hours in the Soxhlet apparatus to which 90 ml of petroleum ether was added, allowing all of the oil to pass into the solvent. At the end of the extraction, petroleum ether was removed from the balloon in a vacuum evaporator under reflux, and the sample was kept in the oven at 103 + 2 °C until it reached constant weight to remove all solvent residue and moisture. The sample removed from the oven was kept in the desiccator until it reached room temperature, and the oil rate was determined by weighing (Andrich et al. 2001). These oil rates were multiplied by seed yields and oil yields were calculated.

Data analysis

The data of adult count obtained in the study were first converted to percentages, and then subjected to analysis of variance (ANOVA) after arc-sin transformation. Comparisons between percent mortality rate and corrected percent mortality effect rate (Henderson and Tilton 1955) means were made using Duncan Multiple Comparison Test. All statistical analyzes were done using SPSS 23.0 package program.

RESULTS AND DISCUSSION

Experiments were conducted in Eskisehir province Tepebasi district and Ankara province Sereflikochisar district to prevent the damage given by *B. planifrons*, which causes a decrease in quality and quantity in the product, as a result of feeding on the safflower plant. The density of the individuals belonging to the post-wintering of the pest was suppressed in the early period and the damage to the product was reduced. The data showing the average of pest adults in the experiments are given in Figure 1.

As a result of the obtained data, there was no difference in the effectiveness of the insecticides used in the experiment according to the locations (insecticide*time*location interaction) where they were applied at different census times. (F=13.450; P=0.000; df=6). Similarly, it was observed that there was no difference between the effect values obtained as a result of the repeated adult census in Ankara and Eskisehir locations (F=4.226; P=0.062; df=1). No difference was found between the effect values (location*insecticide interaction) of the insecticides applied in the treatments (F=1.824; P=0.196; df=3). There is no difference between the effect values of the treatment locations and the repeated census dates (location*time interaction) (F=0.554; P=0.582; df=2).

Differences were determined in the effectiveness of insecticides according to the census times (F=23.268; P=0.000; df=2). The difference between the census dates is expected due to the action mechanisms and duration of action of insecticides. In the study, it was determined that the effect values of insecticides were different according to the census dates. As a result, there was a difference in yield, 1000-grain weight, and oil rate obtained from different insecticide-treated plots.

In both studies, a high effect was observed in decreasing the number of adults, and the average number of adults was observed to one or less. Among the insecticides used, Insect.A and Insect.D showed an effective rate of over 80% as of the 14th day. Insect.D, in particular, had the highest impact in the field of the Institute and reached an impact rate of 91.02% (Table 1).

Due to the lack of studies on safflower in Turkey and worldwide, the effects of insecticides were discussed with studies on other oilseed plants and their effects on the product. It was determined that, the damage is given by *Uroleucon compositae* (Theobald). (Hemiptera: Aphididae) causes loss in yield in safflower and that among the five different insecticides applied for control, thiamethoxam active exhibited the highest effect as 0.83 aphids per 5 cm plot, while an average of 66.13 aphids per 5 cm plot was found in the control plots (Gore et al. 2010). In the study conducted by Gvozdenac et al. (2019) to control wireworm in sunflower [*Helianthus annuus* L. (Asterales: Asteraceae)], 15.8% damaged plants were found in control and 1.86% in thiamethoxam application. Showler and Robinson (2005) stated that the application of insecticides in cotton [*Gossypium* spp. (Malvales: Malvaceae)] against *Anthonomus grandis grandis* (Boheman) (Coleoptera: Curculionidae) with different methods resulted in a 46-56% increase in yield. When different insecticides from neonicotinoid, organophosphate/carbamate, pyrethroid groups were applied against *Thrips tabaci* (Lindeman, 1889) (Thysanoptera: Thripidae) and *Lygus lineolaris* (Palisot de Beauvois 1818) (Hemiptera: Miridae) pests in cotton, both pests were controlled to decrease the economic damage threshold in all periods of the cotton and an average yield increase of 8.51% was achieved in the product (North et al. 2019). Similarly, the lint yield was increased by 8% (111 kg/ha) as a result of applying thiamethoxam to cotton seeds to prevent damage given by thrips (Lahiri et al. 2018). Prathibha et al. (2017) stated that the activities of different insecticides have been tested to control *Leucophalis burmeister* Brenkske (Coleoptera: Scarabaeidae), which causes yield loss in Areca palm, and as a result, bifenthrin insecticide at a dose of 2 kg/ha can be used in the management of this pest due to its mechanism of action and a long stay in the soil.

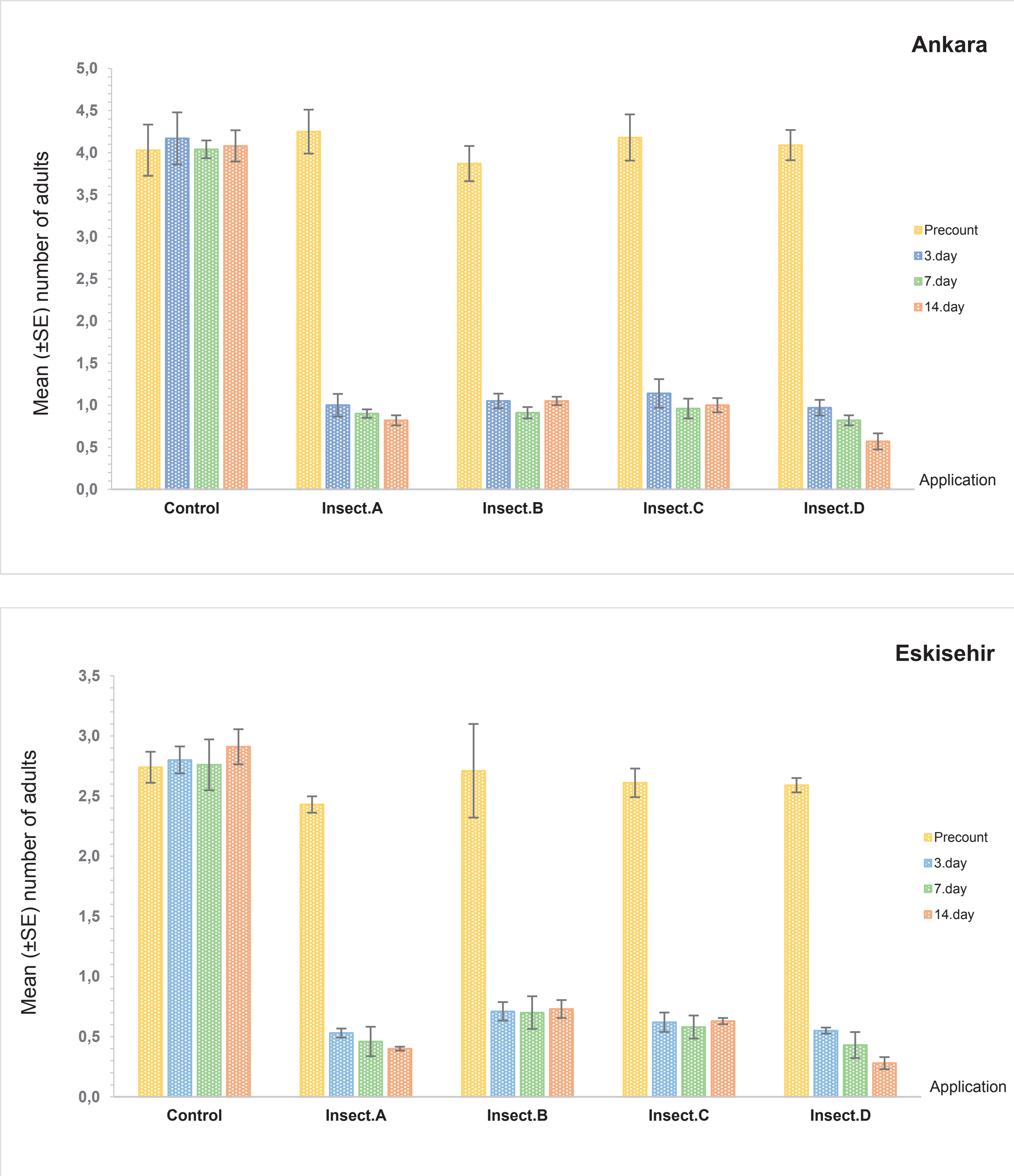

Figure 1. Mean (±SE) number of adults of different insecticides applied against *Bangasternus planifrons* (Brulle) (Coleoptera: Curculionidae) in Ankara and Eskisehir provinces of safflower fields

In other studies, carried out in the world, it is seen that treatments against pests prevented product loss. The differences in the effects of the insecticides applied may be caused by differences in the action mechanisms of insecticides and their molecular structures. The metabolic activities and morphological structure of targeted insects are among the important factors affecting the rate of action of insecticides. In our study, as of the 3rd day, Insect.A (Chlorantraniliprole + Lambda-cyhalothrin) and Insect.D (Thiamethoxam + Lambda-cyhalothrin) showed similar rates of the effect of 77.85% and 78.37%, respectively, while the effect of Insect.D continued in the following period and reached an average impact rate of 88.74%.

The effects on safflower grains were determined by sampling from the application areas during the harvest period. An increase of 60.43-123.18% was achieved in safflower yield

Table 1. Mean (±SE) efficacy of different insecticides applied against *Bangasternus planifrons* (Brulle) (Coleoptera: Curculionidae) in safflower fields

Fields	Days	Insect.A±SE			Insect.B±SE			Insect.C±SE			Insect.D±SE			
Eskisehir	3	78.54±1.97	B*	a**	73.97±1.23	A	a	76.95±2.12	A	a	79.20±0.97	C	a	*F*=2.039; *P*=0,162; df=3
	7	81.10±1.79	AB	ab	75.87±2.73	A	bc	77.75±1.68	A	c	83.61±2.68	B	a	*F*=4.913; *P*=0,019; df=3
	14	84.44±0.93	A	b	73.03±1.75	A	c	74.68±1.77	A	c	91.02±1.64	A	a	*F*=38.823; *P*=0,00; df=3
		F=34.982; *P*=0,081; *df*=2			*F*=0.969; *P*=0,416; *df*=2			*F*=0.940; *P*=0,426; *df*=2			*F*=19.792; *P*=0,001; *df*=2			
Ankara	3	77.16±1.52	A	a	73.74±1.93	A	a	74.22±1.26	A	a	77.54±0.90	B	a	*F*=1.175; *P*=0,360; df=3
	7	79.39±1.34	A	a	77.24±1.46	A	a	78.04±1.27	A	a	80.68±0.72	B	a	*F*=1.519; *P*=0,260; df=3
	14	80.59±2.19	A	b	73.10±1.20	A	c	76.42±0.96	A	bc	86.46±1.73	A	a	*F*=13.119; *P*=0,00; df=3
		F=1.025; *P*=0,397; *df*=2			*F*=2.040; *P*=0,186; *df*=2			*F*=1.656; *P*=0,244; *df*=2			*F*=12.844; *P*=0,002; *df*=2			

* Different capital letters in a column were significantly different (P<0.05)

** Different small letters in a line were significantly different (P<0.05)

compared to the control. Similar increases were found in the 1000 grain weight and oil ratio. There was an increase of 6.28-15.82% in the safflower 1000 grain weight compared to the control. It was determined that there was an increase of 6.33-11.92% in the oil rate compared to the control. While the highest increase was in Insect.D application, a higher increase was detected in Insect.A application compared to other Insect.B and Insect.C applications. Data on yield, oil rate, and 1000 grain weight obtained from the experiments are given in Figure 2.

Linear regression analysis was applied to the data to determine the correlation between the increase in yield, 1000 grain, and oil rate with many adult insects. As a result of the regression analysis, a negative correlation of 83.73% was determined between the number of adults and the increase in yield, while a negative relationship of 75.44% and 75.83% was determined in 1000 grain weights and oil rates, respectively (Figure 3).

Brown et al. (1999) used different insecticides to control pests in canola [*Brassica napus* L. (Brassicales: Brassicaceae)] in their study and stated that while the yield increased by 10-46% compared to the control due to the damage caused by insects. The oil rate increased by 5.4-6.9% and that the oil quality was not affected by insect damage. Gvozdenac et al. (2019) stated that the damage of wireworms in sunflower was reduced with thiamethoxam application when compared to the control and that the applications did not differ from the control in the oil rate

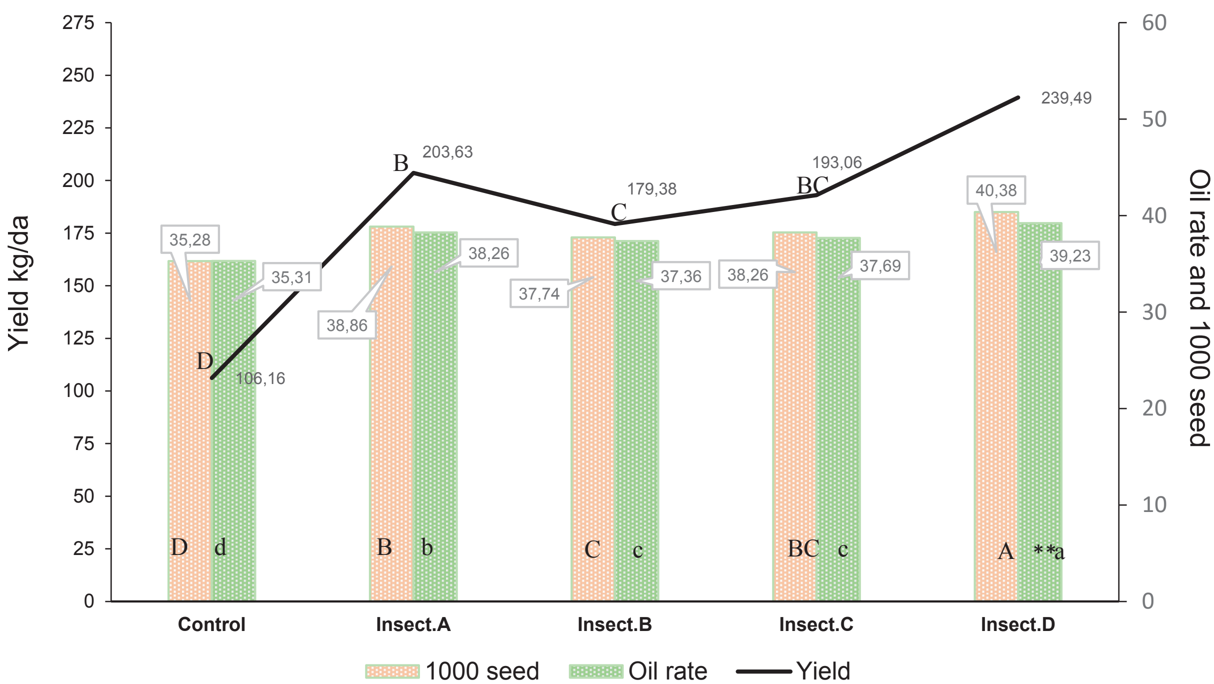

Figure 2. Mean (±SE) yield, oil rate, and 1000 seed weight results obtained from different insecticides applied against *Bangasternus planifrons* (Brulle) (Coleoptera: Curculionidae) in safflower fields. Note 1: *Different capital letters in a column were significantly different, (yield: *F*=86.074, *P*=0.00, *df*=4; 1000 seed: *F*=61.574, *P*=0.00, *df*=4) Note 2: **Different small letters in a line were significantly different, (oil rate: *F*=66.416, *P*=0.000, *df*=4)

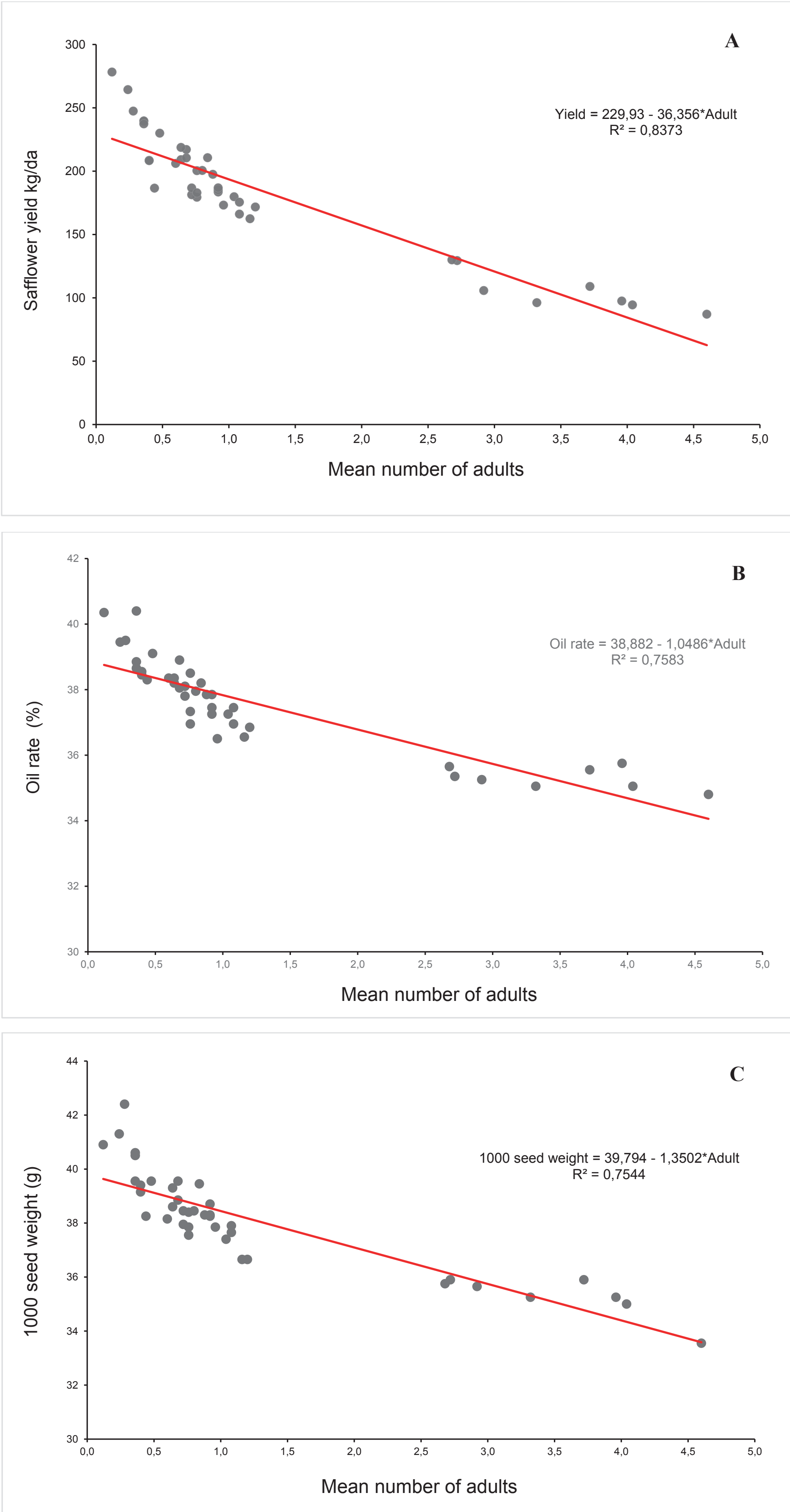

Figure 3. The correlation relationship graphs between *Bangasternus planifrons* (Brulle) (Coleoptera: Curculionidae) adults and seed yield (a), oil rate (b), and 1000 grain weight (c) in safflower fields

and 1000-grain weight. In a study conducted on winter canola, they found that insecticide application increased the yield, but there was no change in 1000 grain weight (Leach et al. 1994). The pest did not only cause yield loss, but it also negatively affected the product quality. It is thought that the decrease in the seed quality of the product, especially the development of safflower seeds in a closed capsule, was a result of the insect's feeding and the enzymes it secretes which prevents the formation of healthy seeds by the destruction of the seed and seed receptacle.

Many factors that cause losses in the products obtained during agricultural activities. One of the most important abiotic and biotic factors is insects. In safflower production, characteristics such as yield, oil ratio, and oil yield are highly affected by insect damage. Application of insecticides has often become inevitable in protecting crops. In this study, the effectiveness of some insecticides in the control of *Bangasternus planifrons*, which causes yield loss as a result of feeding on safflower plant, and the effect of Balcı variety on yield components were investigated. Application of insecticides suppressed the insect pest thereby reducing the damage in the products. Changes in the quality of the product were also determined while assessing the damage found between the areas where the control was made and the areas where no control was done. It was determined that the pest in question causes loss of yield and reduces the amount of oil rate and 1000 grain weight of the product. Among the four insecticides applied, thiamethoxam + lambda-cyhalothrin application provided the highest seed yield, 1000 grain weight, and an increase in oil rate. Current research shows that insecticides applied in safflower against *B. planifrons* can reduce pest damage.

ÖZET

Dünya çapında yetiştiriciliği yapılan aspir (*Carthamus tinctorius* L.) (Asterales: Asteraceae) üretimi böcekler gibi farklı abiyotik ve biyotik faktörlerden önemli ölçüde etkilenmektedir. Aspir üretimi sırasında zararlıları kontrol altına almak için farklı insektisitler uygulanmaktadır. İnsektisitlerin uygulanması, zararlılarla birlikte bitkileri de etkileyebilir. Bu çalışma *Bangasternus planifrons* (Brulle, 1882) (Coleoptera: Curculionidae) zararlısına karşı Balcı aspir çeşidinde dört farklı insektisit kullanılarak Ankara ve Eskişehir illerinde 2016 yılında yapılmıştır. Çalışma sonucunda zararlı popülasyonu insektisit uygulamasının 14. günü itibariyle %73-91 etki oranında azalmış ve verimde kontrol grubuna göre %60.43-123.18 artış sağlanmıştır. Zararlının beslenmesi sonucunda tohum kalitesinde bozulma, yağ oranı kaybı ve 1000 tane ağırlığı kaybı meydana gelmiştir. Zararlıların kontrolü ve zararlı popülasyonunun azaltılması yoluyla kalite ve verimde artış sağlanmıştır. Uygulama yapılan alanlardan elde edilen verilere yapılan regresyon analizi sonucunda zararlının ergin sayısı ile verim, yağ oranı ve 1000 tane ağırlığında da sırasıyla %83.73, %75.83 ve %75.44 oranında negatif ilişki belirlenmiştir. Sonuç olarak *B. planifrons*'un aspir bitkisinde zararı ile verimde, yağ oranında ve 1000 tane ağırlığında kayba neden olan önemli bir etken olduğu, zararlıya karşı yapılacak uygun bir insektisit uygulaması ile oluşan zararın önlenebileceği sonucuna varılmıştır.

Anahtar kelimeler: *Bangasternus*, insektisit, yağ oranı, aspir, verim, Eskişehir

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Original article

Eriophyoid mites (Acari: Eriophyoidea) from İzmir-Turkey

İzmir, Türkiye'deki Eriophyoid akarlar

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A B S T R A C T

A comprehensive faunistic research was carried out in order to detect Eriophyoid mites in İzmir. As a result of the research seventeen species of eriophyoid mites were detected for the mite fauna of Turkey. The samples were collected on fruit and ornamental plants in İzmir province, Turkey 2021. The identified eriophyid species are *Aceria erinea* Nalepa, 1891, *Aceria avanensis* Bagdasarian, 1970, *Aceria salicina* Nalepa, 1911, *Aceria filiformis* Nalepa, 1891, *Aceria ilicis* Canestrini, 1890, *Aceria massalongoi* Canestrini,1890, *Aceria oleae* Nalepa, 1900, *Aceria sheldoni* Ewing, 1937, *Eriophyes pyri* Pagenstecher, 1857, *Eriophyes tiliae* Nalepa, 1890, *Aculus fockeui* Nalepa & Trouessart, 1891, *Phyllocoptes pruni* Soliman & Abou-Awad, 1979, *Aculus schlechtendali* Nalepa 1890, *Aculus mogeri* Farkas, 1960, *Colomerus vitis* Pagenstecher, *Calepitrimerus vitis* Nalepa, 1905, *Rhyncaphytoptus ficifoliae* Keifer, 1939. Information on hosts, damage symptoms and geographical distribution of these species are given in the article.

INTRODUCTION

Eriophyoid mites are obligatory phytophagous invertebrates, with the majority of species being host plant specialists. Weed-associated eriophyoids are considered to have high potential as classical biological control agents because of their high host plant specificity. These mites are important not only as direct pests of the plants but they also act as vectors of some important plant virus diseases. Eriophyoid identification by morphological examination is quite difficult because of their small size and unclear taxonomical characteristics. Eriophyoid mites are very small, obligatory phytophagous invertebrates and the generality of these mites are specialized to the host (Lindquist et al. 1996). Weed-associated eriophyoids are thought out have high potential as biological control agents on account of their high host plant specificity (Smith et al. 2010). They infest yield all over the world and many are important pests. Turkey has rich eriophyoid biodiversity because of the position region biologic history of the country (Ekim and Güner 2000, Karagöz 2003). Many studies were carried out to determine the eriophyoid fauna of Turkey (Denizhan et al. 2006, 2021, Denizhan and Çobanoğlu 2010, Karaca 1956, Petanović and Stanković 1999). Denizhan et al. (2015) listed eriophyoid mites in the catalogue giving a description of the dispersion in Turkey. The aim of this study is to define and provide information on eriophyid in İzmir Eriophyoid fauna which were found.

MATERIALS AND METHODS

The samples were collected from the leaves of cultivated plants and weeds from the province of İzmir (Bayındır, Bergama, Beydağ, Buca, Foça, Menderes, Narlıdere, Ödemiş and Seferihisar) from the beginning of June to the end of September. Eriophyoid mites collected from the plants were directly examined under a dissecting stereo-microscope (Leica ES2) and mounted on microscope slides according to Keifer (1975). The identification studies were made by the help of microscope Leica DM 1000. The morphological nomenclature follows Lindquist et al. (1996), all the measurements accomplished by according to Amrine and Manson (1996). For the systematic studies Amrine et al. (2003) has been followed. Assoc. Dr. Evsel Denizhan made diagnoses. Information on the hosts, damage symptoms, and geographical distribution of these species each sample are provided. The specimens are deposited in the Trakya University, Science Faculty-Department of Biology Turkey.

RESULTS AND DISCUSSION

10 species belonging to Eriophyidae Nalepa family, 4 species belonging to Phyllocoptinae Nalepa subfamily, 2 species belonging to Cecidophyinae Keifer subfamily, 1 subspecies belonging to Rhyncaphytoptinae Roivain family of Diptilomiopidae Keifer family from the samples collected from cultivated plants and weeds in İzmir province between June and September. 17 species have been identified.

Superfamily: Eriophyoidea Nalepa, 1898

Family: Eriophyidae Nalepa, 1898

Aceria erinea Nalepa, 1891

Host: *Juglans regia* L. (Juglandaceae)

Geographic distribution: Antarctic, Australian, Indomalayan, Nearctic, Neotropical, Palaearctic.

Relation to the host plant: Gall-making mite. Infested walnut leaves show a shiny, yellowish-green bulge between two leaf veins on the upper surface with whitish, furry, blister-like erinea on the lower leaf surface.

Dispersion in Turkey: Ankara, Van (Denizhan and Çobanoğlu 2010), İzmir (Buca, Seferihisar)

Aceria avanensis Bagdasarian, 1970

Host: *Juglans regia* L. (Juglandaceae)

Geographic distribution: Palaearctic.

Relation to the host plant: A gall-making mite causing small, protruding pouch-like and warty galls on the leaf lamina which may also appear to be deformed.

Dispersion in Turkey: Ankara, Van, Yalova (Denizhan and Çobanoğlu 2010, Denizhan et al. 2015), İzmir (Foça, Ödemiş, Bergama) (11.06.2021, 17.07.2021, 24.08.2021).

Aceria salicina Nalepa, 1911

Host: *Salix alba* L. (Salicaceae)

Geographic distribution: Nearctic, Palaearctic.

Relation to the host plant: Gall-making mite. This species causes leaf nodules and it was found inside witches' brooms.

Dispersion in Turkey: Ankara, Erzincan, Erzurum (Alaoğlu 1996), Van (Denizhan et al. 2015), İzmir (Foça, Bergama) (19.06.2021, 23.07.2021).

Aceria filiformis Nalepa, 1891

Host: *Ulmus campestris* L.

Geographic distribution: Palaearctic, Nearctic

Relation to the host plant: Gall-making mite.

Dispersion in Turkey: Ankara (Denizhan and Çobanoğlu 2010), İzmir (Bayındır, Menderes) (26.07.2021, 13.08.2021).

Aceria ilicis Canestrini, 1890

Host: *Quercus ilex* L. (Fagaceae)

Geographic distribution: Palaearctic.

Relation to the host plant: Gall-making mite. This species causes rusty-brown erinea on the lower leaf.

Dispersion in Turkey: Ankara (Denizhan and Çobanoğlu 2010), İzmir (Foça, Narlıdere) (19.06.2021, 28.08.2021).

Aceria massalongoi Canestrini,1890

Host: *Vitex agnus-castus* L. (Verbenaceae)

Geographic distribution: Palaearctic.

Damage to the host: Gall-making mite. This species induces small pouch galls of hemispheric shape on the leaves, often on the laminar margins. Leaves become distorted even when hardly galled.

Dispersion in Turkey: İzmir (Denizhan et al. 2015), İzmir (Foça, Bergama) (19.06.2021, 01.09.2021).

Aceria oleae Nalepa, 1900

Host: *Olea europaea* L. (Rutaceae)

Geographic distribution: Palearctic

Damage to the host: This species induces leaf twisting with hair falling and fruit deformation.

Dispersion in Turkey: İzmir Denizhan et al. (2015), İzmir (Foça, Ödemiş) (19.06.2021, 03.09.2021)

Aceria sheldoni Ewing, 1937

Host: *Citrus limon* L.

Geographic distribution: Palearctic, Neotropical, Oriental, Nearctic, Australian, Ethiopian.

Damage to the host: This species distortion of shoot growth, deformation of fruit, discoloration of fruit.

Dispersion in Turkey: Afyon, Bingöl, Erzurum, Isparta, Adana (Denizhan et al. 2015), İzmir (Foça) (19.06.2021).

Eriophyes pyri Pagenstecher, 1857

Host: *Pyrus communis* L. (Rosaceae)

Geographic distribution: Africotropical, Antarctic, Australian, Nearctic, Neotropical, Palaearctic.

Damage to the host: A gall-making, mite causing blisters (pouch galls) on pear leaves.

Dispersion in Turkey: Diyarbakır, Elazığ, Malatya, Tunceli; Ankara, Eskişehir, Konya, Niğde; Çukurova region; in Erzurum-Narman, Tortum, Oltu, İspir, Erzincan, Kemaliye, Kars, Aydın, Çanakkale, Denizli, İzmir, Kütahya; the area of Van Lake Basin (Denizhan and Çobanoğlu 2010), Ankara and the area of Van Lake Basin, Yalova (Denizhan et al. 2015), İzmir (Foça, Ödemiş, Bergama) (08.07.2021, 17.08.2021).

Eriophyes tiliae Nalepa, 1890

Host: *Tilia platyphyllos* Scop.

Geographic distribution: Nearctic, Palaearctic.

Damage to the host: Galls

Dispersion in Turkey: Ankara (Denizhan et al. 2015), İzmir (Bergama, Narlıdere) (17.08.2021, 13.09.2021).

Subfamily: Phyllocoptinae Nalepa, 1892

Aculus fockeui Nalepa & Trouessart, 1891

Host: *Prunus domestica* L. (Rosaceae)

Geographic distribution: Africotropical, Australian, Indomalayan, Nearctic, Neotropical, Palaearctic.

Damage to the host: Vagrant. This species induces yellow leaf spots in spring followed by upper longitudinal curls on young leaves along with silvering and mottling of older leaves.

Dispersion in Turkey: Ankara, Van, Yalova (Denizhan and Çobanoğlu 2010), İzmir (Ödemiş, Bergama) (28.06.2021, 17.08.2021).

Phyllocoptes pruni Soliman & Abou-Awad, 1979

Host. *Prunus domestica* L. (Rosaceae)

Geographic distribution. Antarctic, Australian, Indomalayan, Nearctic, Neotropical, Palaearctic.

Damage to the host. Vagrant.

Dispersion in Turkey. Ankara, Van (Denizhan et al. 2015), İzmir (Foça) (01.09.2021).

Aculus schlechtendali Nalepa 1890

Host: *Malus domestica* Borkh. (Rosaceae)

Geographic distribution: Antarctic, Australian, Indomalayan, Nearctic, Neotropical, Palaearctic.

Damage to the host: Vagrant. This mite causes pitting and rusting of young leaves.

Dispersion in Turkey: Ankara, Van (Denizhan and Çobanoğlu 2010), İzmir (Narlıdere, Bayındır, Foça, Ödemiş) (23.06.2021, 07.07.2021, 01.08.2021, 06.09.2021).

Aculus mogeri Farkas, 1960

Host: *Populus alba* L. (Salicaceae)

Geographic distribution: Palaearctic.

Damage to the host: Vagrant. Mites cause leaf discolouration

Dispersion in Turkey: Ankara (Denizhan and Çobanoğlu 2010), İzmir (Buca, Foça) (04.06.2021, 23.09.2021).

Subfamily: Cecidophyinae Keifer, 1966

Colomerus vitis Pagenstecher

Host: *Vitis vinifera* L. (Vitaceae)

Geographic distribution: Africotropical, Australian, Nearctic, Neotropical, Palaearctic.

Damage to the host: Gall-making mite

Dispersion in Turkey: Ankara, Yalova (Denizhan and Çobanoğlu 2010), İzmir (Foça, Ödemiş, Seferihisar) (15.06.2021, 23.07.2021, 11.08.2021).

Calepitrimerus vitis Nalepa, 1905

Host: *Vitis vinifera* L. (Vitaceae)

Geographic distribution: Ethopian, Nearctic, Neotropical, Palaearctic.

Damage to the host: The mites inhabit lower leaf surfaces, causing some chlorosis

Dispersion in Turkey: Erzurum (Denizhan et al. 2015), İzmir (Foça, Beydağ, Bergama) (03.06.2021, 05.08.2021, 04.09.2021, 13.09.2021).

Family: Diptilomiopidae Keifer, 1944

Subfamily: Rhyncaphytoptinae Roivainen, 1953

Rhyncaphytoptus ficifoliae Keifer, 1939

Host: Ficus sp. (Moraceae)

Geographic distribution: Australian, Indomalayan, Nearctic, Neotropical, Palaearctic.

Damage to the host: Vagrant

Dispersion in Turkey: Ankara (Denizhan and Çobanoğlu 2010), İzmir (Foça, Ödemiş, Bergama, Seferihisar, Buca) (23.07.2021, 11.08.2021, 04.09.2021, 13.09.2021).

As a result, the species found in the fauna study conducted for the detection of Eriophyoid mites in the province of Izmir, which is the main aim of the study, will have an important contribution to the Eriophyoid fauna of Turkey. In addition, the fact that İzmir province has a high diversity and importance in terms of fruit growing will shed an important light on the future studies to be made in this sense.

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ÖZET

İzmir ilinde Eriophyoid akarların tespiti amacıyla kapsamlı bir faunistik araştırma yapılmıştır. Yapılan araştırma sonucunda Türkiye eriophyid akar faunası için 17 tür tespit edilmiştir. Bitki örnekleri İzmir ilinden meyve bahçeleri ve süs bitkilerinden toplanmıştır. Tespit edilen türler *Aceria erinea* Nalepa, 1891, *Aceria avanensis* Bagdasarian, 1970, *Aceria salicina* Nalepa, 1911, *Aceria filiformis* Nalepa, 1891, *Aceria ilicis* Canestrini, 1890, *Aceria massalongoi* Canestrini,1890, *Aceria oleae* Nalepa, 1900, *Aceria sheldoni* Ewing, 1937, *Eriophyes pyri* Pagenstecher, 1857, *Eriophyes tiliae* Nalepa, 1890, *Aculus fockeui* Nalepa & Trouessart, 1891, *Phyllocoptes pruni* Soliman & Abou-Awad, 1979, *Aculus schlechtendali* Nalepa 1890, *Aculus mogeri* Farkas, 1960, *Colomerus vitis* Pagenstecher, *Calepitrimerus vitis* Nalepa, 1905, *Rhyncaphytoptus ficifoliae* Keifer, 1939' dır. Tespit edilen türlerin coğrafik dağılım, konukçusu ve verdiği zarar şekli ile ilgili bilgiler makale içerisinde verilmiştir.

Anahtar kelimeler: Acarina, Eriophyoid, fauna, dağılım.

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Original article

The association of 16SrVI and 16SrI phytoplasma groups with carrot seeds and weeds in Ankara and Konya provinces in Turkey

Türkiye'de Ankara ve Konya illerinde 16SrVI ve 16SrI fitoplazma gruplarının havuç tohumları ve yabancı otlarla ilişkisi

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A B S T R A C T

The inoculum sources of phytoplasmas associated with carrot severe yellowing and reddening symptoms were investigated in Ankara and Konya provinces, Turkey. The presence of 16SrVI and 16SrI-related phytoplasmas in the seeds of seven carrot cultivars that are widely cultivated in the regions, as well as in weeds in the fields, was determined in this study. Sequence analysis was confirmed existing phytoplasma groups in samples were determined by using conventional molecular methods (nested-PCR) and the obtained results were supported by phylogenetic studies. In addition, the obtained nucleotide sequences were compared with the reference phytoplasma sequences by in silico PCR-RFLP analysis. *Daucus carota* wild, *Medicago sativa*, *Conium maculatum*, and *Sinapis arvensis* weeds were infected with the 16SrVI (Clover proliferation) phytoplasma group. In addition, 16SrVI and 16SrI (Aster yellows) phytoplasma groups were identified in seedlings germinated from seeds of seven carrot cultivars: one was a local red carrot cultivar and six were commercially produced cultivars. To our knowledge, this was the first report of carrot seeds infected with the 16SrVI group and the presence of the 16SrVI group in *S. arvensis*, *C. maculatum*, and *D. carota* wild.

INTRODUCTION

Carrot (*Daucus carota* L.), a member of the family Apiaceae, is grown and consumed in almost every geographical region of the world. Turkey produces an average of 588.778 tons of carrot from 10.989 hectares annually, of which about 84% of production is carried out in Ankara and Konya provinces located in central Anatolia (TSI, 2020). Supply for both domestic and foreign market demands is mainly covered by these outputs. Thus, the earnings contribute significantly to the region's economy.

Several pathogens such as spiroplasmas, viruses, and *Candidatus* Liberibacter solanacearum have been reported to cause economic yield losses in carrot production areas (Cebrián et al. 2010, Latham et al. 2004, Satta et al. 2016). These pathogens have been often found together under field conditions (Alfaro-Fernandez et al. 2012, Cebrian et al. 2010, Gamarra et al. 2011, Lee et al. 2006); however, there were also reports for single-pathogen infection (Trkulja et al. 2021, Valiunas et al. 2001). In addition to carrots, up to 100% yield losses in certain vegetables have been reported by phytoplasma-caused epidemics (Ember et al. 2011, Kumari et al. 2019).

Phytoplasmas are prokaryotic organisms without rigid cell walls and are limited to plant phloem tissues and are phylogenetically relevant to G+C Gram-positive bacteria (Weisburg et al. 1989). In general, phytoplasma infections cause some morphological symptoms on carrot plants such as reddening/yellowing of leaves, shoot proliferation, and decreased root quality (Satta et al. 2020). Other than that, phytoplasmas have been shown to also significantly impact the development of flower organs (Pracros et al. 2006).

For several decades, effective methods for identifying and characterizing phytoplasma infections on plants were not available (Kumari et al. 2019). Nowadays, different phytoplasma groups/subgroups can be characterized by utilizing molecular techniques and RFLP analysis methods based on the conserved 16S rRNA gene sequence (IRPCM 2004, Lee et al. 1995). In addition to the extensive use of conventional molecular techniques in the diagnosis of phytoplasma, quantitative-PCR techniques which produce more sensitive results, have also been applied (Liu et al. 2017). Furthermore, the high-quality availability of sequence analyses allows in silico simulations of restriction digestions and high-throughput identification in the classification of various phytoplasma groups (Wei et al. 2007).

So far, phytoplasmas belonging to 16SrI (Aster yellows) and 16SrXII (Stolbur) groups in Europe (Duduk et al. 2008, Satta 2016), the 16SrV (Elm yellows) group in Israel (Weintraub and Orenstein 2004), the 16SrII (Peanut witches'-broom) group in Saudi Arabia (Omar 2017), and the 16SrVI (Clover proliferation) group in the USA (Lee et al. 2006) have been reported to infect carrot in fields. In addition, carrots and weeds (Convolvulus arvensis and Daucus carota wild) were found to be infected with the 16SrXII group in Hatay province located in the Eastern Mediterranean Region of Turkey (Sertkaya 2014).

Insect vectors are considered the main reason for quick phytoplasma spread (Hogenhout et al. 2008). Human activities such as transportation and the use of infected plant propagation materials at the initial stage of cultivation also have been associated with the geographical transmission of phytoplasma groups (Al-Sadi et al. 2012, Mazraie et al. 2019). Several studies showed that phytoplasma groups can also be transmitted via seeds in various plant species, including carrots (Calari et al. 2011, Khan et al. 2002, Satta et al. 2019). In addition, perennial crop plants and weeds or wild plants could serve as reservoirs (Duduk et al. 2018, Kumari et al. 2019). Thus, weeds and wild plants are of utmost importance to understand epidemiological aspects of various diseases associated with phytoplasmas (Banzato et al. 2021).

Various phytoplasma groups have been reported to cause substantial yield reduction on carrot production in several countries, but, in the case of Turkey, the knowledge on both their presence and phylogenetic positions is rather limited. Moreover, there is still a huge gap in our understanding of inoculum sources of phytoplasma diseases. In this study, we investigated the potential of carrot seed and reservoir weed species as sources of phytoplasmas that occurred in carrot fields in the Ankara and Konya provinces.

MATERIALS AND METHODS

Weed and carrot seed sampling

Symptomatic and asymptomatic seven weed species belonging to five different families were sampled from carrot fields and their surrounding areas in Beypazarı and Nallıhan districts of Ankara and Meram district of Konya, Turkey, during growing seasons between April 2018 and September 2020. Nineteen weed samples were collected, including five *Amaranthus retroflexus* (fam: Amaranthaceae), seven *Sinapis arvensis* (Fam: Brassicaceae), three *Conium maculatum* (Fam: Apiaceae), one *Bifora radians* (Fam: Apiaceae), one *Daucus carota* wild (Fam: Apiaceae), one *Medicago sativa* (Fam: Fabaceae), and one *Fumaria officinalis* (Fam: Papaveraceae). Symptoms such as reddening of leaf tips, mosaic, and vein clearing were noted on a few of them while most others were symptomless. Seeds of eight widely grown carrot cultivars were also obtained from farmers during the surveys. Seed samples were taken into 50 ml glass tubes and stored at 4 oC until sowing.

For the germination of carrot seeds, a sterile and insect-free growing environment was provided under greenhouse conditions. Since the number of carrot seeds was quite limited, only approximately 200-300 seeds per cultivar were sown on two sets of pots. First, sterilized soil was placed into 9 cm width sterile pots, and then the seeds were sown on the shallow upper surface of the soil. The sown seeds were regularly irrigated with distilled water. The seedlings, including their roots, were harvested at the cotyledons leaf stage (before the true leafing stage) and then prepared for nucleic acid isolation.

Total nucleic acid (TNA) isolation

TNA was isolated from the shoot tissues of 19 weed samples and 32 freshly harvested seedlings of eight carrot cultivars at the cotyledon stage using CTAB protocol with some modifications (Li et al. 2008). Freshly harvested seedlings were cleaned of soil particles using 70% alcohol and sterile water. Two hundred mg tissue from each weed sample and 1000 mg tissue from seedlings of each cultivar were homogenized to form the isolation starting materials. The tissue was well crushed in CTAB solution buffer (2%

CTAB, 2% PVP-40, 100 mM Tris-HCL pH 8.0, 0.5 M EDTA, 1.4 M NaCl, 0.02% MCE) at a ratio of 1:5 (w:v) and then incubated at 65 oC for 20 min before centrifuged at 17,000 rpm for 10 min. The supernatant (850 µl) was treated with an equal volume of chloroform/isoamyl alcohol (24:1) and then centrifuged at 17,000 rpm for 20 min. The fluid phase on the upper surface was transferred to a sterile tube and treated with 0.7 volume of isopropanol, and the nucleic acid pellet obtained after centrifugation was washed with ice-cold 70% alcohol. The quality and purity of the genomic DNA obtained after the pellet was dissolved with nuclease-free water were measured using NanoDrop 2000 (Thermo Scientific, U.S.A). TNA was stored at -20 oC until further used in the nested-PCR assay.

Nested-PCR amplification

Nested-PCR experiments were done using R16mF2/R16mR2 or R16F2n/R2 primer pairs (Gundersen and Lee 1996) followed by fU5/rU3 (Lorenz et al. 1995) primer pairs to identify phytoplasma from its 16S rRNA gene. DNA concentrations from within TNAs for the first PCR reaction were made using NanoDrop 2000 (Thermo Scientific, U.S.A) and about 0.05 µg of gDNA was used for each reaction tube. The first amplification products from Nested-PCR assays were diluted 1:30 with nuclease-free water before being utilized in subsequent reactions.

The reactions were implemented in 25 µl PCR tubes containing 0.05 µg genomic DNA (first PCR) and 1 µl of 30 µl diluted PCR product (second PCR), 1 µl of 10 mM dNTPs, 0.2 µl of 25 mM MgCl2, 1 µl of 10 mM phytoplasma universal primers, 2.5 µl of 10X PCR buffer, and 0.25 U of Taq DNA polymerase (5 U/µl) (Ampliqon, Denmark). The amplification conditions were carried out as stated by Gundersen and Lee (1996). The PCR products were separated on 1.2% agarose gel stained with ethidium bromide. The amplicon's size was measured using a UV light imaging system (Genegenius, U.K.).

Phylogenetic analysis and sequencing

If there were more than one positive nested-PCR amplification result from the same cultivar in the seedling experiments, two results were chosen for nucleotide sequencing, and one sample was chosen when there was only one positive result. The Sanger method was applied by a commercial firm (BM lab, Ankara, Turkey) to reveal bidirectional sequencing of a total of 16 PCR products from four weed samples and seedlings. BLAST (Basic Local Alignment Search Tool) analysis of the NCBI (the National Center for Biotechnology Information) was used to confirm the nucleotide (nt) data obtained by sequencing with those of other phytoplasma isolates in the GenBank. The sequences were then submitted to the NCBI database under acc. nos. MZ463005-MZ463020.

The sequences obtained from weeds and seedlings were aligned with other sequences of different phytoplasma groups from across the world using ClustalW in MEGAX software (Kumar et al. 2018). The best models for nt substitution were selected using the lowest BIC (Bayesian Information Criterion) scores. The phytoplasma phylogenetic tree was generated by the Maximum likelihood method (Felsenstein 1981) with 1000 bootstrap repetitions using the Tamura-Nei parameter model (TN93) (Tamura and Nei 1993)+Gamma distributed (G). The *Spiroplasma citri* 16S rRNA gene, isolate Qualubia (acces no AM157769) was used as the out-group.

In silico PCR-RFLP analyses

Computer-simulated PCR-RFLP (the restriction fragment length polymorphism) analyses were performed using the Snapgene software (GSL Biotech; http://www.snapgene.com) to improve the accuracy of phytoplasma classification based on partial of the 16S rRNA gene sequences. For this purpose, a total of 16 nt sequences obtained from carrot seedlings and weeds were digested in silico with RsaI enzyme, among 17 RFLP enzymes (*RsaI*, *SspI*, and *TaqI*, *BfaI*, *BstUI* (Tha*I*), *AluI*, *BamHI*, *DraI*, *HinfI*, *HpaI*, *EcoRI*, *HaeIII*, *HhaI*, *Sau3AI* (*MboI*), *HpaII*, *KpnI*, *MseI*) that were widely used in the determination of phytoplasma groups (Lee et al. 1998). A virtual 4% agarose gel electrophoresis image was plotted to the computer screen automatically after in silico restriction digestion. In addition, the partial sequences of 16Sr gene fragments of Ca. P. trifolii 16SrVI-A (acces no AY390261), Brinjal little leaf (BLL) 16SrVI-D (acces no EF186820), Oenothera phytoplasma 16SrI-B (acces no M30790), Aster yellows phytoplasma (ACLR-AY) 16SrI-F (acces no AY265211), Clover phyllody phytoplasma (CPh) 16SrI-C (acces no AF22065), Elm yellows phytoplasma (EY1) 16SrV-A (acces no AY197655), Peanut witches-broom phytoplasma 16SrII-A (access no L33765), Ca. P. solani (Stolbur) 16SrXII-A (access no AJ964960), and Australian grapevine yellows 16SrXII-B (access no L76865) isolates were used in silico analyses to compare the patterns obtained from RFLP analyses with Turkish weed and seedlings isolates.

RESULTS

Symptomology of seedlings and weeds

No symptom was observed on 15 weed samples. However, vein clearing, reddening at the leaf tips, yellowing, and severe reddening/purpling were observed on one of each *S. arvensis*, *C. maculatum*, *M. sativa*, and *D. carota* wild, respectively (Figures 1 and 2, Table 2). There was also no symptom caused by phytoplasma observed on all seedlings of eight cultivars planted on 16 pots up to harvest time at the cotyledon stage. However, germination rates of cultivars were different, particularly, seeds of a local cultivar (TS1) germinated rate below 50% and resulted in the death of some seedlings (Table 1). In addition, germination rates of other commercial carrot seeds differed between 70% and over 90% (Table 1 and Figure 3).

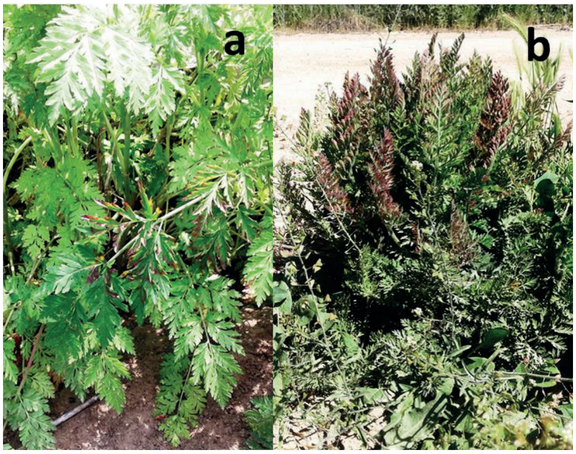

Figure 1. **(a)** redding of leaf tips symptoms on *Conium maculatum* (b) severe redding or purpling symptoms on *Dacus carota* wild

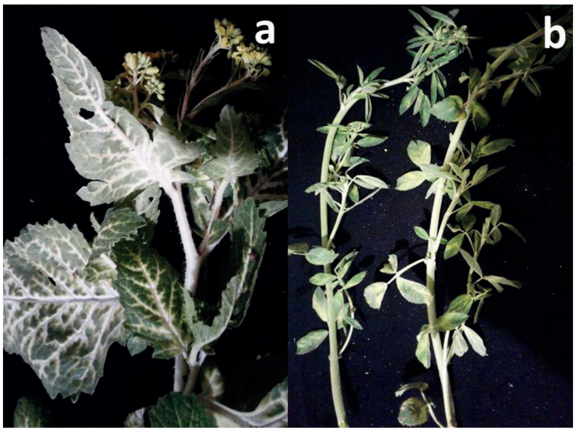

Figure 2. **(b)** Vein clearing symptoms on Sinapis arvensis (b) yellowing symptoms on Medicago sativa

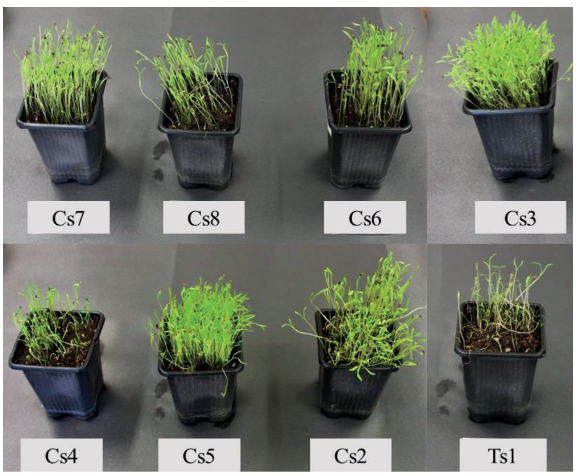

Figure 3. Germination performances of different carrot seed varieties (Cs: Commercial seed, Ts: Traditional seed)

Table 1. Germination and infection status of carrot seedlings germinated in a controlled environment

Carrot seed cultivars	Total no of DNA samples	Total no of infected samples	Germination results	Phytoplasma infection rRNA
TS1*	4	4	Germination rate below 50% and dead seedlings	16SrVI
CS2**	5	3	Germination over 80%	16SrVI
CS3**	8	5	Germination over 90%	16SrVI
CS4**	3	3	Germination about 70%	16SrVI
CS5**	2	0	Germination over 90%	-
CS6**	3	1	Germination over 80%	16SrVI
CS7**	5	3	Germination over 80%	16SrVI
CS8**	2	1	Germination about 70%	16SrI
Total	32	20		

*TS: Local seed CS**: Commercial Seed

Table 2. Symptoms observed in weeds and status of phytoplasma infections

Weed sample	Total number of sample	Total no of infected sample	Symptoms	Phytoplasma infection rRNA
Amaranthus retroflexus	5	0	5=asymptomatic	-
Sinapis arvensis	7	1	5=asymptomatic 1=vein clearing 1=chlorotic discoloration	16SrVI
Conium maculatum	3	1	2=asymptomatic 1=redding of leaf tips	16SrVI
Medicago sativa	1	1	1=yellowing	16SrVI
Fumaria officinalis	1	0	1=asymptomatic	-
Daucus carota wild	1	1	1=severe redding or purpling	16SrVI
Bifora radians	1	0	1=asymptomatic	-
Total	19	4		

TNA isolation and molecular assays

TNAs were successfully obtained using the CTAB nucleic acid extraction protocol, which had been modified to also obtain genomic DNAs. Spectrophotometric measurements confirmed the quality and purity of the obtained TNAs as suitable templates for PCR studies. Successful amplifications were produced in the nested-PCR experiments using fU5/rU3 (Lorenz et al. 1995) primer pairs and the expected 883 bp long amplicons were obtained (Figure 4). Positive findings were acquired from 20 out of 32 carrot seedlings and 4 out of 19 weed samples. Tables 1 and 2 details the molecular detection results for both seedlings and weeds.

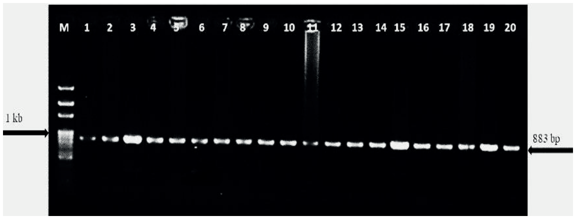

Figure 4. Image of fragments of about 883 bp in agarose gel obtained from weeds and carrot seedlings PCR studies using fU5/rU3 primers (1:*Sinapis arvensis*, 2:*Dacus carota*, 3:*Medicago sativa*, 4:*Conium maculatum* , 5:Ts1a, 6:Cs2c, 7:Cs3c, 8:Cs3a, 9:Cs2e, 10: Ts1b, 11:Cs4a, 12:Cs7c, 13:Cs6a, 14:Cs7b, 15:Cs8c, 16:Cs4c, 17:Ts1c ,18:Cs3b , 19: Cs7a, 20:Positive control (M: Marker 100 bp; Solis Biodyne, Estonia)

Phylogenetic analysis and sequence similarities

To compare the sequences of the partial fragment of the 16S rRNA gene region, a total of 16 isolates obtained from carrot seedlings and weeds were analysed with other phytoplasma strains available in the GenBank. The nt sequence of CS8c isolate obtained from a commercial cultivar was 99.65% similar to an Iranian isolate 'Bajgah periwinkle little phytoplasma' (acc. no. DQ266089) of 16SrI 'Aster Yellows' group. The rest of the isolates (fifteen out of 16) showed 99.78-99.89% nt sequence identities with 'Eggplant phyllody phytoplasma' (acc. no. MT240537), '*Candidatus* Phytoplasma trifolii-grape5' (acc. no. MK392485), and 'Brinjal Leaf Phytoplasma-BLL' (acc. no. MT071396) of 16SrVI 'Ca. P. trifolii' group.

The phylogenetic tree was constructed based on an 883 bp fragment of the 16S rRNA using sequences of different phytoplasma groups/subgroups herein obtained and also retrieved from the GenBank (Figure 5). Four weed and eleven seedlings (from both local and commercial cultivars, Table 1) isolates were clustered within the 16SrVI group in the phylogram (Figure 5). On the other hand, the CS8c isolate obtained from a commercial carrot cultivar was clustered in the 16SrI group (Figure 5). Major clades in which the weed and seedling isolates clustered were supported by high bootstrap reliability values (>90).

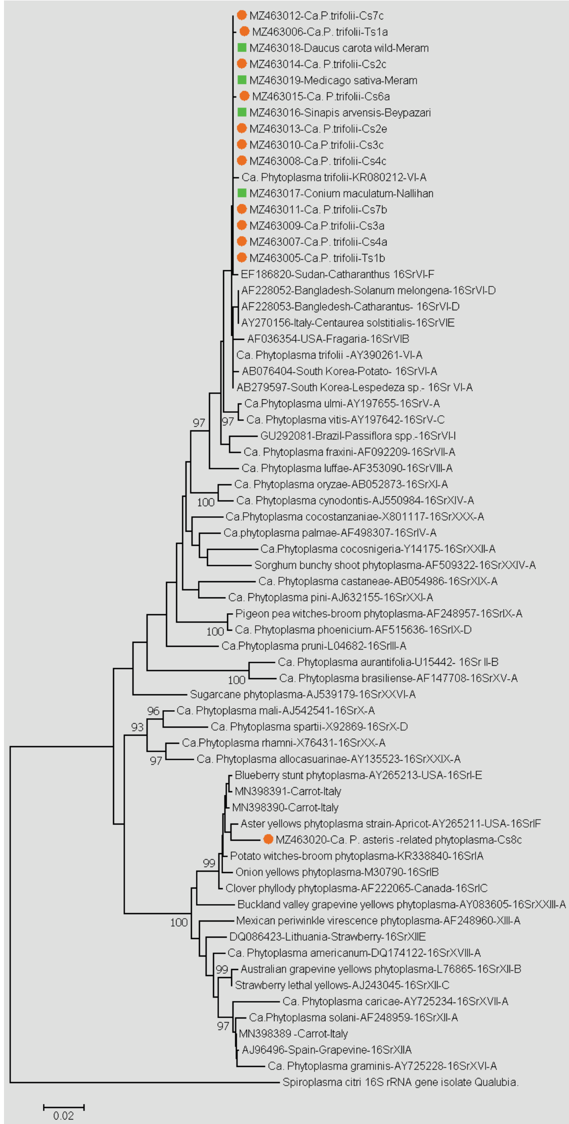

Figure 5. Phylogenetic cladogram constructed by Maximum likelihood test of nt of partial 16S rRNA gene. Fifty-nine isolates were applied in the 16S rRNA comparison. Turkish weed and seedling isolates are marked with circle and square symbols. Bootstrap values on each branch were supported by 1000 replicates; only values greater than 90% were shown. The *Spiroplasma citri* 16S rRNA gene, isolate Qualubia (access no AM157769) was used as the out-group

As a result, the phytoplasma isolates obtained in this study were clustered in the same evolutionary lineages with phytoplasma strains, namely 16SrVI and 16SrI strains, showing the monophyletic feature. Nt similarity and phylogenetic analyses also suggested that the 15 isolates in 16SrVI could be more specifically classified into the 16SrVI-A subgroup while the CS8c isolate has belonged to the 16SrI-F subgroup.

In silico PCR-RFLP

The phylogram and nt similarity results were supported by the in silico RFLP analysis. After comparing computer-simulated PCR-RFLP analysis of virtual patterns of the partial 16S rRNA gene of sixteen isolates was determined that distinctive RFLP profiles with *RsaI* enzymes according to reference strains. The *RsaI* enzyme-digested profiles of fifteen isolates identified as related to the 16SrVI and 16SrI groups according to their nt similarity ratios were consistent with 16SrVI-A/D and 16SrI and were different from other references (16SrI-B/F/C, 16SrV-A, 16SrII-A, and 16SrXII-A/B) (Figure 6). The RFLP virtual pattern of the other 16SrI group-associated Cs8c isolate with the *RsaI* enzyme was consistent with the 16SrI-F subgroup, whereas it was different with the 16SrI-B/C subgroup and other groups/subgroups (16SrVI-A/D, 16SrV-A, 16SrII-A, and 16SrXII-A/B) (Figure 6).

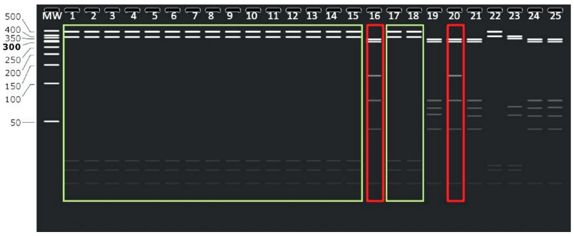

Figure 6. Virtual RFLP gel (4.0%) patterns from in *silico* digestion of partial 16S rRNA fU5/rU3 amplicons of phytoplasmas infecting carrot seedlings and weeds with *RsaI* restriction enzyme. The green rectangles demonstrate the digestion pattern of the 16SrVI-A/D reference strains. The red rectangles demonstrate the digestion pattern of the 16SrI-B/F/C reference strains. [1: Cs4c, 2: Cs7b, 3: Cs7c, 4: Cs4a, 5:Ts1a, 6:Cs2c, 7:Cs3c, 8:Cs3a, 9:Cs2e, 10: Ts1b, 11: *Conium maculatum*, 12: *Medicago sativa*, 13:Cs6a, 14: *Dacus carota*, 15:*Sinapis arvensis* 16: Cs8c] Weed and seedling isolates, [17] 16SrVI-A (AY3902619), [18] 16SrVI-D (EF186820), [19] 16SrI-B (M30790), [20] 16SrI-F (AY265211), [21] 16SrI-C AF222065), [22] 16SrV-A (AY197655), [23] 16SrII-A (L33765), [24] 16SrXII-B (L76865), [25] 16 SrXII-A (AJ964960). (MW; Qiagen, Gelpilot 50 bp ladder)

DISCUSSION

This study aimed to reveal seed and weeds as inoculum sources that play crucial roles in the spread of phytoplasma diseases causing symptoms such as redness/browning, yellowing, and reduced root quality which were widely observed in the largest carrot growing areas of Turkey (Ankara and Konya).

Transmission of phytoplasmas through seeds was somewhat a controversial topic and has long been considered impossible due to the lack of a direct connection between the phloem system and embryos (Menon and Pandalai 1960). However, it was later proved for the first time by Khan et al. (2002) that seeds of phytoplasma-infected symptomatic alfalfa mother plants transmitted this pathogen. Since then, there have been reports of seed transmission of phytoplasmas in other vegetables, such as tomato and winter oilseed rape (Calari et al. 2011), as well as *Brassica napus* (Satta et al. 2019) at the seedling stage. The presence of phytoplasmas on carrot seeds was first demonstrated by Carminati et al. (2019), and it was confirmed that these seeds were contaminated with the 16SrI 'Aster Yellows' group. In a later study, Satta et al. (2020) found that seedlings obtained from carrot seeds were infected at the cotyledon stage with one of the groups 16SrI and 16SrXII, and also seedlings of a batch belonging to the same group were infected with both groups of phytoplasmas. Therefore, this present study confirmed 16SrI group infection, while also detecting the 16SrVI group in the seedlings of carrot cultivars for the first time. The two phytoplasmas groups were not found together in any infected seedlings grown from the same cultivar according to our observation.

Abnormalities and malformations in the floral organs and fruits of various species infected with phytoplasmas resulted in the formation of seeds with reduced viability (McCoy et al. 1989). Accordingly, seed production in infected mother plants was affected both quantitatively and qualitatively due to the presence of phytoplasmas (Satta et al. 2019). More importantly, it has been reported that phytoplasma infection affects the expression of some flower development genes in the whole flower meristem of tomato and hydrangea plants (Himeno et al. 2011, Kitamura et al. 2009, Pracros et al. 2006). Therefore, late infections usually still produce a normal number of seeds, but these seeds could be infected and thus give the pathogen a greater chance of transmission, conversely, early infection causes such serious changes in the mother plant that seed production and viability of the seeds as next-generation planting materials are highly reduced (Satta et al. 2020). In our study, while the germination rates and development of seedlings of commercial cultivars stayed at a normal level, very weak germination, growth, and even death of some seedlings were observed on a non-commercial/local red carrot cultivar (named TS1) which is regularly cultivated and harvested under uncontrolled field environment every year by farmers themselves. This could indicate that the TS1 mother plants were infected by phytoplasma at the early growing season and exposed to the pathogen for a long time before flowering.

At least 43 weed species with phytoplasma infections have been documented from around the world (Mall et al. 2010). The most prominent symptoms on infected plants were the proliferation of axillary shoots, small leaves, extensive chlorosis, witches' broom, and yellowing (Mall et al. 2010). The phytoplasmas found in weeds around the world mostly belong to the 16SrI, 16SrII, 16SrXI, 16SrXII, and 16SrXIV groups, but some members of the 16SrIII, 16SrIV, 16SrV, 16SrVI, 16SrVII, 16SrIX, 16SrX, and 16SrXXIX groups have also been identified (Duduk et al. 2018). Also, studies have shown that while *A. retroflexus* weed is infested with the 16SrI group, and 16SrV-B and 16SrXII-A subgroups (Credi et al. 2006, Wu et al. 2010, Yang et al. 2011), phytoplasmas have not been found in *F. officinalis* and *B. radians* weeds. Similarly, phytoplasma infection was not detected in any of the asymptomatic *A. retroflexus*, *F. officinalis*, and *B. radians* weeds collected from inside and around carrot fields in our study. On the other hand, this present study revealed that symptomatic *M. sativa*, *S. arvensis*, *D. carota* wild, and *C. maculatum* weeds were infected by the 16SrVI group. Other studies determined that M. sativa was infected with 16SrII-C/D and 16SrVI-A subgroups, and 16SrXII groups (Credi et al. 2006, Esmailzadeh Hosseini et al. 2016a, 2016b). Infections by 16SrIX-C 'withes`s broom', 16SrI-(B/AJ), and 16SrXII-A phytoplasma subgroups have been reported in wild plants *S. arvensis*, *C. maculatum*, and *D. carota* wild, respectively (Casati et al. 2016, Fernández et al. 2020, Sertkaya 2014). Therefore, according to our best knowledge, this was the first report of the presence of 16SrVI group phytoplasma in the *S. arvensis*, *C. maculatum*, and *D. carota* wild.

According to Satta et al. (2020) and the results of our study, no symptomological finding could be related to phytoplasma infected seedlings. However, poor growth and even death were observed on seedlings germinated from a local cultivar. In future studies, factors affecting the germination performance of infected seeds should be evaluated in detail, both genetically and physiologically. Insect vector's role in epidemiology also needs to be investigated as they could spread phytoplasma further from infected weeds and cultured plants under field conditions, which in turn could accumulate the pathogen population in cultivation areas, thus increasing future sources of inoculum.

Seedlings were obtained from carrot seeds in the cotyledon leaf stages, and the phytoplasma contamination of these seeds was indirectly revealed by conventional molecular techniques in a short period of time. The methodology that may use in the detection of phytoplasmas from seedlings obtained from seeds is recommended to be applied to different vegetable seeds, an algorithmic method should be developed by determining certain parameters to detect the effects of infections on seed germination. Furthermore, population genetic structures should be revealed using phytoplasma genes in the future to better understand the spread of phytoplasmas from one place to another by seed and their adaptation to weeds in nature.

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ÖZET

Havuçta şiddetli sararma ve kızarıklık belirtileri ile ilişkili fitoplazma inokulum kaynakları Ankara ve Konya illerinde araştırılmıştır. Bu çalışma kapsamında bölgelerde yoğun olarak ekimi yapılan yedi havuç çeşidinin tohumlarında ve tarlalarda yer alan yabancı otlarda 16SrVI ve 16SrI ilişkili fitoplazmaların varlığı tespit edilmiştir. Konvansiyonel moleküler yöntemler (nested-PCR) kullanılarak dizi analizleri ile fitoplazma gruplarının varlığı doğrulanmış ve filogenetik analizler ile desteklenmiştir. Ayrıca elde edilen nükleotid dizileri, *in silico* PCR-RFLP analizi ile referans fitoplazma dizileri ile karşılaştırılmıştır. *Daucus carota* wild, *Medicago sativa*, *Conium maculatum* ve *Sinapis arvensis* yabancı otlarında 16SrVI (Clover proliferation) fitoplazma grubu ile enfeksiyon belirlenmiştir. Ayrıca yedi havuç çeşidinin tohumlarından çimlenen fidelerde 16SrVI ve 16SrI (Aster yellows) fitoplazma grupları tespit edilmiştir: Biri yerel kırmızı havuç çeşidi ve altısı ticari olarak üretilmiş çeşitlerdir. Bilgilerimize göre elde edilen bulgular; 16SrVI grubu ile enfekte olmuş havuç tohumlarının ve *S. arvensi*s, *C. maculatum* ve *D. carota* wild'da 16SrVI grubunun varlığının ilk raporudur.

Anahtar kelimeler: fitoplazma, fide, yabancı ot, Nested-PCR, *in silico* analiz, filogenetik

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Original article

Beneficial mite fauna of Hevsel Gardens-Diyarbakır

Hevsel Bahçelerinin (Diyarbakır) faydalı akar faunası

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A B S T R A C T

This study was conducted to determine beneficial mite species of Hevsel Gardens in Diyarbakir province in 2018-2019. These historical gardens, which have been cultivated continuously for thousands of years, are listed on UNESCO World Heritage List. Survey studies were carried out periodically every 15 days from April to November with the random sampling method. In the study, samples were taken from 66 plants species consisting of fruit trees, vegetables, and weeds. As a result of the study, 15 predator mite species from families Tydeidae, Cheyletidae, Stigmaeidae, Triophtyidae, Raphignathidae, Iolinidae, and Phytoseiidae were identified on 33 plants species. Among these families, 7 from Phytoseiidae, 3 from Iolinidae, and one species from other families were determined. *Phytoseius finitimus* (Ribaga, 1904) (Mesostigmata: Phytoseiidae) was the dominant species with 37.5%. The highest number of mite species were found on *Rubus* sp. with 7 species. In the study, more beneficial mites were found in regions where agricultural activity is less. Predator mites were mostly found on fruit trees, and less frequently on vegetables and fragrant plants. Poplar and mulberry trees have a high population in Hevsel Gardens. Although many samples were taken from these two plants during the surveys, only *P. finitimus* was found. With this two-year study, it has been revealed that Hevsel Gardens, which are important for the world, are rich in beneficial mites. Important predator species used in the world for biological control against harmful mites and insects have been identified. With study, beneficial species that create new records for the region were determined.

INTRODUCTION

Hevsel Gardens is a unique delta land with an average area of 7.000 da, lying near to the historical Diyarbakir Walls (Figure 1). Despite the fact that Diyarbakir is the main grain production there has been a rise in the production of various fruits and vegetables in recent years. In Hevsel Gardens, the Tigris River passes in the middle, most vegetables are grown and especially the leaves of the city meet a significant part of the edible vegetables. There are various fruit trees in the area, but there is no economic production. No study on beneficial mite fauna has been carried out in the area with a special ecology so far. Although mites are millimeters, they are very important creatures for nature. About 50.000 species of these creatures have been described so far, and most of them are beneficial, but there are also important species that cause serious damage to agricultural products. They are highly effective against beneficial mites, pests, and mites. Therefore, identifying and revealing beneficial mites is extremely important in terms of pest control. This study is aimed to reveal the beneficial mite fauna of Hevsel Gardens in the World Heritage List and to contribute to the mite fauna of Turkey. In the provinces of the region, as a few studies have been conducted to determine the mite fauna (Ayata 2015, Bolu 2002, Çıkman et al. 1996, Geçer and Denizhan 2015, Kaplan and Yücel 2014, Karaca et al. 2007, Yaman et al. 2017), but no such study has been carried out in the area in question. In addition, the research was mostly aimed at determining the pest species. This study is important in terms of being the first in the historical Hevsel Gardens and revealing beneficial species.

Figure 1. Hevsel Gardens

MATERIALS AND METHODS

Cultivated plants, weeds and trees, and plants growing naturally in non-agricultural areas were the main material of the study.

Field studies

As of April, field surveys were started, and samples were taken periodically every 15 days until the end of November, with random sampling. For this purpose, the leaves, flowers, fruits, and twigs of the cultivated plants in the gardens and the other plants in the dense population were sampled. The number of samples taken was determined according to the total number of plants in the area. While taking samples from trees, samples were taken from four sides of the tree to represent the whole tree. Vegetables and weeds were sampled from the lower, middle, and upper parts of the plant. The samples, together with the comprehensive label number, were first taken into a paper bag and then into a small polyethylene bag and after being tied up, they were placed in ice containers and brought to Directorate of Diyarbakir Plant Protection Research Institute, Entomology Laboratory.

Laboratory studies

The upper and lower surfaces of the leaves were examined with a stereoscopic binocular microscope, and any mites observed were taken into 70% ethanol with a brush. Berlese Funnel was used to remove mites found on plant parts such as flowers, short branches, and fruit. According to the size of the leaves, 30-60 leaves were placed in the Berlese Funnels. To prevent the mites from escaping, liquid petroleum jelly was applied to the upper part of the Berlese Funnel and kept under 100-watt light for 24-48 hours (depending on the drying condition of the leaves). Accordingly, the mites escaping from the light fell into the glass tubes containing 70% ethyl alcohol at the bottom of the funnel.

Slide preparation

The preparation of slides was done according to Düzgüneş (1980). To see the diagnostic characteristics of the mites in the preparation phase, the mites were kept on the hot plate at 50 °C in Syracuse containers until their color was cleared by checking every 15 minutes in an average of 5 ml of lactophenol solution. Then, 3-5 drops of acid fuchsin dye were added to the mites and left for 1-3 hours. The mites were taken from the Syracus cups with the help of a pointed preparation needle, then placed on a slide with a drop of Hoyer on it. A dorso-ventral position was carefully placed on the slide with the help of the preparation needle under the Leica S8 APO Stereo Microscope, and after it was brought to the appropriate position for diagnosis, a coverslip was placed on it. The preparations were then kept in an oven at 50 °C for one week. Finally, the coverslip was fixed with transparent nail polish and the preparations were sent to the subject specialist for diagnosis after sorting.

RESULTS

To reveal the beneficial mite species of Hevsel Gardens; 65 plants consisting of vegetables, fruit trees, weeds, and other groups found in the area were sampled for two years (Table 1). As a result of the surveys, 15 species of beneficial mites were determined on 33 plants (Table 2). These species are given in the table below.

As can be seen from the table, a total of 15 predatory mite species were determined from the families Tydeidae, Cheyletidae, Stigmaeidae, Triophtyidae, Raphignathidae, Iolinidae, and Phytoseiidae.

Beneficial mites determined in Hevsel Gardens

Family: Phytoseiidae

Species: *Phytoseius finitimus* (Ribaga, 1904)

Economic importance: It is an important predator, it is common and dense in Turkey (Figure 2).

Table 1. Plant species sampled in Hevsel Gardens in 2018-2019

Plants			
1.	*Malus domestica Borkh*	2.	*Cucumis sativus L.*
3.	*Vitis vinifera L.*	4.	*Cucumis melo var. Flexuosus L.*
5.	*Prunus avium L.*	6.	*Lycopersicon lycopersicum L.*
7.	*Cydonia oblonga Miller*	8.	*Solanum melongena L.*
9.	*Diospyros kaki L.*	10.	*Capsicum annuum L.*
11.	*Pyrus sp.*	12.	*Eruca vesicaria Miller*
13.	*Rubus fructicocus L.*	14.	*Lactuca sativa L.*
15.	*Punica granatum L.*	16.	*Lepidium sativum L.*
17.	*Prunus cerasus L.*	18.	*Petroselinum crispum Miller*
19.	*Prunus armeniaca L.*	20.	*Ocimum basilicum L.*
21.	*Prunus persica L.*	22.	*Spinacia oleracea L.*
23.	*Pyrus communis L.*	24.	*Allium sativum L.*
25.	*Prunus amygdalus L.*	26.	*Allium ampeloprasum L.*
27.	*Ficus carica L.*	28.	*Allium cepa L.*
29.	*Citrullus lanatus (Thunb.) Matsum.&Nakai*	30.	*Cucurbita pepo L.*
31.	*Juglans regia L.*	32.	*Phaseolus vulgaris L.*
33.	*Morus alba L.*	34.	*Cucurbita sp.*
35.	*Fragaria sp.*	36.	*Cucurbita sp.*
37.	*Sorghum halepense L.*	38.	*Abelmoschus esculentus L.*
39.	*Chenopodium album L.*	40.	*Cucurbita sp.*
41.	*Amaranthus retroflexus L.*	42.	*Abelmoschus esculentus L.*
43.	*Xanthium strumarium L.*	44.	*Beta vulgaris var. cicla L.*
45.	*Solanum nigrum L.*	46.	*Brassica sp.*
47.	*Portulaca oleracea L.*	48.	*Mentha piperita L.*
49.	*Populus alba L.*	50.	*Brassica sp.*
51.	*Rhus coriaria L.*	52.	*Mentha pulegium L.*
53.	*Fraxinus excelsior L.*	54.	*Lamium alba-album L.*
55.	*Pinus sp.*	56.	*Medicago sativa L.*
57.	*Platanus sp.*	58.	*Verbascum thapsus L.*
59.	*Salix alba L.*	60.	*Euphorbia cyparissias L.*
61.	*Maclura pomifera (Rafinesque)*	62.	*Echinops sp.*
63.	*Elaeagnus angustifolia L.*	64.	*Centaurea sp.*
65.	*Laurus nobilis L.*	66.	*Alcea sp.*

Table 2. Beneficial mite species determined in Hevsel Gardens in 2018-2019

Order	Family	Species
Prostigmata	Tydeidae	*Tydeus californicus* (Banks)
	Cheyletidae	*Acaropsis sollers* (Kuzin)
	Stigmaeidae	*Zetzellia mali Ewing*
	Triophtyidae	*Triophtydeus triophthalmus* (Oudemans)
	Raphignathidae	*Raphignathus gracilis* (Rack)
	Iolinidae	*Neopronematus neglectus* (Kuznetzov)
		Pronematus sextoni (Baker)
		Pronematus sp.
Mesostigmata	Phytoseiidae	*Typhlodromus recki* (Wainstein)
		Phytoseius finitimus (Ribaga)
		Euseius finlandicus (Oudemans)
		Amblyseius andersoni (Chant)
		Typhlodromus pyri (Scheuten)
		Neoseiulus barkeri (Hughes)
		Proprioseiopsis messor (Wainstein)

Figure 2. Dorsal view of *Phytoseius finitimus*

Distribution: It was recorded for the first time in Italy. It is quite common in the Mediterranean region. It has been most commonly reported in Greece and Israel. Iran, Israel, Italy, Morocco, Portugal, Spain, Egypt, France, Greece, Tunisia, and the USA (Demite et al. 2014). Phytoseius finitimus is especially abundant in vineyard areas of the Mediterranean Region. In the researches in Turkey; Adana, Adapazarı, Amasya, Ankara, Antalya, Aydın, Bolu, Burdur, Bursa, Çanakkale, Edirne, Erzincan, Erzurum, Giresun, Gümüşhane, Hakkari, Mersin, Isparta, İstanbul, İzmir, Kastamonu, Konya, Muğla, Niğde, it has been reported that it is widely seen in the future such as Rize, Tekirdağ, Tokat and it is quite effective (Akçakoyunluoğlu 2017, Faraji et al. 2011, Göven et al. 2002, 2009, Kasap et al. 2013). The plants on which it was determined in this study are given in Table 3.

Table 3. Plants on which *Phytoseius finitimus* is found

Sample	Host
1	*Malus domestica*
2	*Morus sp.*
3	*Rubus fructicocus*
4	*Ficus carica*
5	*Petroselinum crispum*
6	*Portulaca oleracea*
7	*Amaranthus retroflexus*
8	*Cydonia oblonga*
9	*Pyrus sp.*
10	*Populus alba*
11	*Mentha pulegium*
12	*Prunus avium*
13	*Punica granatum*
14	*Prunus armeniaca*
15	*Rhus coriaria*
16	*Vitis vinifera*
17	*Juglans sp.*
18	*Mentha piperita*
19	*Spinacia oleracea*
20	*Capsicum annuum*
21	*Pyrus sp.*
22	*Lamium sp.*
23	*Xanthium sp.*
24	*Platanus sp.*
25	*Eruca vesicaria*
26	*Laurus nobilis*

Species: *Euseius finlandicus* (Oudemans, 1915)

Economic importance: Euseius finlandicus is an economically important species (Figure 3). It is known to play an important role in the natural control of mites such as red spiders, Eriophids, and others. This species is a general predator feeding on specific pollen (McMurtry and Croft 1997).

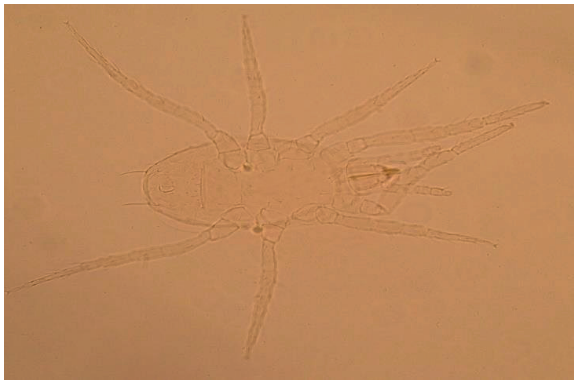

Figure 3. Dorsal view of *Euseius finlandicus*

Distribution: It has been found in many countries such as Austria, Canada, England, Finland, France, Georgia, Germany, Greece, Hungary, Iran, Italy, Poland and United States (Demite et al. 2014). Adana, Amasya, Antalya, Ankara, Bitlis, Ordu, Erzurum, Hakkari, Tokat, Samsun and Kahramanmaraş in Turkey (Alaoğlu 1996, Çobanoğlu 1993, Çobanoğlu and Kumral 2014, Faraji et al. 2011, Göven et al. 2009, İnal 2005, İncekulak and Ecevit 2002, Kasap and Çobanoğlu 2007, 2009, Kumral and Kovancı 2007, Özşişli and Çobanoğlu 2011, Yanar and Ecevit 2005, Yanar and Erdoğan 2013).

Euseius finlandicus has also been detected in trees in previous studies. It was found in the study from April to November. It has been detected in both fruits and vegetables but is more commonly found on fruit trees (Table 4).

Table 4. Plants on which *Euseius finlandicus* is found

Sample	Host
1	*Prunus avium*
2	*Punica granatum*
3	*Malus domestica*
4	*Malus domestica*
5	*Cydonia oblonga*
6	*Morus sp.*
7	*Pyrus sp*
8	*Capsicum annuum*
9	*Mentha piperita*
10	*Cydonia oblonga*

Species: *Typhlodromus pyri* (Scheuten, 1857)

Economic importance: The predator is a species; it is an important predator of *Panonychus ulmi* (Koch) (Prostigmata: Tetranychidae) (Gerson et al. 2003).

Distribution: Netherlands, France, England, Belgium, Sweden, Denmark, Russia, Moldavia, Azerbaijan, America and Turkey (Chant and Yoshida-Shaul 1986, Çobanoğlu 1993). Tokat, İstanbul, Ordu, Bursa, İzmir, Manisa, and Ankara in Turkey (Akyol 2019, Göven et al. 2009, Kumral 2005, Kumral and Çobanoğlu 2015, Yeşilayer 2009, Yeşilayer and Çobanoğlu 2011, Yanar and Ecevit 2005).

As can be seen from Table 5, T. recki has been found on 15 plants.

Table 5. Plants on which *Typhlodromus pyri* is found

Sample	Host
1	*Morus sp.*
2	*Punica granatum*
3	*Pyrus sp.*
4	*Prunus persica*
5	*Cydonia oblonga*
6	*Mentha piperita*
7	*Rosa sp.*
8	*Malus domestica*
9	*Platanus sp.*
10	*Ficus carica*
11	*Populus alba*
12	*Vitis vinifera*
13	*Rubus fructicocus*
14	*Juglans sp.*
15	*Amaranthus retroflexus*

Species: *Typhlodromus* (*Anthoseius*) *recki* (Wainstein, 1958)

Economic importance: *Typhlodromus recki*, is a predatory species commonly found in Turkey (Figure 4).

Figure 4. Dorsal view of *Typhlodromus recki*

Distribution: Turkey, Iran, Algeria, Tunisia, Russia, Moldova, Italy, Caucasus Region, Armenia, Lebanon, Azerbaijan, Georgia, Greece, Croatia, Israel and Kazakhstan (Kreiter et al. 2000, Moraes et al. 1986, Rahmani et al. 2010). In Turkey, Ankara, Amasya, Burdur, Bursa, Edirne, Erzurum, Gümüşhane, İçel, Isparta, İstanbul, Adapazarı, Amasya, İzmir, Kars, Kastamonu, Konya, Muğla, Nevşehir, Niğde, Tekirdağ, Tokat and Zonguldak (Akçakoyunluoğlu 2017, Bayram and Çobanoğlu 2007, Çobanoğlu 1989, 1991, 2004, Faraji et al. 2011, Kumral 2005, Madanlar 1992, Swirski and Amitai 1982, Şekeroğlu 1984).

As can be seen from Table 6, *T. recki* has been found on more than ten plants, and these plants are mostly fruit trees.

Table 6. Plants in which *Typhlodromus recki* has been detected

Sample	Host
1	*Prunus persica*
2	*Prunus armeniaca*
3	*Punica granatum*
4	*Morus sp.*
5	*Mentha pulegium*
6	*Rubus fructicocus*
7	*Ficus carica*
8	*Malus domestica*
9	*Vitis vinifera*
10	*Capsicum annuum*
11	*Amaranthus retroflexus*

Species: *Amblyseius andersoni* (Chant, 1957)

Economic importance: This species; *Phythonemus pallidus* (Banks) (Prostigmata: Tarsenomidae), is a predator that can be used to control mites such as *T. urticae* and *Aculops lycopersici* (Massee) (Prostigmata: Eriophyidae). It gives good results in a low pest population (Figure 5). This predator also feeds on small arthropods and pollen and is active at lower temperatures than other predators (Anonymous 2020). This species generally prefers Gal mites (Eriophyidae) as prey (Dicke et al. 1988).

Distribution: Italy, Poland, Serbia, England, France, Germany, Greece, Hungary, Ukraine and America. This species is mostly found in the west of Turkey. Samsun, Tokat, Erzurum, Adana, Adapazarı, Antalya, Bartın, Bolu, Bursa, Edirne, Giresun, Hatay, İstanbul, Kırklareli, Rize, Sakarya, Tekirdağ, Tokat, Trabzon, Kahramanmaraş and Çanakkale (Akçakoyunluoğlu 2017, Akyazı and Ecevit 2003, Demite et al. 2014, Döker et al. 2020, Faraji et al. 2011, İnal 2005, Kasap et al. 2013, Özşişli and Çobanoğlu 2011, Yanar and Erdoğan 2013, Yanar and Ecevit 2005).

A.andersoni has been found on 7 plants (Table 7).

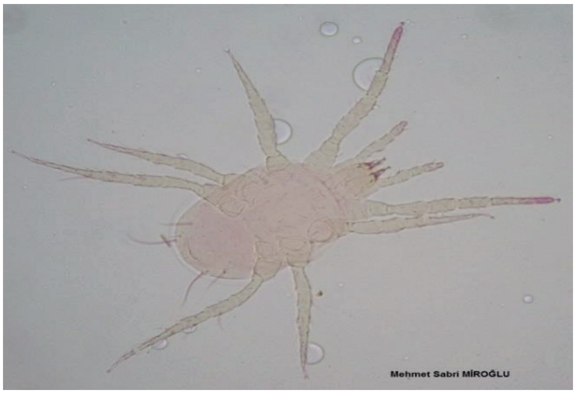

Figure 5. Dorsal view of *Amblyseius andersoni*

Table 7. Plants in which *Amblyseius andersoni* has been detected

Sample	Host
1	*Vitis vinifera*
2	*Pyrus sp.*
3	*Rubus fructicocus*
4	*Populus alba*
5	*Mentha pulegium*
6	*Fraxinus excelsior*
7	*Xanthium sp.*

Species: *Proprioseiopsis messor* (Wainstein, 1960)

Economic importance: It is an important predator, feeding on harmful mites as well as thrips (Anonymous 2020).

Distribution: Argentina, Brazil, China, Egypt, England, Finland, France, Georgia, Germany, Greece, Iran, Israel, Italy, Japan, Netherlands, Norway, Portugal, Russia, South Africa, South Korea, Spain, Sweden, Ukraine and America. Antalya, Şanlıurfa, Aydın, Samsun, İzmir, Çanakkale, Ankara and Bursa in Turkey (Çakmak et al. 2003, Çıkman et al. 1996, Çobanoğlu 1989, Çobanoğlu and Kumral 2014, Demite et al. 2014, İnal 2005, Kasap et al. 2013, Kılıç et al. 2012, Kumral and Çobanoğlu 2015).

Species: *Neoseiulus barkeri* (Hughes, 1948)

Economic importance: It is an important predator species (Figure 6). In this study, it was found on *Amaranthus retroflexus* plant.

Distribution: Argentina, Brazil, China, Egypt, England, Finland, France, Georgia, Germany, Greece, Iran, Israel, Italy, Japan, Netherlands, Norway, Portugal, Russia, South Africa, South Korea, Spain, Sweden, Ukraine and America. Antalya, Şanlıurfa, Aydın, Samsun, İzmir, Çanakkale, Ankara and Bursa in Turkey (Kumral and Çobanoğlu 2015, Çobanoğlu and Kumral 2014, Demite et al. 2014, Kasap et al. 2013, Kılıç et al. 2012, İnal 2005, Çakmak et al. 2003, Çıkman et al. 1996, Çobanoğlu 1989).

Figure 6. Adult of *Neoseiulus barkeri*

Family: Cheyletidae

Species: *Acaropsis sollers* (Kuzin, in Rohdendorf, 1940)

Economic importance: It is an important predator of economic importance (Figure 7).

Distribution: Greece, India, America, China, Russia, Turkmenistan (Eliopoulas and Papadoulis 2001, Gupta and Chatierjee 2004, Volgin 1989). İzmir and Edirne in Turkey (Çobanoğlu 1996, Madanlar and Kısmalı 1991, Özer et al. 1989).

Figure 7. Dorsal view of *Acaropsis sollers*

A. sollers has been found on 19 plants (Table 8).

Table 8.

Family: Stigmaeidae

Species: *Zetzellia mali* (Ewing, 1960)

Economic importance: It is an important agent in the biological control of pest mites and insects.

Distribution: Detected in most countries such as USA, Canada, France, Germany, Italy, England, Netherlands, Switzerland, Africa and Iran (González-Rodrigeuz 1965,

Table 8. Plants on which *Acaropsis sollers* was detected

Sample	Host
1	*Chenopodium album*
2	*Ficus carica*
3	*Beta vulgaris var. cicla*
4	*Portulaca oleracea*
5	*Solanum nigrum*
6	*Cydonia oblonga*
7	*Pyrus sp.*
8	*Juglans sp.*
9	*Capsicum annuum*
10	*Prunus armeniaca*
11	*Rubus fructicocus*
12	*Vitis vinifera*
13	*Rubus fructicocus*
14	*Malus domestica*
15	*Rosa damascena*
16	*Prunus armeniaca*
17	*Prunus persica*
18	*Platanus sp.*
19	*Pyrus communis*

Khanjani and Ueckermann 2002, Lindquist et al. 1996, Meyer 1969, Meyer and Ueckermann 1989, Yeşilayer and Çobanoğlu 2013). It is common available in Turkey. It has been recorded in both fruit and vegetables in Ankara, Bilecik, Bitlis, Samsun, Ordu and Van (Akyazı and Ecevit 2003, Akyol 2019, Çobanoğlu et al. 2003, Denizhan and Çobanoğlu 2008, 2009, Doğan 2007, Düzgüneş 1963, Kasap and Çobanoğlu 2007, Özkan et al. 1988, Sağlam and Çobanoğlu 2010, Soysal and Akyazı 2018). In this study, it was detected in apple, quince and blackberry (Table 9).

Table 9. Plants on which *Zetzellia mali* has been detected

Sample	Host
1	*Malus domestica*
2	*Rubus fructicocus*
3	*Cydonia oblonga*

Family: Raphignathidae

Species: *Raphignathus gracilis* (Rack, 1962)

Economic importance: Species of the genus *Raphignathus* are predators. They live under debris, soil, moss, lichen, storage product, house dust, bird's nest, and tree bark (Doğan and Erman 2019).

Distribution: United States, Germany, China, South Africa, Azerbaijan, Iran, Israel, Japan, Crimea, Egypt, Poland, New Zealand. Afyonkarahisar, Artvin, Denizli, Erzincan, Erzurum, Istanbul, Izmir, Kelkit Valley, and Kutahya in Turkey (Miroğlu 2020).

Family: Triophtyidae

Species: *Triophtydeus triophthalmus* (Oudemans, 1929)

Economic importance: There are different opinions about eating habits (Tempfli et al. 2015).

Distribution: Germany, Switzerland, Italy, and Hungary (Tempfli et al. 2015). It has been found in the Black Sea Region of Turkey and especially in the provinces of Ordu and Samsun.

Family: Tydeiade

Species: *Tydeus californicus* (Banks, 1904)

Economic importance: There are different opinions about eating habits.

Distribution: It is distributed all over the world. Adana, İstanbul, Çanakkale, Adana, Ankara, Bursa, Hatay, Kahramanmaraş and Mersin in Turkey (Da Silva et al. 2016, Düzgüneş 1963, İnak 2017, Kasap et al. 2014, Yeşilayer 2009).

Family: Iolinidae

Species: *Pronematus sextoni* (Baker, 1968)

Economic importance: A predator is a species.

Distribution: Moldova, Ukraine, India, Cuba, and South Africa. It was found for the first time in Turkey, in Ordu Province, on cucumber and beans. (Soysal 2017). In this study, P. sextoni, F. carica was found on July 10, 2018.

Species: *Neopronematus neglectus* (Kuznetzov, 1972)

A predator is a species. Neopronematus neglectus; Pyrus sp., R. fructicocus, and P. armeniaca found on plants. In Turkey, this species was recorded for the first time in tomatoes in Bursa (Çobanoğlu and Kumral 2014).

DISCUSSION

Mites in the Phytoseiidae family have been successfully used in biological control against many plant-feeding mites and important insects including thrips. (Kazak et al. 1989, Zhang and Rhode 2003). As in this study, a large number of species belonging to the aforementioned family have been identified in many studies conducted in Turkey (Faraji et al. 2011, İnak 2017, Öksüz 2019, Özcan 2019). *Phytoseius finitimus* (37.5%) was determined as the dominant predatory mite species in the study. This species is most commonly detected on *Rubus* sp. and *Malus* sp., and was encountered from May to November. Males of the species were found at a very low rate (2.3%). This species also is an efficient predator, has been frequently recorded in studies conducted in Turkey (Akçakoyunluoğlu 2017, Faraji et al. 2011, Göven et al. 2002, 2009, Kasap et al. 2013). This study is promising because previous research in the region has extensively detected spider mites and eriophyid mites, as well as pests such as aphids and thrips (Ayata 2015, Bolu 2002, Çıkman et al. 1996, Geçer and Denizhan 2015, Kaplan 2014, Karaca et al. 2007, Yaman et al. 2017).

Acaropsis sollers, determined from the Cheyletidae family in the study, was the second dominant predator species. This mite has been found in the products in the warehouses in most of the studies conducted at home and abroad (Çobanoğlu 1996, Madanlar and Kısmalı 1991, Özer et al. 1989). However, in this study, 17 plants, mostly fruit trees, were found from April to November. This was important in terms of showing that the predatory species in question lived in different ecologies.

Members of the Iolinidae family have been found rare in Turkey in previous studies. In this study, 3 species were identified. All 3 species were rare in Turkey and reported for the first time in the Southeastern Anatolia Region. *Pronematus sextoni* determined from this family, *Eutetranychus orientalis* (Klein) (Prostigmata: Tetranychidae) has been reported to feed on (Dhooria 1982). This species has also been reported to be a predator of *Polyphagotarsonemus latus* (Banks) (Prostigmata: Tarsonemidae) (Singh 2017).

There are different opinions about *T. californicus*; some researchers have stated that it can be a predator or feed on phytophage or pollen. Kasap et al. (2014) stated that *T. californicus* is known to be neutral however, Yeşilayer (2009) reported that it forms a leathery layer under the leaves of its host plant. The aforementioned type was detected in the study, especially in the samples in the area where the pesticide was not applied. It was recorded for the first time in the region with this study.

The Stigmaeidae family has been identified as the *Z. mali*. Species of the *Zetzellia* genus have been reported to be predators of plant pest mites such as Eriophyidae, Tetranychidae, and Tenuipalpidae. (Gerson and Smiley 1990, Koç and Madanlar 1998). In the study, this species was mostly recorded in fruit trees. For the first time, its existence was revealed in the Southeastern Anatolia Region with this study.

Although the feeding behavior of the Triophtyidae family is not known exactly, it has been reported that they feed on the eggs of some insects and spider mites (Da Silva et al. 2014, Tempfli et al. 2015). In the study, *T. triophthalmus* determined from this family was reported especially in the Black Sea Region, its presence was detected for the first time in the Southeastern Anatolia Region with this study. There is not enough information about nutrition habits (Tempfli et al. 2015).

According to studies on the Raphignathidae family, most of them are predators. (Fan and Zhang 2005). Raphognathis gracilis, which was determined from this family in the study, was recorded in our country for the first time in Istanbul (Yeşilayer 2009). It was determined for the first time in the Southeastern Anatolia Region by this study.

Although most of the mites are beneficial, more harmful species were determined in the studies conducted in the Southeastern Anatolia Region (Ayata 2015, Bolu 2002, Geçer and Denizhan 2015, Kaplan 2014, 2020, Taşçıoğlu et al. 1969, Yaman et al. 2017). In this study, beneficial species were found. The determined species were mostly detected on fruit trees, and the highest number of species was recorded on *R. fructicocus* with seven species. This can be attributed to the fact that there is no economical fruit growing in the area. Therefore very little application of pesticides. Few species and individuals were found in vegetables and fragrant plants.

The rich fauna of Hevsel Gardens should be protected, for this, the farmers here should be informed and educated about beneficial mites. Inspections in the gardens should be carried out periodically, and the entry of environmental pollutants into the gardens should be prevented. As a result, this study formed the basis for the beneficial mite fauna of the region, common and rare beneficial species were found and revealed that Hevsel Gardens is rich in beneficial mites.

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ÖZET

Bu çalışma, 2018-2019 yıllarında Diyarbakır ili Hevsel Bahçeleri'nde bulunan faydalı akar türlerinin belirlenmesi amacıyla yapılmıştır. Binlerce yıldır kesintisiz olarak ekilen bu tarihi bahçeler, UNESCO Dünya Mirası Listesi'nde yer almaktadır. Sürvey çalışmaları tesadüfi örnekleme metodu ile nisan ayından kasım ayına kadar her 15 günde bir periyodik olarak yapılmıştır. Çalışmada meyve ağaçları, sebzeler ve yabancı otlardan oluşan 66 bitki türünden örnekler alınmıştır. Çalışma sonucunda 33 bitki türü üzerinde Tydeidae, Cheyletidae, Stigmaeidae, Triophtyidae, Raphignathidae, Iolinidae ve Phytoseiidae familyalarından 15 predatör akar türü tespit edilmiştir. Bu familyalardan

Phytoseiidae familyasından 7 tür, Iolinidae familyasından 3 tür ve diğer familyalardan bir tür tespit edilmiştir. *Phytoseius finitimus* (Ribaga, 1904) (Mesostigmata: Phytoseiidae) %37.5 oran ile baskın tür olmuştur. En fazla akar türü 7 tür ile *Rubus* sp.(böğürtlen)'de bulunmuştur. Araştırmada, tarımsal faaliyetin az yapıldığı bölgelerde daha fazla sayıda faydalı akar bulunmuştur. Predatör akarlar, daha çok meyve ağaçlarında bulunmuş bunun yanında sebze ve kokulu bitkilerde daha az oranda bulunmuştur. Hevsel Bahçeleri'nde kavak ve dut ağaçları yüksek bir popülasyona sahiptir. Bu iki bitkiden çok sayıda örnekleme yapılmasına rağmen sadece *P. finitimus* saptanmıştır. İki yıllık bu araştırma ile Dünya için önemli olan Hevsel Bahçeleri'nin faydalı akar yönüyle zengin olduğu ortaya çıkartılmıştır. Zararlı akar ve böceklere karşı biyolojik mücadelede dünyada kullanılan önemli avcı türler tespit edilmiştir. Çalışma ile bölge için yeni kayıt oluşturan faydalı türler belirlenmiştir.

Anahtar kelimeler: Diyarbakır, predatör, Phytoseiidae, *Phytoseius finitimus*

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