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# Bitki Koruma Bülteni / Plant Protection Bulletin

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Original article

## Scale insect (Hemiptera: Sternorrhyncha: Coccoomorpha) species on medicinal and aromatic plants in Adana (Turkey)

Adana (Türkiye)'da tıbbi ve aromatik bitkilerdeki Kabuklubit (Hemiptera: Sternorrhyncha: Coccoomorpha) türleri

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### ABSTRACT

Medicinal and aromatic plants are used primarily in the medicine, food and cosmetic industries. Scale insect species belonging to Coccoomorpha (Hemiptera: Sternorrhyncha) feed on medicinal and aromatic plants, reducing the yield and commercial value of these crops. This study aimed to determine the scale insect species on these plants in Adana Province between 2015 and 2017. Scale insect samples were collected from leaves, trunks, branches and fruits of 28 medicinal and aromatic plant species examined and identified in the laboratory. In the study, a total of 16 species were identified from the families of Coccidae (5 species), Diaspididae (7 species), Monophlebidae (1 species) and Pseudococcidae (3 species) belonging to this infraorder.

### INTRODUCTION

Turkey is one of the leading countries in the production of medicinal and aromatic plants (MAP) because of its unique geographic location, climate and soil characteristics, agricultural potential, large surface area, having rich plant diversity. Medicinal and aromatic plants are being used in alternative or complementary medicinal science, which has become more widespread in recent years worldwide. Besides food and medicine industries, MAPs are used in many fields, such as the beverage, perfume and cosmetic industries (Baydar 2016, Yücer and Altıntaş 2012). In Turkey, about 20 species of MAPs are cultivated on an area of 1.3 million decares. Among these plants, poppy, cumin, anise, thyme and rose (oil) are cultivated the most. Turkey is one of the leading countries in thyme production and ranks first in world thyme

exports. In addition, approximately 90% of the world's dry bay leaf production and 50% of the world's rose oil demand comes from Turkey. Turkey is an important supplier for MAPs such as thyme and laurel, as well as capers and cumin. It is reported that medicinal and aromatic plant exports amounted to 280 million dollars in 2015 (Öztürk et al. 2014, Temel et al. 2018). One of the wide usage areas of MAPs is to be used as organic plant protection products in the control of pest insect and mite species (Aydın and Mammadov 2017, Bakkali et al. 2008, Isman 2006, Isman et al. 2008, Kim et al. 2015, Lengai et al. 2020, Topuz and Madanlar 2006, Ujvary 1999). In recent years, natural products of botanical origin instead of synthetic ones have been increased worldwide and in Turkey (Arnason et al. 2012, Karaca et al. 2017, Misra 2014). Due to the enhancement

in the consumption of natural products, the world market volume of MAPs shows a rapid increase. With the increase in demand for these plants, which were previously collected from nature, studies on the production of MAPs have also been accelerated (Şekeroğlu and Gezici 2020). In order to evaluate sustainable production and market potential as competently, medicinal and aromatic plants must be of the required quantity and quality. One of the important issues affecting yield and quality in MAPs are the pests feeding on these plants (Abro et al. 2016, Marimuthu et al. 2018, Simova-Tosia et al. 1997). Sarma et al. (2008), reported that there are 40-45 main pest species in MAPs and they may cause 50-60% damage. Pests reduce the yield as well as the commercial value of crops, causing damages, such as stubbornness, shortening between nodes, colour change in flower and body, decrease in number and diameter of flowers, loss of absorption, leaf curling, leaf deformation and spillage in MAPs. They reduce the medicinal value of these plants by impairing their quality, and also have negative effects on secondary metabolites in plant parts used as drugs (Abo-Zaed Amal et al. 2019, Milek and Simala 2010, Taşkın 2015, Verma 2006). A group of pest species in MAPs is species related to scale insects (Hemiptera: Sternorrhyncha: Coccoomorpha). Scale insects are important pest groups that settle on the trunk, branch, leaf, fruit, flower and even roots and feed by sucking the plant sap. As a result, effecting in plant development, early leaf fall, fumagine formation, deterioration in fruit quality, shape and colour disorders are observed. High pest populations can cause the host plant to die back completely (Kaydan et al. 2007, Kosztarab 1996, Yaşar 1990). Some species can transmit plant virus diseases (Cabaleiro and Segura 2006). Species belonging to this infraorder have a wide number of hosts. They can cause significant damages and economic losses especially in fruit, vineyard and forest trees, park and ornamental plants (Kozstarab and Kozár 1988). Worldwide, the presence of 8.194 species belonging to 50 families were reported, while Diaspididae, Pseudococcidae and Coccidae are the three largest families of Coccoomorpha (Garcia Morales et al. 2016). In Turkey, 359 species belonging to 18 families has been identified (Kaydan et al. 2013a). It is reported that species of Coccoomorpha cause damage by feeding on the reproductive and vegetative organs of medicinal and aromatic plants and symptoms of sooty mold (Abo-Zaed Amal et al. 2019, Conti 2003, Kondo et al. 2018). Upto now, scale insect species on many plant species in different geographic areas of Turkey were investigated (Çalışkan Keçe and Ulusoy 2017, Çalışkan et al. 2017, Develioğlu et al. 2018, Karsavuran et al. 2001, Karsavuran et al. 2004, Kaydan et al. 2005, Kaydan et al. 2013b, Kaymak and Yaşar 2017, Ülgentürk and Toros 1996, 1999, Ülgentürk et al. 2008, Yaşar 1990, Yaşar and Küçükçakal 2013). It is known that there are limited number of studies to reveal the scale insect fauna in medicinal and aromatic plants, whose

importance, use and production is increasing in Turkey. The previously recorded pest species are the ones from the general faunistic studies. The present study was undertaken to record the scale insect species associated with various medicinal and aromatic plants in Adana province.

## MATERIALS AND METHODS

### Study sites

The studies were conducted in Karaisalı and Sarıçam districts located at Adana province, Turkey. Species were collected from MAPs by non-periodical surveys in 2015 to 2017. Infected plant materials were gathered from Ali Nihat Gökyiğit Botanical Garden in Sarıçam (37° 3'2.53"N; 35° 21'15.49"E, 109 m above sea level) and experimental field of Çukurova University, Karaisalı Vocational School (37° 15'12.96"N; 35° 47.28"E, 235 m above sea level) and around the district.

### Sampling and laboratory processing of specimens

In total 208 samples were collected from plants by visual check of which 178 species from Sarıçam and 30 species from Karaisalı. Each infected sample was put into a plastic bag, labeled and taken to the laboratory. Scale insect samples on plants were put into Eppendorf tubes with 70% alcohol for further examination. Samples were prepared under the light microscope by using the slide-mounting method described by Kosztarab and Kozár (1988). The collection data (host plants, host locality, collection date) are recorded. Previously recorded distribution and host-plant data were taken from Kaydan et al. (2013a) and Garcia Morales et al. (2016). All the species were identified by the second author.

## RESULTS AND DISCUSSION

Totally 16 Coccoomorpha species belonging to Coccidae (5), Diaspididae (7), Monophlebidae (1) and Pseudococcidae (3) families were identified. These species were; *Anapulvinaria pistaciae* (Bodenheimer), *Ceroplastes floridensis* Comstock, *C. rusci* (Linnaeus), *Coccus hesperidum* Linnaeus, *Parthenolecanium corni* (Bouché) (Coccidae), *Aonidiella aurantii* (Maskell), *Aonidia lauri* (Bouché), *Aspidiotus nerii* (Bouché), *Epidiaspis leperii* (Signoret), *Lepidosaphes pistaciae* Archangelskaya, *Melonaspis inopinata* (Leonardi), *Parlatoria oleae* (Colvée) (Diaspididae), *Icerya purchasi* Maskell (Monophlebidae), *Phenacoccus madeirensis* Green, *P. solenopsis* Tinsley, *Planococcus citri* (Risso) (Pseudococcidae). Locality, collection date and host plants for the determined species are given below.

Family: Coccidae

*Anapulvinaria pistaciae* (Bodenheimer, 1926)

Material examined: 2 ♀♀, Karaisalı, ex *Pistacia lentiscus* L. (Anacardiaceae), 20.XII.2015, Coll: N.Z. Elekcioglu.

*Anapulvinaria pistaciae* has Palaearctic distribution (García Morales et al. 2016). It was recorded in Ankara, Isparta, Gaziantep and Urfa in Turkey on *P. vera* L. and *Pistacia* sp. (Bodenheimer 1953, Yanık and Yücel 2001, Ülgentürk and Çanakçıoğlu 2004, Kaydan et al. 2013a).

This species is mainly found on seven plants belonging to Anacardiaceae (especially *Pistacia* spp.) in Turkey (Kaydan et al. 2013a) and on some plants from Juglandaceae and Tamaricaceae families in Iran (Moghaddam 2013).

*Ceroplastes floridensis* Comstock, 1881

Material examined: 2 ♀♀, Sariçam, ex *Artemisia annua* (L.) Cass. Spach (Asteraceae) 11.IX.2017, Coll: N.Z. Elekcioglu; 2 ♀♀, Sariçam, ex *A. dracuncululus* L., 18.IX.2017; Coll: N.Z. Elekcioglu; 2 ♀♀, Sariçam, *Laurus nobilis* L. (Lauraceae), 10.VIII.2017, Coll: N.Z. Elekcioglu; 2 ♀♀, Sariçam, *Myrtus communis* L. (Myrtaceae), 04.IX.2017, Coll: N.Z. Elekcioglu; 2 ♀♀, Sariçam, *Koelreuteria paniculata* Laxm. (Sapindaceae), 11.IX.2017; 2 ♀♀, Sariçam, ex *Rhus coriaria* L. (Anacardiaceae), 18.IX.2017, Coll: N.Z. Elekcioglu; 2 ♀♀, Sariçam, ex *Senna x floribunda* (Cav.) H.S. Irwin & Barneby (Fabaceae), 18.IX.2017, Coll: N.Z. Elekcioglu.

This species has worldwide distribution (it is recorded in 102 countries) and regarded as cosmopolit species (CABI 2020). *C. floridensis* has been found on 257 plant species belonging to 70 families (García Morales et al. 2016). In Turkey it was found in Mediterranean, Southeast Anatolian and Aegean Regions on *Cedrus libani* A. Rich. (Pinaceae), *Arbutus unedo* L. (Ericaceae), *L. nobilis* and *M. communis* (Kaydan et al. 2013a, Ülgentürk et al. 2013).

*Comment.* This species was determined on *Nerium oleander* L. (Apocynaceae), *Cinnamomum* spp. (Lauraceae), *L. nobilis*, *L. azorica* (Seub.) Franco, *Coffea* spp. (Rubiaceae), *Aloysia citrodora* Paláu (Verbenaceae), *Citrus* spp. (Rutaceae) and *Camellia sinensis* (L.) Kuntze (Theaceae) as MAPs (García Morales et al. 2016, Kaydan et al. 2013a).

*Ceroplastes rusci* (Linnaeus, 1758)

Material examined: 2 ♀♀ Karaisalı, ex *Myrtus communis* L. (Myrtaceae); 27.VII.2015, Coll: N.Z. Elekcioglu; 2 ♀♀, Karaisalı, ex *Nerium oleander* L. (Apocynaceae), 20.XII.2015, Coll: N.Z. Elekcioglu; 2 ♀♀, Karaisalı, ex *N. oleander*, 23.V.2017, Coll: N.Z. Elekcioglu; 2 ♀♀ and *Pistacia lentiscus* L. (Anacardiaceae), 22.VII.2015, Coll: N.Z. Elekcioglu.

It is known that *C. rusci* is a cosmopolit species and recorded in 59 countries all over the world (Anonymous 2020a). *C. rusci* has been recorded 131 plant species belonging to 48 different families. Akşit et al. (2003), Kaydan et al. (2013a) and Ülgentürk et al. (2013) recorded *C. rusci* on

*M. communis*, *N. oleander*, *P. palaestina* Boiss, *P. vera* L., *Populus* sp. (Salicaceae), *Salix* sp. (Salicaceae), *Olea europea* L. (Oleaceae) and *Ficus carica* L. (Moraceae).

*Rhus coriaria* L. (Anacardiaceae), *N. oleander*, *Ruscus aculeatus* L. (Nolinaceae), *Artemisia monosperma* L. (Asteraceae), *Laurus nobilis* L. (Lauraceae), *M. communis*, *Piper nigrum* L. (Piperaceae) and *Citrus* spp. (Rutaceae) were recorded as host plants of *C. rusci* in terms of MAPs (Kaydan et al. 2013a, Ülgentürk et al. 2013).

*Coccus hesperidum* Linnaeus, 1758

Material examined: 2 ♀♀, Sariçam, ex *Hyssopus officinalis* L. (Lamiaceae), 18.IX.2017, Coll: N.Z. Elekcioglu.

This species is considered as cosmopolit species which was determined in 145 countries (CABI 2020). *C. hesperidum* has been recorded in Mediterranean, Blacksea, Middle Anatolia Regions in Turkey (Kaydan et al. 2013a). It has been recorded on 130 plant species of which *Nerium oleander* L. (Apocynaceae), *Plumeria* spp. (Apocynaceae), *Ruscus hypophyllum* L. (Asparagaceae), *Artemisia vulgaris* L. (Asteraceae), *Rhododendron* sp. (Ericaceae), *Ricinus communis* L. (Euphorbiaceae), *Pelargonium* sp. (Geraniaceae), *Ocimum basilicum* L. (Lamiaceae), *Rosmarinus officinalis* L. (Lamiaceae), *Teucrium* sp. (Lamiaceae), *Thymus* sp. (Lamiaceae), *Vitex agnus-castus* L. (Lamiaceae), *Cinnamomum camphora* (L.) J. Presl. (Lauraceae), *Laurus nobilis* L. (Lauraceae), *Alcea rosea* L. (Malvaceae), *Hibiscus* spp. (Malvaceae), *Myrtus communis* L. (Myrtaceae), *Portulaca oleracea* L. (Portulacaceae), *Rosa* sp. (Rosaceae), *Rubus* spp. (Rosaceae) and *Coffea* spp. (Rubiaceae) are MAPs all over the world (García Morales et al. 2016). It was recorded on *Cedrus libani* A. Rich. (Pinaceae), *Acer pseudoplatanus* L. (Sapindaceae), *Crateagus monogyna* Jacq. (Rosaceae), *L. nobilis*, *Lonicera caprifolium* L. (Caprifoliaceae), *N. oleander*, *Pistacia atlantica* Desf. (Anacardiaceae), *Quercus* spp. (Fagaceae) and *Pinus* sp. (Pinaceae) (Alkan 1957, Ülgentürk and Toros 1999, Uygun et al. 2001, Kaydan et al. 2013a, Ülgentürk et al. 2013).

*Parthenolecanium corni* (Bouché, 1844)

Material examined: 2 ♀♀, Sariçam, ex *Lycium barbarum* L. (Solanaceae), 18.IX.2017, Coll: N.Z. Elekcioglu.

*Parthenolecanium corni* is recorded in 73 countries in the world and considered as cosmopolit species (CABI 2020). It was found on 225 plant species belonging to 48 families (García Morales et al. 2016). *P. corni* is known in East Anatolian, Aegean, Black Sea, Mediterranean and Middle Anatolia Regions in Turkey (Kaydan et al. 2013a). In Turkey it was found on *Morus alba* L. (Moraceae), *Robinia pseudoacacia* L. (Fabaceae), *Vitis vinifera* L. (Vitaceae), *Prunus armeniaca* L., *P. persicae* L. (Rosaceae), *Corylus*



*avellana* L. (Betulaceae), *Elaeagnus* sp. (Elaeagnaceae), *Fagus* sp. (Fagaceae), *Quercus* sp. (Fagaceae), *Fraxinus* sp. (Oleaceae), *Salix* sp. (Salicaceae), *Ulmus* sp. (Ulmaceae) and *Crateagus* sp. (Rosaceae) (Ülgentürk and Toros 1999, Kaydan et al. 2013a, Kaydan et al. 2014). Among the host plant species *Rosmarinus officinalis* L. (Lamiaceae), *Mentha* sp. (Lamiaceae), *Thymus* sp. (Lamiaceae), *Pelargonium* sp. (Geraniaceae), *Tilia* spp. (Malvaceae) and *Urtica* spp. (Urticaceae) are known as MAPs (Kaydan et al. 2013a).

Family: Diaspididae

*Aonidiella aurantii* (Maskell, 1879)

Material examined: 2 ♀♀, Sariçam, ex *Ceratonia siliqua* L. (Fabaceae), 04.IX.2017, Coll: N.Z. Elekcioglu; 2 ♀♀, Karaisalı, ex *Rosa* sp. (Rosaceae), 22.XII.2017, Coll: N.Z. Elekcioglu.

*Aonidiella aurantii* is one of the most important citrus pest all over the world. It is a cosmopolit species and is present in 89 countries on 180 species from 86 families mainly Arecaceae, Asparagaceae, Fabaceae, Moraceae, Oleaceae, Rosaceae, Rutaceae (García Morales et al. 2016), and it has been found in Mediterranean and Aegean Region of Turkey (Kaydan et al. 2013a). In Turkey it was recorded on citrus, acacia, rosa, *Amaranthus viridis* L. (Amaranthaceae), *C. siliqua* and *Laurus nobilis* L. (Lauraceae) (Kaydan et al. 2013a, Kaydan et al. 2014). It was recorded on MAPs as; *Nerium oleander* L. (Apocynaceae), *Ruscus hypophyllum* L. (Asparagaceae), *Calendula officinalis* L. (Asteraceae), *Berberis* sp., (Berberidaceae), *Rhododendron* sp. (Ericaceae), *C. siliqua*, *Salvia* sp. (Lamiaceae), *L. nobilis*, *Hibiscus* sp. (Malvaceae), *Myrtus* sp. (Myrtaceae), *Rosa* sp., *Aloe vera* (L.) Burm.f. (Asphodelaceae) and *Citrus* spp. (Rutaceae) (Kaydan et al. 2013a, García Morales et al. 2016).

*Aonidia lauri* (Bouche, 1833)

Material examined: 2 ♀♀, Sariçam, ex *Laurus nobilis* L. (Lauraceae), 21.VII.2017, Coll: N.Z. Elekcioglu.

This species is a Palaearctic species but found in 2 countries in Nearctic Region although in Palearctic Region it is recorded on generally Mediterranean subregion (García Morales et al. 2016). *A. lauri* is recorded on *L. nobilis* in Mediterranean, Aegean, South East Anatolian and Marmara Region in Turkey (Kaydan et al. 2013a).

*Aspidiotus nerii* Bouche, 1833

Material examined: 2 ♀♀, Sariçam, ex *Nerium oleander* L. (Apocynaceae), 19.VII.2017, Coll: N.Z. Elekcioglu; 2 ♀♀, Sariçam, ex *Ceratonia siliqua* L. (Fabaceae), 22.VII.2017; Coll: N.Z. Elekcioglu; 2 ♀♀, Karaisalı, ex *Rhododendron* sp. (Ericaceae), 10.III.2016, Coll: N.Z. Elekcioglu; 2 ♀♀, Karaisalı, ex *C. siliqua*, 27.VII.2017, Coll: N.Z. Elekcioglu.

*Aspidiotus nerii* is a cosmopolit species which has been found in 76 countries all over the world (García Morales et al. 2016). It's host plants has a quite wide range and can be on Anacardiaceae, Apocynaceae, Araliaceae, Arecaceae, Asparagaceae, Ericaceae, Euphorbiaceae, Fabaceae, Oleaceae, Proteaceae and Rutaceae (García Morales et al. 2016). In Turkey it was found on *Acacia cultiformis* A. Cunn. ex G. Don, *A. cyanophylla* Mill. (Fabaceae), *Aucuba japonica* Thunb. (Garryaceae), *Asparagus acutiformis* L. (Asparagaceae), *Campsis radicans* Seem. (Bignoniaceae), *Canna indica* L. (Cannaceae), *Cedrus libani* A. Rich. (Pinaceae), *Citrus limon* (L.) Osbeck (Rutaceae), *Cycas revoluta* Thunb. (Cycadaceae), *Hedera helix* L. (Araliaceae), *Jasminum* sp. (Oleaceae), *Laurus nobilis* L. (Lauraceae) (Kaydan et al. 2013a) of which some of them MAPs such as *Pistacia lentiscus* L., *P. terebinthus* L. (Anacardiaceae), *Foeniculum vulgare* Mill. (Apiaceae), *N. oleander*, *Ruscus aculeatus* L. (Nolinaceae), *Senecio* sp. (Asteraceae), *Berberis* sp. (Berberidaceae), *Capparis* spp. (Capparidaceae), *Cistus* spp. (Cistaceae), *Rhododendron ponticum* L. (Ericaceae), *Ricinus communis* L. (Euphorbiaceae), *Salvia* sp. (Lamiaceae), *Sideritis* sp. (Lamiaceae), *Teucrium* spp. (Lamiaceae), *L. nobilis* and *Myrtus communis* L. (Myrtaceae) (García Morales et al. 2016).

*Epidiaspis leperii* (Signoret, 1869)

Material examined: 2 ♀♀, Sariçam, ex *Pistacia terebinthus* L. (Anacardiaceae), 24.VII.2017, Coll: N.Z. Elekcioglu; 2 ♀♀, Karaisalı, 22.VII.2015, *P. lentiscus* L., Coll: N.Z. Elekcioglu.

Although this species has been regarded as Palaearctic species it was found in 42 countries worldwide. *E. leperii* is a polyphagus species which found on the plants belonging to Rosaceae Oleaceae, Juglandaceae, Moraceae, Sapindaceae and Lauraceae (CABI 2020). It has been recorded at all regions of Turkey (Kaydan et al. 2013a), found on *Aesculus hippocastanum* L. (Sapindaceae), *Cactus* spp. (Cactaceae), *Opuntia ficus-indica* (L.) Mill. (Cactaceae), *Pistacia* sp., *Prunus domestica* L. (Rosaceae), *Prunus* sp., *Pyrus communis* L. (Rosaceae), *Malus* sp. (Rosaceae) and *Robinia pseudoacacia* L. (Fabaceae) (Erözmen and Yaşar 2018, Kaydan et al. 2013a).

*Lepidosaphes pistaciae* Archangelskaya, 1930

Material examined: 2 ♀♀, ex *Pistacia terebinthus* L. (Anacardiaceae); Karaisalı, 22.VII.2015, Coll: N.Z. Elekcioglu.

This species is almost a monofag species and found on *Pistacia* spp. which found in Palaearctic and Oriental Zoogeographic Regions (Kaydan et al. 2013a, Kozár 1998). In Turkey it is recorded on *P. terebinthus*, *P. lentiscus* L. and *P. vera* L. as MAPs (Kaydan et al. 2013a).

*Melonaspis inopinata* (Leonardi, 1913)

Material examined: 2 ♀♀, Karaisalı, *Pistacia terebinthus* L. (Anacardiaceae), 22.VII.2015. Coll: N.Z. Elekcioglu.

This Palaearctic species has been recorded in Palaearctic Region including Turkey (García Morales et al. 2016). It is recorded on *P. khinjuk* Stocks, *P. mutica* F. & M., *Acer cinerascens* Boiss. (Aceraceae), *Anabasis* sp. (Brassicaceae), *Celtis* sp. (Cannabaceae), *Arbutus unedo* L. (Ericaceae), *Ricinus communis* L. (Euphorbiaceae), *Bauhinia* sp. (Fabaceae), *Cercis siliquastrum* L. (Fabaceae), *Astragalus* sp. (Fabaceae), *Quercus* sp. (Fagaceae), *Juglans regia* L. (Juglandaceae), *Fraxinus excelsior* L. (Oleaceae), *Acacia* sp. (Fabaceae), *Malus communis* Lam., *M. domestica* Borkh. (Rosaceae), *Prunus communis* L., *P. avium* L., *Prunus* sp. (Rosaceae) and *Salix* sp. (Salicaceae) in Turkey (Anonymous 2020b). It is recorded on *Astragalus* sp. as MAPs in Turkey (Kaydan et al. 2013a).

Family: Monophlebidae

*Icerya purchasi* Maskell, 1879

Material examined: 2 ♀♀, Karaisalı, ex *Rosmarinus officinalis* L. (Lamiaceae), 19.IX.2017, Coll: N.Z. Elekcioglu; 2 ♀♀ Karaisalı, *Origanum onites* L. (Lamiaceae), 19.IX.2017, Coll: N.Z. Elekcioglu; 2 ♀♀ Karaisalı, *O. majorana* L., 12.IX.2017, Coll: N.Z. Elekcioglu; 2 ♀♀, Karaisalı, *O. majorana*, 22.XII.2017, Coll: N.Z. Elekcioglu; 2 ♀♀ Karaisalı, *Rosa* sp. (Rosaceae), 22.XII.2017, Coll: N.Z. Elekcioglu.

*Icerya purchasi* is a cosmopolit species, recorded in 148 countries and it was found in Mediterranean, Aegean, Black Sea and Marmara Regions in Turkey (Kaydan et al. 2013a). It has been regarded as an important citrus pest but also found on many MAPs such as *R. officinalis*, *Foeniculum vulgare* Mill. (Apiaceae), *Nerium oleander* L. (Apocynaceae), *Ricinus communis* L. (Euphorbiaceae), *Ocimum basilicum* L. (Lamiaceae), *Laurus nobilis* L. (Lauraceae), *Alcea rosea* L. (Malvaceae), *Passiflora quadrangularis* L. (Passifloraceae), *Portulaca oleracea* L. (Portulacaceae), *Rosa centifolia* L. (Rosaceae), *Lantana camara* L. (Verbenaceae), *Berberis* sp. (Berberidaceae), *Hibiscus* sp. (Malvaceae), *Salvia* sp. (Lamiaceae), *Senna* spp. (Fabaceae) and *Citrus* spp. (Rutaceae) (Kaydan et al. 2013a, CABI 2020).

Family: Pseudococcidae

*Phenacoccus madeirensis* Green, 1923

Material examined: 2 ♀♀, Sarıçam, ex *Stevia rebaudiana* (Bertoni) Bertoni (Asteraceae), 03.VII.2017, Coll: N.Z. Elekcioglu; 2 ♀♀, Sarıçam, ex *S. rebaudiana*, 16.VII.2017, Coll: N.Z. Elekcioglu; 2 ♀♀, ex *Pelargonium crispum* (P.J.Bergius) L'Her (Geraniaceae), 03.VII.2017, Coll: N.Z. Elekcioglu.

The Madeira mealybug, *P. madeirensis*, is an important pest which has invading potential through new areas worldwide. It is a polyphagous insect species which has been recorded from 135 genera in 51 families. It is a pest of many fruit trees, fibre plants, food crops, vegetables, cacao and ornamental

plants (Wang et al. 2019). It has a Neotropical origin, although it was first described on the island of Madeira in 1923 (Green 1923). It has spread to 88 countries and regions across five continents (CABI 2020, Garcia Morales et al. 2016). It has been recorded in Turkey first time in 2012 in Çanakkale, Adana and Antalya (Kaydan et al. 2012). As MAPs host plant it is recorded on *Petroselinum crispum* Hill (Apiaceae), *Matricaria chamomilla* L. (Asteraceae), *Ricinus communis* L. (Euphorbiaceae), *Ocimum basilicum* L. (Lamiaceae), *Salvia coccinea* Buc'hoz ex Etl. (Lamiaceae), *Passiflora edulis* Sims. (Passifloraceae), *Capsicum annuum* L. (Solanaceae), *Aloysia citrodora* Paláu (Verbenaceae), *Lantana camara* L. (Verbenaceae), *Pelargonium* spp., *Hibiscus* spp. (Malvaceae), *Mentha* sp. (Lamiaceae), *Datura* sp. (Solanaceae), *Rumex* sp. (Polygonaceae), *Althaea* sp. (Malvaceae), *Rosa* sp. (Rosaceae), *Citrus* sp. (Rutaceae) and *Calendula* sp. (Asteraceae) (García Morales et al. 2016).

*Phenacoccus solenopsis* Tinsley, 1898

Material examined: 2 ♀♀, Sarıçam, ex *Schinus molle* L. (Anacardiaceae), 08.XI.2015, Coll: N.Z. Elekcioglu; 2 ♀♀, Sarıçam, ex *S. molle*, 15.V.2017, Coll: N.Z. Elekcioglu; 2 ♀♀, ex *Brassica rapa* subsp. *nipposinica* L. (Brassicaceae), Coll: N.Z. Elekcioglu; 2 ♀♀, Karaisalı, ex *Conyza* sp. (Asteraceae), 10.X.2015, Coll: N.Z. Elekcioglu; 2 ♀♀, Karaisalı, ex *Conyza* sp., 21.X.2016, Coll: N.Z. Elekcioglu; 2 ♀♀, Karaisalı, ex *Capsicum* sp. (Solanaceae), 14.IX.2017, Coll: N.Z. Elekcioglu.

The solenopsis mealybug is regarded as an important plant pest worldwide and recorded in 52 countries (CABI 2020, Hodgson et al. 2008). This mealybug may reach high populations and cause reduced plant growth or plant death and has the potential to inflict significant damage to field crops in all growing regions (Fand and Suroshe 2015). Although this species was found only on ornamental plants in Turkey it may have wider distribution in the open field and could become an important pest in all areas. It has been recorded on MAPs all over the world as *Nerium oleander* L. (Apocynaceae), *Plumeria rubra* L. (Apocynaceae), *Achillea* sp. (Asteraceae), *Calendula officinalis* L. (Asteraceae), *Tagetes erecta* L., *T. patula* L. (Asteraceae), *Capparis decidua* (Forssk.) Edgew. (Capparaceae), *Capsicum annuum* L., *Momordica charantia* L. (Cucurbitaceae), *Ricinus communis* L. (Euphorbiaceae), *Pelargonium* sp. (Geraniaceae), *Mentha spicata* L. (Lamiaceae), *Ocimum basilicum* L. (Lamiaceae), *Salvia officinalis* L. (Lamiaceae), *Thymus vulgaris* L. (Lamiaceae), *Vitex agnus-castus* L. (Lamiaceae), *Alcea rosea* L. (Malvaceae), *Althaea* sp. (Malvaceae), *Hibiscus* spp. (Malvaceae), *Passiflora edulis* Sims. (Passifloraceae), *Portulaca oleracea* L. (Portulacaceae), *Citrus* spp. (Rutaceae), *Nicotiana tabacum* L. (Solanaceae), *Physalis alkekengi* L. (Solanaceae), *Lantana camara* L. (Verbenaceae) and *Elettaria cardamomum* (L.) Maton (Zingiberaceae) (García Morales et al. 2016).

*Planococcus citri* (Risso, 1813)

Material examined: 2 ♀♀, Sariçam, ex *Ceratonia siliqua* L. (Fabaceae), 17.VII.2017, Coll: N.Z. Elekcioglu; 2 ♀♀, Sariçam, ex *C. siliqua*, 21.VII.2017, Coll: N.Z. Elekcioglu; 2 ♀♀, Karaisalı, ex *C. siliqua*, 21.VII.2017, Coll: N.Z. Elekcioglu.

This polyphagous and cosmopolit species has been recorded all over the world and many different MAP species as *Rhus* sp. (Anacardiaceae), *Nerium oleander* L. (Apocynaceae), *Ruscus aculeatus* L. (Nolinaceae), *Artemisa dracunculul* L. (Asteraceae), *Senecio* sp. (Asteraceae), *Tagetes* sp. (Asteraceae), *Ricinus communis* L. (Euphorbiaceae), *C. siliqua*, *Mentha spicata* L. (Lamiaceae), *Ocimum basilicum* L. (Lamiaceae), *Rosmarinus officinalis* L. (Lamiaceae), *Laurus nobilis* L. (Lauraceae), *Hibiscus* sp. (Malvaceae), *Myrtus communis* L. (Myrtaceae), *Passiflora edulis* Sims. (Passifloraceae), *Portulaca oleracea* L. (Portulacaceae), *Nicotiana tabacum* L. (Solanaceae), *Coffea* spp. (Rubiaceae) and *Citrus* spp. (Rutaceae) (Bodenheimer 1953, CABI 2020, Kaydan et al. 2013a).

The present study shows that the regional faunal studies of scale insects are important to better understand their relationship with host plants. Medical and aromatic plant production has been increasing in Turkey in recent years and scale insects are one of the important agents affecting yield and quality. As a result, it is thought that more detailed studies on scale insects and other pest species at the regional and national level in the future regarding plant preferences will guide both the determination of agricultural control strategies and other studies to be carried out in Turkey.

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#### ÖZET

Tıbbi ve aromatik bitkiler öncelikle ilaç, yiyecek ve kozmetik endüstrisinde kullanılmaktadır. Kabuklubit (Hemiptera: Coccoomorpha: Sternorrhyncha) türleri tıbbi ve aromatik bitkilerle beslenerek, bu ürünlerin verim ve ticari değerini düşürmektedirler. Bu çalışma Adana ilinde tıbbi ve aromatik bitkilerdeki kabuklubit türlerini belirlemek amacıyla 2015-2017 yılları arasında yürütülmüştür. Kabuklubit örnekleri 28 tıbbi ve aromatik bitki türünün yaprak, gövde, dal ve meyvelerinden toplanmış, laboratuvarında incelenmiş ve tanımlanmıştır. Çalışmada, bu infratakıma bağlı Coccidae (5 tür), Diaspididae (7 tür), Monophlebidae (1 tür) ve Pseudococcidae (3 tür) familyalarından toplam 16 tür saptanmıştır.

Anahtar kelimeler: Coccidae, Diaspididae, Monophlebidae, Pseudococcidae, tıbbi bitki, aromatik bitki

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Original article

## Population structure and gene-flow among *Tetranychus urticae* populations collected from different geographic regions of Turkey

Türkiye'nin farklı coğrafi bölgelerinden toplanan *Tetranychus urticae* popülasyonlarındaki gen akışı ve popülasyon yapıları

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### ABSTRACT

*Tetranychus urticae* Koch (Acari: Tetranychidae) is a devastating agricultural pest that can feed on more than 1000 host plants. This extremely polyphagous nature of this pest may allow random disperse of them. Although population movement and structure are of vital importance to design area-wide pest control programs, there is no such study focusing on this issue in Turkey. The present study showed that there was no genetic subdivision among *T. urticae* the populations collected from four geographic regions of Turkey ( $F_{ST}=0.090$ ,  $p>0.05$ ), based on *cytochrome c oxidase subunit I (COI)*. In addition, the haplotype network supported these results since no clustering pattern was present. However, Black Sea populations had high genetic differentiation with other populations. This might be due to its isolated geography, different climate conditions, and limited sampling area. A high level of gene-flow between the Mediterranean and Aegean/Central Anatolian populations was determined. It is known that geography alone is not enough to explain population structure and genetic variation when excluding other ecological factors. Therefore, other factors such as current and historical climate data should be integrated to assess gene-flow in future studies.

### INTRODUCTION

Turkey is geographically divided into seven regions, and each of them is of great importance for agricultural production. These zones normally limit the dispersal activity of agricultural pests due to their different climatic features, geographical structure, and plant diversity (Bebber et al. 2014, Mazzi and Dorn 2012). However, the knowledge is quite limited if this hypothesis is still valid for extremely polyphagous pests such as *Tetranychus urticae* Koch (Acari: Tetranychidae), considering the abundance of host plants.

*T. urticae*, the two-spotted spider mite, is a harmful agricultural pest that can feed on more than 1100 host plants that contribute to its worldwide dispersal (Migeon and Dorkeld 2021, Van Leeuwen et al. 2010). Although there are many studies about biological and chemical control of this pest (Attia et al. 2013, Van Leeuwen et al. 2009) as well as the potential of local entomopathogenic fungus isolates (Yucel 2021), there is limited information on its movement preference during dispersal and consequent gene-flow among populations in certain geographical areas.

*T. urticae* can spread via active and passive mechanisms (Hussey and Parr 1963, Kennedy and Smitley 1985). Active mechanisms such as moving often cause short-distance dispersal (Hussey and Parr 1963), on the other hand, passive dispersal by wind may result in the long-distance spread of spider mites (Osakabe et al. 2008). A better understanding of the dispersal ability of *T. urticae* populations across different geographic regions may contribute to pest control (Stinner et al. 1983).

The mitochondrial *cytochrome c oxidase subunit I* (COI) has been used in many studies to uncover relationships between and within spider mites including phylogeographic patterns (Ros and Breeuwer 2007). In this study, COI sequences belonging to *T. urticae* populations collected from four different geographic regions of Turkey were used to assess gene flow and genetic differentiation between and within the regions. Additionally, COI haplotypes were determined and network analysis was performed to further elucidate the population structures.

## MATERIALS AND METHODS

### *Tetranychus urticae* populations

*T. urticae* populations were collected from three different geographic regions of Turkey: Black Sea (BS), Aegean (A), and Central Anatolia (CA) (Table 1). Mites were transferred into 70% and 90% alcohol for morphological and molecular identification, respectively. Prior to molecular analysis, morphological identification was performed using Hoyer's medium for permanent slides (Zhang 2003).

All the sequences obtained in this study were submitted to NCBI GenBank (accession numbers MZ824594-MZ824619). Besides the sequences herein obtained, some additional sequences, belonging to *T. urticae* populations in the Mediterranean (M) and CA region, from NCBI GenBank were used for further analyses (Table 1).

### Genomic DNA extraction and gene amplification

Total DNA was extracted from pools of 10 adult female mites using Qiagen DNeasy Blood & Tissue Kit following the manufacturer's instructions. DNA extracts were stored at -20°C until further process. After the final washing step, genomic DNA was eluted with 100 µl of elution buffer per sample. The quality and purity of DNA were checked by agarose gel electrophoresis (1.5%) and UV spectrophotometer (Thermo Scientific NanoDrop 2000).

A partial fragment of COI gene was amplified by PCR using the following primers: 5'-TGATTTTTGGTCACCCAGAAG-3' and 5'-TACAGCTCCTATAGATAAAAAC-3' (Navajas et al. 1994). PCR conditions were as follows: 3 min at 95°C, 40 cycles of 30 s at 95°C, 30 s at 48°C and 60 s at 72°C, and a final extension of 7 min at 72 °C.

The PCR reaction was performed in a total volume of 30 µl, containing 5 µl of mite DNA, 0.5 µl of each primer, 18 µl of PCR grade water, and 6 µl of FIREPol Master Mix (Solis Biodyne, Estonia). Purification of PCR products was conducted using HighPrep PCR clean-up system (MagBio Genomics Inc.), according to the supplier's protocol and subsequent sequencing of amplicons was performed at Macrogen Inc. (Seoul, South Korea).

### Population structure and gene-flow analyses

The number of haplotypes, haplotype diversity, nucleotide diversity was calculated using DnaSP v6.12.03 (Rozas et al. 2017) and PopArt v1.7 (Leigh and Bryant 2015) was used Analysis of Molecular Variance (AMOVA). Analyses of genetic differentiation and gene flow were assessed using DnaSP v6.12.03 (Rozas et al. 2017).

### Haplotype network analysis

All sequences were aligned using MAFFT v.7 with default settings (Kato et al. 2019). DnaSP v6.12.03 (Rozas et al. 2017) was used to generate haplotype data, and the haplotype network was constructed from 337 base pair (bp) long alignment using PopArt v1.7 (Leigh and Bryant 2015).

**Table 1.** Collection site and accession numbers of *Tetranychus urticae* populations

Region	City	Accession Numbers	Reference
Black Sea	Zonguldak	MZ824606-MZ824613	This study
Aegean	Aydin	MZ824614-MZ824619	This study
Central Anatolia	Ankara, Aksaray, Nevsehir, Konya, Karaman, Eskisehir	MZ824594-MZ824605; MW542505-MW542515	This study; İnak 2021
Mediterranean	Antalya, Mersin	MK508712-MK508722	İnak et al. 2019

**Table 2.** Number of samples, segregating sites, haplotypes, haplotype diversity, and nucleotide diversity among *Tetranychus urticae* populations from different geographic regions

Region	N	Segregating sites	Number of haplotypes (h)	Haplotype diversity	Nucleotide diversity ( $\pi$ )
Central Anatolia	22	31	7	0.74	0.032
Black Sea	8	4	4	0.75	0.004
Mediterranean	8	26	7	0.96	0.035
Aegean	6	15	5	0.93	0.025
Total	44	44	19	0.88	0.030

**Table 3.**  $F_{ST}$  and  $Nm$  values between *Tetranychus urticae* populations

CA*	-7.13	4,5	0,28	
BS	0,27	0,57		0,47
M	6,74		0,31	0,05
A		0,04	0,48	-0.03
	A	M	BS	CA

\*CA: Central Anatolia, BS: Black Sea, M: Mediterranean, A: Aegean;  $F_{ST}$  values in the lower matrix.  $Nm$  values in the upper matrix.

## RESULTS

### Population structure

After alignment, a total of 337 bp COI fragment was used for further analyses. All obtained results related to haplotypes and nucleotides are presented in Table 2.

Overall, 293 out of 337 bp was conserved, and nucleotide diversity ( $\pi$ ) was 0.030 (=3% genetic variation) among all sequences. Nucleotide diversity varied from 0.025 to 0.035 for various regions, except sequences belonging to the Black Sea region that has the lowest nucleotide diversity (0.004).

A total of nineteen different haplotypes were found, and the haplotypes' diversity values ranged from 0.74 to 0.96 based on a partial fragment of mitochondrial COI sequences. The diversity of haplotypes were the highest in Mediterranean populations; on the other hand, sequences belonging to Central Anatolia had the lowest haplotype diversity.

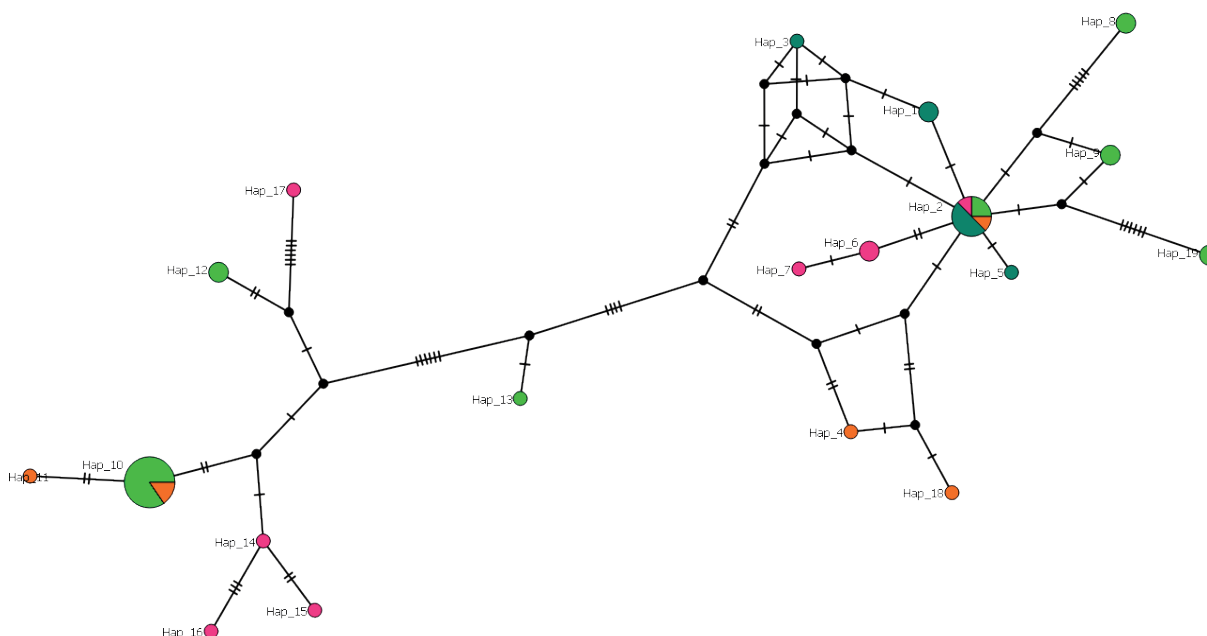
### Genetic differentiation ( $F_{ST}$ ) and gene-flow ( $Nm$ ) between populations

The highest genetic differentiation was between the Black Sea and Aegean sequences (0.48), followed by Black Sea and Central Anatolia, in line with low  $Nm$  values between these regions that are 0.27 and 0.28, respectively (Table 3).

There was no genetic subdivision between the populations from Central Anatolia and Aegean regions, based on  $F_{ST}$  value. Mediterranean populations had also very low genetic differentiation with Aegean and Central Anatolia populations.

### Haplotype network analysis and AMOVA

A total of 19 COI haplotypes were determined in *T. urticae* populations across Turkey. However, network analysis showed



**Figure 1.** Haplotype network analysis of *Tetranychus urticae* populations from four geographic regions of Turkey. The sizes of the circles are proportional to haplotype frequency



that there is no clustering pattern for a certain region (Figure 1). Hap\_10 and Hap\_2 were the most frequent haplotypes that mostly included CA and A populations, respectively.

Results of AMOVA analysis among and within populations are given in Table 4. AMOVA analysis showed that the fixation index (F<sub>ST</sub>) among all populations was 0.090. Molecular variance among populations was 9.09%, while 90.90% variation was determined within the population (Table 4).

**Table 4.** Analysis of molecular variance (AMOVA) and degrees of freedom (df) of among and within *Tetranychus urticae* populations

Variation	df	Sum of squares	Sigma2	%variation
Among populations	3	522.170	7.057	9.09174
Within populations	40	2822.375	70.559	90.90826
Total	43	3344.545	77.616	

## DISCUSSION

*Tetranychus urticae* is an extremely polyphagous agricultural pest throughout the world (Van Leeuwen et al. 2010). Although population movement and structure are of vital importance to design area-wide pest control, there is no such study focusing on this issue in Turkey. To date, geographic distance and host plant adaptation has been associated with genetic differentiation and similarity, respectively, in *T. urticae* populations (Tsagkarakou et al. 1998, Tsagkarakou et al. 1999, Uesugi et al. 2009). In addition, since many parameters affecting geographic structure could be unique for the regions, studies should be performed with a special focus on target regions to investigate genetic differentiation and gene-flow among pest populations. In this study, genetic variation and movement of *T. urticae* populations collected from different geographic regions of Turkey were investigated.

Overall, 293 out of 337 nucleotides (86.9%) were conserved among all *COI* sequences and a total of 19 haplotypes were determined using 44 sequences, showing high haplotype diversity in Turkish *T. urticae* populations. In addition, AMOVA analysis showed 9% and 90.9% variation among and within populations, respectively. Low variation among populations showed the absence of subdivision among populations. The fixation index (F<sub>ST</sub>) of all populations was not significant (p=0.065), indicating low genetic differentiation again. These results were also supported by haplotype network analysis which showed that no correlation existed between population diversity and geographic regions (Figure 1).

Estimation of F<sub>ST</sub> value (Fixation index =  $\Phi_{st}$ ) that can range from 0 to 1, is the most frequent method to assess differentiation among populations since its first development in 1965 (Wright 1965). A higher degree of genetic differentiation among populations leads to a higher F<sub>ST</sub> value (Meirmans 2006). On the contrary, a negative F<sub>ST</sub> value has been considered equal to zero, indicating no population structure (Meirmans 2006). Wright (1978) suggested that F<sub>ST</sub> values ranging from 0.05 and 0.15 indicate moderate genetic differentiation between pairs of populations. On the other hand, Nm (=number of migrants) estimates the gene-flow between populations (Whitlock and Mccauley 1999). A higher Nm value means higher gene migration, and when Nm>4, the presence of very high-level migration can be assumed (Beals et al. 2000).

The nucleotide diversity of the populations from various regions was close to each other, except the ones from the Black Sea region having very low diversity (0.004). This might be due to its isolated geography, different climate conditions, and limited sampling area. In line with this, a high level of genetic differentiation and low level of migration between BS and other regions have been determined based on F<sub>ST</sub> and Nm values. In spite of having isolated geography, nucleotide and haplotype diversity were the highest in the Mediterranean region. It is known that geography alone is not enough to explain population structure and genetic variation when excluding other ecological factors (Jin et al. 2020). Therefore, other factors such as current and historical climate data should be integrated to assess gene-flow in future studies. In addition, human activities such as the importation of ornamental plants might cause the introduction of plant pests together with them and contribute to the increased genetic variation in the Mediterranean region.

Populations from the Aegean region showed low genetic differentiation with other populations (except BS). In addition, moderate differentiation between Mediterranean and Central Anatolian populations has been detected that might be explained by substantial differences in their climate regimes. However, since geographic regions do not match with the regions for climate regimes of Turkey (İyigün et al. 2013), more detailed studies considering climates regime zones, rather than geographic regions, are needed to get a better understanding of climate-migration relationships between *T. urticae* populations.

In conclusion, *T. urticae* populations collected from four different geographic regions showed low genetic differentiation and high gene-flow between each other, with the exception of Black Sea populations. However, more samplings from wider areas should be obtained in future studies. In addition, other ecological factors should be integrated to reveal gene-flow among *T. urticae* populations across the country.

## ÖZET

*Tetranychus urticae* Koch (Acari: Tetranychidae) 1000'den fazla konukçu bitkiden beslenebilen tahrip edici bir tarımsal zararlıdır. Bu aşırı polifag doğası, bu zararlının rastgele dağılmasına olanak sağlayabilmektedir. Popülasyon hareketi ve yapısı geniş alanlarda zararlı kontrolü programları dizayn edilmesinde çok önemli olmasına rağmen, Türkiye'de bu konuda gerçekleştirilmiş bir çalışma bulunmamaktadır. Bu çalışmada, farklı coğrafik bölgelerden toplanan *T. urticae* popülasyonları arasında *sitokrom oksidaz c altünite I (COI)* genine dayanarak genetik alt bölünme olmadığını göstermektedir ( $F_{ST}=0.090$ ,  $p>0.05$ ). Ayrıca, haplotip network ağ analizinde kümelenme yapısı olmaması bu sonucu desteklemektedir. Ancak, Karadeniz popülasyonlarının diğer popülasyonlar ile yüksek genetik farklılığa sahip olduğu gösterilmiştir. Bu durum, bölgenin sahip olduğu izole coğrafyasından, farklı iklim koşullarından ve örnekleme yapılan alanın sınırlı olmasından dolayı olabilir. Akdeniz Bölgesi popülasyonları ile Ege ve İç Anadolu Bölgesi popülasyonları arasında yüksek gen akışı belirlenmiştir. Coğrafyanın tek başına popülasyon yapısı ve genetik varyasyonu açıklamada yeterli olmadığı bilinmektedir. Bu nedenle, güncel ve tarihsel iklim verileri gibi diğer faktörler ileri gen akışı çalışmalarında birleştirilmelidir.

Anahtar kelimeler: kırmızı örümcekler, genetik farklılaşma, *sitokrom oksidaz c altünite I*, haplotip ağ

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# Bitki Koruma Bülteni / Plant Protection Bulletin

<http://dergipark.gov.tr/bitkorb>

Original article

## Prevalence and molecular characterization of Cucumber mosaic virus isolates infecting tomato plants in Marmara region of Turkey

Marmara Bölgesi domates üretim alanlarında Cucumber mosaic virus izolatlarının yaygınlığı ve moleküler karakterizasyonu

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### ABSTRACT

There are many studies carried out on tomato viruses in Turkey. However, there is no study on the prevalence and genetic diversity of *Cucumber mosaic virus* (CMV), one of the most important viral diseases of tomatoes. In this context, surveys were conducted in the tomato production areas of the Marmara region, and samples were taken from 113 tomato plants showing virus and virus-like symptoms, and tests were carried out to determine CMV infection by DAS-ELISA. As a result of the tests, CMV infection was detected in 34 plants. And, 10 CMV isolates were selected for further studies. Coat protein (CP) and movement protein (MP) genes of selected isolates were amplified by RT-PCR and sequenced. As a result of multiple sequence analysis, CMV isolates from the Marmara region showed 97-100% similarity in nucleotide and amino acid levels within themselves, 77-100% in nucleotide level, and 79-100% in amino acid level in the world isolates according to the CP gene. According to the MP gene region, it was determined that the CMV isolates showed 97-100% and 96-100% similarities at the nucleotide and amino acid levels in each other, respectively. The similarity rates with world isolates were determined as 79-100% at the nucleotide level and 81-100% at the amino acid level. As a result of the phylogenetic analyses performed, tomato CMV isolates were closely related to each other according to both gene regions and were in the la subgroup.

### INTRODUCTION

*Cucumber mosaic virus* (CMV) was first reported on the cucumber (*Cucumis sativus* L.) plant by Price in the USA in 1934. CMV is a viral agent belonging to the *cucumovirus* genus of the *Bromoviridae* family. The host range of the causal agent is probably the largest among plant viruses. CMV can infect more than 1200 plants belonging to 500 genera from 100 families (Jacquemond 2012, Zitter

and Murphy 2009). The causal agent can be transmitted mechanically by plant sap, non-persistent by aphids, and by seeds (Palukaitis and Garcia-Arenal 2003, Palukaitis et al. 1992).

CMV has a positive susceptibility single-stranded and triple-segmented RNA genome. Of these RNA segments, RNA1 and RNA2 contain complex genes related to replicase

and movement, while RNA3 encodes movement (MP) and coat (CP) protein genes (Palukaitis and Garcia-Arenal 2003, Zitter and Murphy 2009). It has also been reported that the agent is transmitted by more than 75 aphid species (Palukaitis et al. 1992).

CMV, one of the most important viral diseases of tomatoes, has been reported to be divided into 2 groups based on their serological relationships and genetic diversity rates. In addition, these two groups are symbolized as I and II (Palukaitis et al. 1992). As a result of the studies carried out in the world in recent years, it was stated that subgroup I was divided into two as Ia and Ib, with the analysis of the CP gene and non-protein-coding regions at the 5' end of the agent (Roossinck et al. 1999).

It has been reported that CMV infections in different hosts have been detected previously in Turkey (Balsak et al. 2021, Güneş and Gümüş 2019, Kurtoğlu and Korkmaz 2018, Özdemir and Erilmez 2012, Usta et al. 2020). In a limited number of studies, it was stated that subgroup IA isolates are commonly seen as a result of molecular studies performed with CMV (Çağlar 2006, Ergün et al. 2013, Ohshima et al. 2016). In recent years, the presence of group II and subgroup IB has been reported in Turkey (Karanfil and Korkmaz 2017, Sarı 2015). However, although it has been reported that CMV infection is frequently detected in tomato production areas of our country, the genetic diversity of these isolates is not known.

In this context, no research has been conducted on the identification and genetic diversity of CMV in the tomato production areas of Çanakkale, Balıkesir, Bursa, Tekirdağ, and Edirne provinces in the Marmara region of Turkey. For this purpose, the presence and genetic diversity of CMV were investigated by taking samples from plants showing virus and virus-like symptoms from the specified areas.

## MATERIALS AND METHODS

### Field and virus diagnostic studies

Field studies were carried out in the tomato production areas of Çanakkale, Balıkesir, Bursa, Tekirdağ, and Edirne provinces and their districts in 2020-2021. During the

production season, the plants were visually examined by making land exits to the tomato production areas, and samples were taken from the plants showing viral disease-like symptoms. The selection of production areas were made randomly. The collected samples were brought to the laboratory in the cold chain in silica gels and stored at 4°C for further analysis.

The presence of CMV in the collected samples was determined by DAS-ELISA test. For this purpose, tests were carried out in line with the recommendations of the company from which the ELISA kits were purchased, based on the method specified by Clark and Adams (1977) (Bioreba, Switzerland).

### Molecular characterization studies

As a result of DAS-ELISA tests, a total of 10 isolates, two isolates for each province, were selected among the isolates infected with CMV, considering their geographical origins, and were used within the scope of molecular characterization studies. Molecular characterization studies were performed based on the coat protein (CP) and movement protein (MP) gene regions of the isolates.

### Reverse transcriptase-polymerase chain reaction

Total RNA was first isolated from the selected samples, which were found to be infected with CMV, using the CTAB method (Li et al. 2008). Total RNAs obtained were first synthesized as complimentary DNA (cDNA) for PCR studies using RevertAid First Strand cDNA Synthesis Kit (Thermo Scientific, USA). Using the obtained cDNAs, the CP and MP genes of the CMV isolates were amplified in 2X Emerald Master Mix (Takara, Japan) and MJ Mini Thermal Cycler (Biorad, USA) using the primer pairs in Table 1.

### Sequencing studies

RT-PCR products containing the amplified CP and MP genes were directly sequenced in a bidirectional manner by purchasing service from BM Labosis (Ankara, Turkey). The obtained raw sequence data were assembled in the CLC Main Workbench V.20 program and deposited into the GenBank (Table 2).

**Table 1.** Primer pairs used in molecular characterization of *Cucumber mosaic virus* isolates

Code	Primer Sequence	Sense	Target Gene	Amplicon Size	Reference
CMV_MPF	ATGGCTTTCCAAGGTACCAG	Forward	Movement Protein	1165 bp	Karanfil and Korkmaz 2021
CMV_MPR	YAGCAYTGGGAGATYCAGA	Reverse			
CMV_CPF	ATGGACAAATCTGAATCAACC	Forward	Coat Protein	638 bp	Karanfil and Korkmaz 2017
CMV_CPR	GATGTGGGAATGCGTTGGTGC	Reverse			



**Table 2.** Information of Turkish tomato *Cucumber mosaic virus* isolates obtained in this study

Isolate Name	Origin	GenBank Accession Number	
		CP	MP
CNK-3	Çanakkale	MZ711448	MZ711458
CNK-13		MZ711449	MZ711459
BLK-27	Balıkesir	MZ711450	MZ711460
BLK-38		MZ711451	MZ711461
BRS-51	Bursa	MZ711452	MZ711462
BRS-63		MZ711453	MZ711463
TKR-72	Tekirdağ	MZ711454	MZ711464
TKR-81		MZ711455	MZ711465
EDR-91	Edirne	MZ711456	MZ711466
EDR-97		MZ711457	MZ711467

Based on the nucleotide (nt) and amino acid (aa) sequences contained in the CP and MP genes of the CMV isolates selected using the consensus sequences obtained, the similarity ratios of the isolates with the isolates obtained from different countries of the world, retrieved from the GenBank (Table 3), were determined in the Sequence Demarcation Tool V 1.2 program (Muhire et al. 2014). Phylogenetic relationships of the selected isolates were determined in CLC Main Workbench V. 20.

Table 3.

## RESULTS AND DISCUSSION

As a result of the field studies, a total of 113 samples showing virus and virus-like symptoms were collected from tomato production areas in the Marmara region. The distribution of the collected samples according to the provinces was obtained by taking 22 samples from Çanakkale, 24 samples from Bursa, 23 samples from Balıkesir, 25 samples from Tekirdağ, and 19 samples from Edirne.

As a result of testing the collected 113 samples with the DAS-ELISA test, 34 samples were found to be infected with CMV. The distribution of infected sample numbers based on the

provinces was determined as 8 for Çanakkale, 5 for Balıkesir, 7 for Bursa, 8 for Edirne, and 6 for Tekirdağ. With this result, the infection rate in the collected samples was determined as 30.08%. While the highest infection rate was found in the province of Edirne with 42.10%, the lowest infection rate was obtained from the province of Balıkesir with 21.73% (Table 4).

In a study conducted in Çanakkale, Bilecik, and Bursa

**Table 4.** The numbers of virus infected and collected samples and infection rate of *Cucumber mosaic virus* in the collected tomato samples from Marmara region of Turkey

Province	Infected sample	Collected sample	Infection rate (%)
Çanakkale	8	22	36.36
Balıkesir	5	23	21.73
Bursa	7	24	29.16
Tekirdağ	8	19	42.10
Edirne	6	25	24.00
Total	34	113	30.08

provinces of the Marmara region in 2013, researchers determined that 67 of the 77 samples were infected with CMV and reported the infection rate as 87% (Uzunoğulları and Gümüş 2015). In a study conducted in the West-Mediterranean region, it was reported that 53 (38.40%) of the 138 tomato samples collected were infected with CMV (Yardımcı and Eryigit 2006). It is thought that the different infection rates obtained between studies vary in parallel with the year of sampling, the availability of aphid vectors, and the sanitation measures applied.

Although all the samples collected within the scope of this study showed very typical virus and virus-like symptoms, the presence of CMV infection in 69.92% of the collected samples strengthens the possibility of at least one viral disease infection in these collected samples. As a matter of fact, the reports of infections of some begomovirus species (Sertkaya and Yılmaz 2017, Yılmaz and Sipahioğlu 2020), *Tobacco brown rugose fruit virus* (Fidan et al. 2019), *Tomato*

**Table 3.** The world *Cucumber mosaic virus* isolates used for references in molecular characterization studies

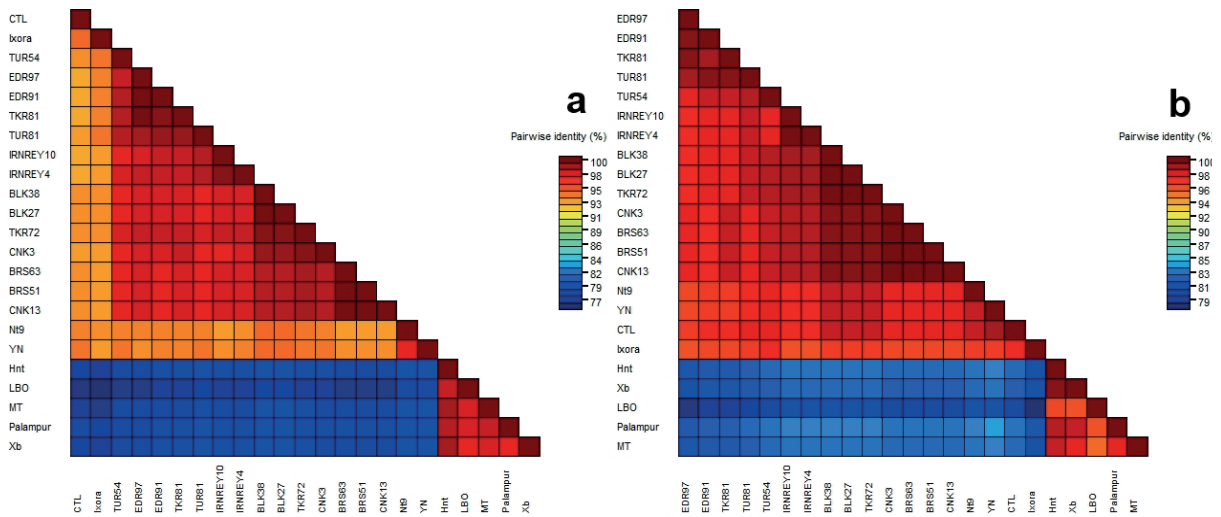
Isolate Name	Origin	Phylogenetic Group	GenBank Accession Number
IRN-REY4	Iran		LC066467
IRN-REY10	Iran		LC066473
TUR-54	Turkey	Ia	LC066503
TUR-81	Turkey		LC066506
Ixora	USA		U20219
CTL	China	Ib	EF213025
Nt9	Taiwan		D28780
YN	China		EF216865
LBO	The Netherlands		AJ304397
Hnt	China	II	KC407999
Palampur	India		HE583224
MT	Japan		AB189917

*chlorosis virus* (Yeşilyurt and Çevik 2019), and *Tomato spotted wilt virus* (Çulal-Kılıç et al. 2017) as a result of studies carried out in the tomato production areas of our country in recent years also confirm this idea.

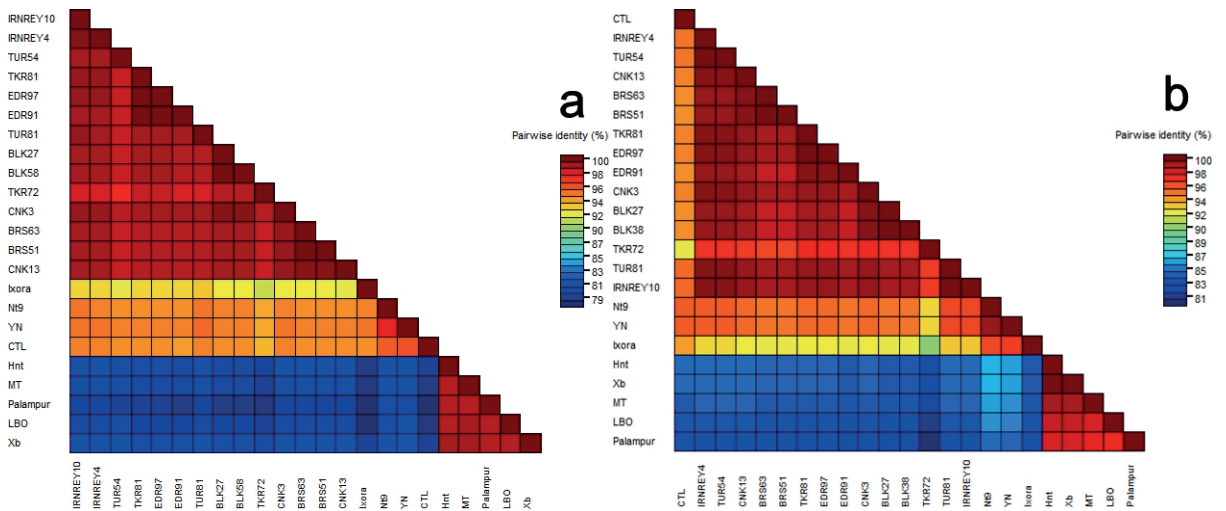
As a result of the molecular characterization studies, it was determined that CMV isolates from the Marmara region showed 97-100% similarity with each other at the level of nucleotides and amino acids according to the CP gene region. As a result of the comparisons made with world isolates according to this gene region, it was determined that the isolates showed 77-100% similarity with each other at the nucleotide level and 79-100% at the amino acid level (Figure 1).

The similarity analyses of CMV isolates from the Marmara region based on the MP gene region, it was determined that the isolates showed similarities between themselves at the rate of 97-100% at the nucleotide level and 96-100% at the amino acid level. The similarity rates of CMV isolates from the Marmara region with the world isolates according to the MP gene region were determined as 79-100% at the nucleotide level and 81-100% at the amino acid level (Figure 2).

As a result of the studies carried out previously on the molecular characterization of CMV isolates in our country, it was stated that the CMV isolates in our country were generally 80-100% similar to the world isolates (Güneş and Gümüş 2019, Karanfil



**Figure 1.** Similarity rates of *Cucurbit mosaic virus* isolates infecting tomato plants from Marmara region of Turkey at the nucleotide (a) and amino acid (b) levels based on the coat protein gene region



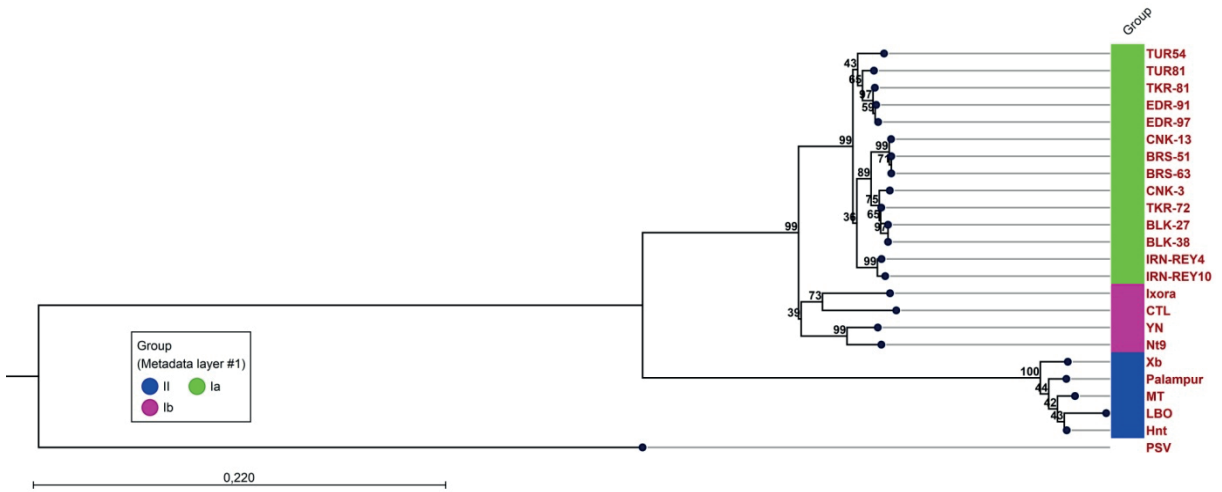
**Figure 2.** Similarity rates of *Cucurbit mosaic virus* isolates infecting tomato plants from Marmara region of Turkey at the nucleotide (a) and amino acid (b) levels based on the movement protein gene region

and Korkmaz 2017). In this context, the results obtained within the scope of this study show parallelism with previous studies.

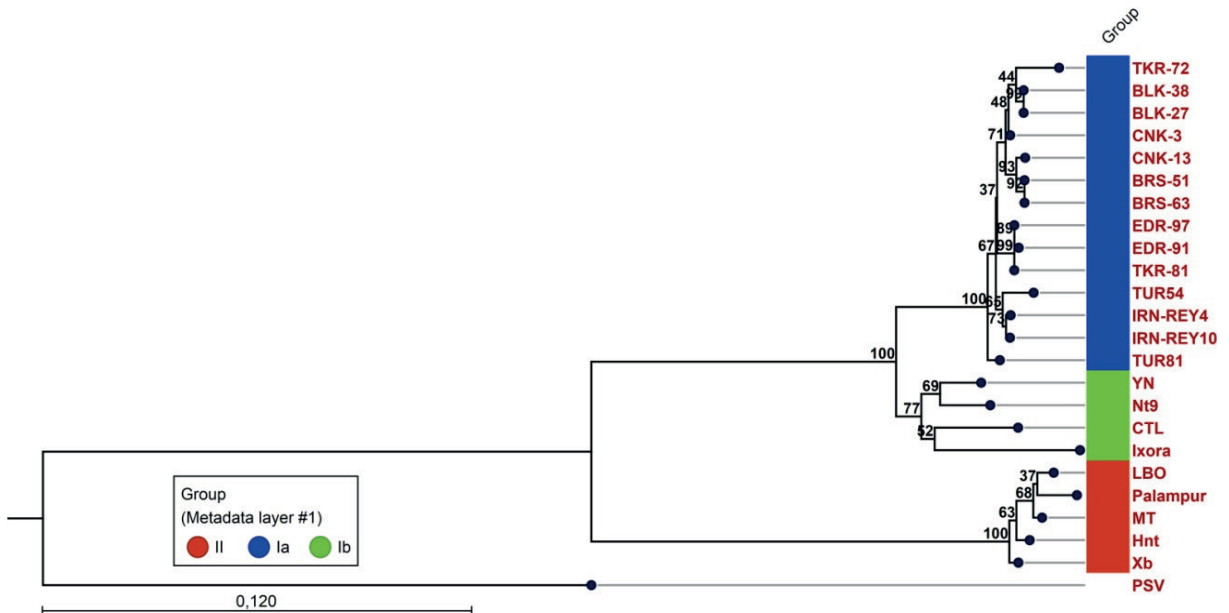
In addition, in general, it is seen that 3 colours are dominant in nt-based similarity matrices and 2 colours are dominant in aa-based similarity matrices. In the nucleotide-based similarity matrix, it is seen that the CMV subgroups IA, IB, and II are clearly separated, while in the aa-based similarity matrix, IA and IB converge to each other in terms of sequence similarity due to the increasing similarity rates, and the matrices are divided into I and II (Figure 1 and 2).

As a result of the phylogenetic analyses, it was determined that the selected tomato CMV isolates were in subgroup IA according to both CP and MP gene regions (Figure 3 and 4).

As a result of previous studies with CMV in different hosts in our country, it has been reported that subgroup IA isolates are common (Karanfil and Korkmaz 2021, Ohshima et al. 2016). In addition, the existence of subgroup IB and II isolates is also known in our country (Karanfil and Korkmaz 2017, Sari 2015). However, the presence of tomato CMV isolate as subgroup IA within the scope of a study carried



**Figure 3.** The phylogenetic tree of *Cucumber mosaic virus* isolates based on the coat protein gene region [The phylogenetic tree was constructed by the maximum likelihood method using HKY+G+T parameters and Peanut stunt virus (PSV) was used an out-group (Genbank accession no: JN135292)]



**Figure 4.** The phylogenetic tree of *Cucumber mosaic virus* isolates based on the movement protein gene region [The phylogenetic tree was constructed by the maximum likelihood method using HKY+G+T parameters and Peanut stunt virus (PSV) was used an out-group (Genbank accession no: JN135292)]



out in the Mediterranean region in our country (Caglar 2006), leads to the conclusion that CMV isolates that cause infection in tomato production areas of our country are predominantly Ia group. In addition, it was determined that CMV isolates in the Marmara region were identified as Ia group with this study, it is thought that geographical origins may affect the phylogenetic groups although it was reported that geographical origins are not very dominant in the phylogenetic classification of CMV isolates and that isolates from the same region may show the distribution in different phylogenetic groups (Ohshima et al. 2016). With this study, the molecular characterization of CMV isolates obtained from tomato production areas for the first time in Turkey was carried out according to two different gene regions. In addition, by depositing the sequences of the CP and MP genes of tomato CMV isolates to the GenBank, it was ensured that tomato CMV isolates originating in our country were included in the GenBank for the first time. Although the rate of CMV infection was found to be low in tomato production areas in the study area, it is thought that the genetic diversity of all segments of the isolates obtained by CMV surveys to be carried out at a national level should be determined. Thus, molecular population structures of CMV isolates in tomato production areas of Turkey will be able to be fully revealed.

## ÖZET

Türkiye’de domates virüsleri ile ilgili olarak gerçekleştirilmiş çok sayıda çalışma vardır. Ancak domatesin en önemli virüs hastalıklarından bir tanesi olan Cucumber mosaic virus (CMV)’nin geniş alanlarda yaygınlığı ve genetik çeşitliliği üzerine gerçekleştirilmiş bir çalışma bulunmamaktadır. Bu bağlamda Marmara Bölgesi domates üretim alanlarında surveyler düzenlenerek virüs ve virüs benzeri simptom gösteren 113 domates bitkisinden örnekler alınarak DAS-ELISA testi ile CMV enfeksiyonunun belirlenmesi amacı ile testlemeler gerçekleştirilmiştir. Gerçekleştirilen testlemeler sonucunda 34 bitkide CMV enfeksiyonu tespit edilmiştir. Enfekteli örnekler içerisinde elde edildikleri iller temel alınarak 10 CMV izolatu moleküler karakterizasyon çalışmaları için seçilmiştir. Seçilen bu izolatların kılıf protein (CP) ve hareket protein (MP) genleri RT-PCR ile amplifiye edilmiş ve sekanslanmıştır. Elde edilen sekanslar kullanılarak gerçekleştirilen çoklu dizi analiz çalışmaları sonucunda, Marmara Bölgesi CMV izolatlarının CP genine göre kendi içlerinde nükleotid ve amino asit düzeyinde %97-100, dünya izolatları ile nükleotid düzeyinde %77-100, amino asit düzeyinde ise %79-100 oranlarında benzerlikler gösterdiği tespit edilmiştir. MP gen bölgesine göre ise CMV izolatlarının kendi içlerinde nükleotid

düzeyinde %97-100, amino asit düzeyinde ise %96-100 oranlarında benzerlikler gösterdiği belirlenmiştir, dünya izolatları ile gösterdikleri benzerlik oranları ise nükleotid düzeyinde %79-100, amino asit düzeyinde ise %81-100 olarak belirlenmiştir. Gerçekleştirilen filogenetik analizler sonucunda Marmara Bölgesi CMV izolatlarının hem CP hem de MP gen bölgesine göre birbirleri ile yakın ilişkili olduğu ve Ia alt grubunda olduğu belirlenmiştir.

Anahtar kelimeler: CMV, DAS-ELISA, RT-PCR, sekanslama

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# Bitki Koruma Bülteni / Plant Protection Bulletin

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Original article

## Periodic and annual changes in body weight and fat ratio of sunn pest, *Eurygaster maura* L. 1758 (Hemiptera: Scutelleridae)

Sünenin, *Eurygaster maura* L. 1758 (Hemiptera: Scutelleridae) vücut ağırlığında ve yağ oranında dönemsel ve yıllık değişimler

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### ABSTRACT

This study, which aims to predict the outbreaks of sunn pest; was carried out in two overwintering sites for six life cycles in 2013-2019. Three critical periods in each life cycle during the study; at the beginning of the estivation, hibernation and active life periods, the densities of the overwintering population, the fat ratios, weights, and the relationships between them were determined. It has been determined that the winter population of sunn pest varies in terms of both weight and fat depending on gender, life cycles and critical periods, and there is a positive relationship between body mass and fat ratios. Lipids ratio is an important indicator that we can use in estimates of sunn pest epidemic with appropriate climatic conditions and nutrient abundance. Especially in female individuals 27% and above, suitable for climatic conditions during active life; It has been demonstrated that in conditions where there is high temperature, low proportion humidity, wind speed and precipitation and proper nutrient abundance for 2 consecutive years, there is a significant increase in population size and pest can cause an epidemic.

### INTRODUCTION

The main pest of wheat, sunn pest (*Eurygaster* spp.), is widespread in the Near and Middle East, Northern and Eastern Europe and North Africa. The pest causes up to 100% damage in the quality and quantity of wheat by feeding on leaves, stems, and grains (Anonymous 2008, Özkan and Babaroğlu 2015, Babaroğlu et al. 2020). The life cycle of the sunn pest, which gives offspring once a year is divided into three main periods including aestivation, hibernation and active life periods. The aestivation period is from July to October-November following the harvest, the hibernation covers the period from October to November and from

March to April of the following year. This period, which lasts about 8-9 months, is also called the passive life period, and during this period, it spends in obligatory diapause usually at 1200-1600 m altitude (overwintering site) without feeding. The passive period ends when the winter passes pest begins to fly from the overwintering site to the plains with the increase of the air temperature in the spring, and the active period, which is the breeding and development period, begins. Adults, who come to the plain in the active period, feed and mate for 1.5-2 months and lay eggs. At the end of this period, adults die. The eggs hatch within 2-3

weeks depending on the climatic conditions and nymphs show up. Nymphs that spend 5 periods within an average of one month become adult. Before returning to overwintering sites, adults are fed voraciously and store the necessary energy during hibernation, aestivation, and active life periods a year later (Anonymous 2008, Özkan and Babaroğlu 2015, Babaroğlu et al. 2020).

In addition to appropriate climatic conditions and abundance of nutrients, the presence of physiologically strong individuals that have stored high amounts of nutrients is also an important factor in the occurrence of harmful outbreaks. Fats play a major role in pest's basic vital activities. In most insects, accumulated fat stores are of primary importance in diapause and post-diapause activities. Most insects end the diapause with a smaller amount of fat than at the beginning of diapause. This indicates that fats are the primary source used during diapause (Hahn and Denlinger 2011, Sinclair 2015, Wipking et al. 1995). Fat reserves are the most important resource used by diapause insects to meet their energy needs during their diapause (Hahn and Denlinger 2007, Estela and Soulages 2010, Sinclair 2015). In addition to the important role of fats in pest survival in the passive period (Fedotow et al. 1955, Popov 1979, 2002; Sinclair 2015, Williams et al. 2015a, Wipking et al. 1995), they also play an important role during migration from overwintering site to the plain (Beenackers et al. 1984, Canavoso et al. 2003, Estela and Soulages 2010, Gade and Auerswald 2002, Kaufmann and Briegel 2004, Ziegler and Schulz 1986) and reproduction (Beenackers et al. 1985, Briegel 1990, Troy et al. 1975, Van Handel 1993).

Increased body mass is associated with increased nutrient reserves. Therefore, individuals with larger bodies generally have more fat reserves than small ones (Sinclair and Marshall 2018, Williams et al. 2015b). Although there are some opposite examples, they usually show higher performance (survival rate, reproduction, spread) of large body individuals in diapause and post- diapause processes compared to small ones (Hahn and Denlinger 2007, Irwin and Lee 2003, Williams et al. 2015b).

Sunn pest causes great damage by creating periodic outbreaks. In pest control, it is very important to take the necessary precautions by predicting outbreaks that will occur. Demonstrating the usability of changes in the rate of sunn pest fat content in explaining the outbreak course has been discussed in this study.

## **MATERIALS AND METHODS**

The studies were carried out in Ekecik (Aksaray province) and TV tower (Kırşehir province) overwintering sites. To

determine the body weights and fat ratios of the adult at the beginning of aestivation, hibernation, and active life periods from both overwintering sites during 6 life cycles in 2013-2019, adult males (3857) and females (4337) were collected and brought to the laboratory in the ice container, and initial body mass was determined by weighing individually. Weighed individuals were taken to the fat analysis as soon as possible without waiting. The determination of fat ratios is based on the Association of Official Analytical Chemists, Official Method 991.36. Firstly, sunn pests were kept in the desiccator at 105 °C for 4-5 hours, after their moisture was removing, they were ground and homogenized. 5-10 g sample was taken into the cartridge and placed in the extractor; the fats of the sunn pests were extracted for 5-6 hours (Anonymous 2000).

Meteorological data including temperature, proportional humidity, and wind speed were obtained from meteorological stations (IMETOS PRO 250) in the study sites.

### *Statistical analysis*

Analysis of variance was used to determine if there were differences and, following Tukey test was used to determine significant differences according to their importance regression analysis was used to determine the relationships between the characters. Data were analyzed using SPSS 24 software.

## **RESULTS AND DISCUSSION**

### *Fat ratio*

In the study, fat ratios in critical periods in the life cycle of the sunn pest were determined at first. From Ekecik (Aksaray province) and TV tower (Kırşehir province) overwintering sites, 6 life cycles (2013-2019) and 3 critical periods in each cycle. It has been determined that the fat content of male and female individuals collected at the beginning of aestivation, hibernation, and active life periods was 30.10% (19.92% - 43.00%) on average (Table 1).

As a result of the statistical analysis made considering life cycle, critical stage, and gender, no interaction was detected between the characters ( $F= 0.432$ ;  $df= 10$ ;  $P= 0.893$ ), but the main characters; it has been determined that fat ratios differ according to the life cycle, critical period, and gender.

It was determined that female individuals (32.01%) have higher rates of fat than male individuals (28.18%) in both locations ( $F= 81.668$ ;  $df= 1$ ;  $P= 0.00$ ). Similar to the results we obtained, it was also expressed by Fedotow (1945), Andreev (1963), Vinogradova (1969), Gospodinov (1973), Paulian et al. (1973), Mustatea et al. (1979), Paulian and Popov (1980), Kılınçer et al. (1987), Popov (2002), and Amiri et al. (2010) that females have a higher rate of fat than males.

**Table 1.** Fat ratio (%) of male and female individuals collected from Ekecik (Aksaray province) and TV tower (Kırşehir province) overwintering sites during aestivation, hibernation and active life periods in 2013-2019

Overwintering site	Critical Period	Gender	Fat ratio (%)					
			Life cycle 1 2013-2014	Life cycle 2 2014-2015	Life cycle 3 2015-2016	Life cycle 4 2016-2017	Life cycle 5 2017-2018	Life cycle 6 2018-2019
Ekecik (Aksaray province)	Beginning of aestivation	♀	39.57	40.36	39.00	37.21	40.18	39.00
		♂	35.29	36.42	35.50	34.09	39.33	33.18
	Beginning of hibernation	♀	28.60	29.98	27.75	26.86	34.98	30.54
		♂	27.68	26.36	27.48	24.47	28.74	25.43
	Active life periods	♀	26.66	23.57	24.75	24.67	32.72	29.02
		♂	24.30	21.35	23.63	22.91	25.77	23.17
TV Tower (Kırşehir province)	Beginning of aestivation	♀	38.94	38.92	37.18	38.26	42.83	39.07
		♂	34.37	36.08	33.09	32.88	37.98	33.32
	Beginning of hibernation	♀	27.96	31.02	31.42	28.52	33.58	32.61
		♂	25.49	29.46	24.23	25.03	26.67	25.30
	Active life periods	♀	23.47	27.87	26.18	26.11	28.31	28.84
		♂	21.19	22.24	19.92	22.00	21.53	23.97

When examined according to the critical periods, it was determined that the sunn pest had the highest fat content at the beginning of summer (Table 2). During the aestivation, there was a decrease in the amount of fat, and the lowest amount of fat was detected in individuals that have just come out of hibernation ( $F= 334.59$ ;  $df= 2$ ;  $P= 0,000$ ).

**Table 2.** Fat ratios (%) of individuals collected from Ekecik and TV tower overwintering sites in 2013-2018 at the beginning of aestivation, hibernation and active life periods

Fat ratios (%)		
mean $\pm$ standard error of mean		
Beginning of aestivation	Beginning of hibernation	Active life periods
37.17 $\pm$ 0.56 a <sup>1</sup>	28.34 $\pm$ 0.59 b	24.76 $\pm$ 0.62 c

<sup>1</sup>Values with different minuscule in the same row are statistically different from each other.

According to Tauber et al. (1986), Danks (1987), insects entering diapause decrease their stored reserves during this time and contain fewer reserves after diapause. It has also been demonstrated by other studies that the new generation adults because of wings maximize their fat reserves by feeding in the wheat fields until to the overwintering site for aestivation and have the lowest fat rate at the beginning of the active period (Bel'kevich 1957, Choumakow et al. 1954, Karaca et al. 2007, Kılınçer et al. 1987, Lazarov et al. 1969, Strogaia 1950, 1955).

A difference has been noted in fat ratios depending on the life cycle of sunn pest ( $F= 10.449$ ;  $df= 5$ ;  $P= 0.000$ ). The highest fat ratio was determined in the 5th life cycle, followed by the

2nd and 6th life cycles, and the 4th life cycle was found to have the lowest fat ratio (Table 3). The situation in question arises from the inconsistency between the wheat phenology and the biology of the pheasant, and the fact that the pest cannot be fed well and cannot accumulate enough fat reserves due to the adverse development of the climatic conditions during feeding. On the contrary, the phenology of the wheat and the climatic conditions are suitable for sunn pests, which is due to the accumulation of sufficient fat reserves.

Strogaia (1955) stated that the body fat ratio of the sunn pest (*E. integriceps*) varied over the years and emphasized that this was due to climatic conditions that affect feeding time and performance. Doronina and Makarova (1973) reported that the body fat ratio varied according to years, and the fat ratio at the beginning of hibernation was 30% in stout individuals and 25% in weak individuals. Paulian et al. (1973) emphasized that when both the phenology of wheat and the climatic conditions were suitable for nutrition, they can accumulate high fat reserves. Areshnikov et al. (1977) reported that the fat content of sunn pests varied according to years and overwintering sites. Similarly, the fat ratios of female individuals differed according to their life cycles depending on the feeding and hibernation conditions (Popov 1977).

One of the most important reasons for the differences in fat rates between cycles was the nutritional performance of the new generation adults. The new generation adult fat ratios in overwintering (Table 4) showed a parallel course to the cycle average fat ratios ( $r = 91.80$ ;  $r^2 = 84.27$ ).

**Table 3.** Fat rates (%) of individuals collected from Ekecik and TV tower overwintering sites in 2013-2019

Fat ratios (%)					
mean $\pm$ standard error of mean					
Life cycle 1 2013-2014	Life cycle 2 2014-2015	Life cycle 3 2015-2016	Life cycle 4 2016-2017	Life cycle 5 2017-2018	Life cycle 6 2018-2019
29.46 $\pm$ 1.76 bc	30.30 $\pm$ 1.87 b	29.17 $\pm$ 1.73 bc	28.58 $\pm$ 1.62 c	32.72 $\pm$ 1.91 a	30.29 $\pm$ 1.55 b

<sup>a</sup>Values with different minuscule in the same row are statistically different from each other.



**Table 4.** Fat rates (%) of individuals collected from Ekecik and TV tower beginning of aestivation in 2013-2019

Fat ratios (%)					
mean ± standard error of mean					
Life cycle 1	Life cycle 2	Life cycle 3	Life cycle 4	Life cycle 5	Life cycle 6
2013-2014	2014-2015	2015-2016	2016-2017	2017-2018	2018-2019
37.04±1.29	37.94±1.02	36.19±1.26	35.61±1.27	40.80±1.27	36.14±1.67

Differences in fat ratios in their life cycles at the beginning of aestivation, it changed depending on the climatic conditions during the period from the new generation adults to its exit to the overwintering site for aestivation. Temperature (average; min.; max.), proportional humidity, and wind speed in the process leading up to the migration of new generation adult individuals to the overwintering sites affect the feeding performance of the pest (Table 5). As shown in Table 5, high increases in fat rates occurred in parallel with temperature increases, but the increase in fat rates was low because pests cannot feed at high proportional humidity, and wind speed.

Changes in body fat rates during diapause were given in Table 6. As seen in the table, 35% of the body fat amount

was used during the passive period. The important part of body fat; approximately 25% was consumed during aestivation and 13% during hibernation. They did not feed during the aestivation period, which was an important part of the sunn pest's passive period. At the same time, the average temperatures in this period were both high and showed irregular changes. As a result, we believe that fat consumption was also high, as there was no slowdown in metabolic activities. Similarly, the fat consumption rates of insects during their diapause depend on both the average and the variability of the temperature (Sinclair 2015). Williams et al. (2012) stated that the variable temperatures in fall and spring caused disproportionately high-fat consumption in these seasons (Jensen inequality).

**Table 5.** Relationship between fat ratios (%) and some climate data

Variables	Statistical data				
	r	r <sup>2</sup>	Sign.		
Fat ratios (%)	Mean	0.812	0.659	0.000*	
	Temperature (°C)	Minimum	0.729	0.531	0.000*
	Maximum	0.833	0.694	0.000*	
Average humidity (%)	-0.686	0.471	0.000*		
Average wind speed (km/h)	-0.517	0.267	0.008*		

\*The correlation is significant at the 0.01 level.

**Table 6.** Fat change rates (%) of male and female individuals collected from Ekecik and TV tower overwintering sites in aestivation, hibernation and active life periods beginning in 2013-2019

Life cycle	Gender	Fat change rates (%)					
		Beginning of aestivation- Beginning of hibernation		Beginning of hibernation- Beginning of active life periods		Beginning of aestivation- Beginning of active life periods	
		Ekecik	TV tower	Ekecik	TV tower	Ekecik	TV tower
Life cycle 1 2013-2014	♀	27.72	28.20	6.78	16.06	32.62	39.73
	♂	21.56	25.84	12.21	16.87	31.14	38.35
Life cycle 2 2014-2015	♀	25.72	20.30	21.38	10.15	41.60	28.39
	♂	27.62	18.35	19.01	24.51	41.38	38.36
Life cycle 3 2015-2016	♀	28.85	15.49	10.81	16.68	36.54	29.59
	♂	22.59	26.78	14.01	17.79	33.44	39.80
Life cycle 4 2016-2017	♀	27.82	25.46	8.15	8.45	33.70	31.76
	♂	28.22	23.87	6.38	12.11	32.80	33.09
Life cycle 5 2017-2018	♀	12.94	21.60	6.46	15.69	18.57	33.90
	♂	26.93	29.78	10.33	19.27	34.48	43.31
Life cycle 6 2018-2019	♀	21.69	16.53	4.98	11.56	25.59	26.81
	♂	23.36	24.07	8.89	5.26	30.17	28.06
Average	♀	24.06	21.30	9.69	13.14	31.42	31.64
	♂	25.09	24.81	11.88	16.22	33.99	37.01
	♂+♀	24.55	22,95	10.73	14.55	32.67	34.16

Fats play an important role in the life cycle of sunn pests. The development of sunn pests consists of two periods as fat accumulation and fat consumption. Approximately 1/3 of the accumulated fat is used in the passive period, and 2/3 is used in the active period. Fat consumption is high in metabolic activities and has no nutrition aestivation. It was also reported by different researchers that it was consumed in low amounts in the active life period, which was a period of growth and development, and in the hibernation period, when the metabolism was very slow. It has been reported by different researchers that similar results were observed from other studies (Choumakow et al. 1954, Fedotow 1945, Fedotow et al. 1955, Karaca et al. 2007, Karel 1958, Lazarov et al. 1969, Ouchatinskaya 1953, 1955; Popov 1979, 2002; Strogaia 1950, 1955).

Although there was no statistically significant difference in the fat consumption of female and male individuals ( $t=0.582$ ;  $df=70$ ;  $p=0.234$ ), it was observed that fat consumption was generally higher in male individuals (Table 6). While the amount of fat consumed during aestivation period did not differ according to generations ( $F=0.648$ ;  $df=5$ ;  $P=0.667$ ), the amount of fat consumed during hibernation varied according to generations ( $F=3.375$ ;  $df=5$ ;  $P=0.025$ ) (Table 7). Karel (1958) and Popov (1979, 2002) report that fat consumption differs both genders and generations, with fat consumption higher in male individuals.

Another criterion is also examined in the study is the bodyweight of sunn pest. The average initial body mass was determined as 109.09 mg as a result of the weighing of the adults collected from two overwintering sites at the beginning of aestivation, hibernation, and active life during 6 life cycles in 2013-2019. Initial mass for females and males were 110.59 mg and 107.42 mg, respectively. Sunn pest population had a very heterogeneous structure in terms of mass (Table 8). As a result of the variance analysis, it was determined that initial body mass varied depending on the life cycle, critical period, gender, and location ( $F=3.459$ ;  $df=10$ ;  $P=0.001$ ).

Although the male and female individuals collected from Ekecik and TV tower overwintering sites varied based on their life cycle, the highest body mass was detected at the beginning of aestivation.

Between the beginning of hibernation and the beginning of the active period, in the male individuals in the first life cycle in the Ekecik overwintering site and differences in the mass of the male and female individuals in the second and sixth life cycles in both overwintering sites were determined (Table 8). In other life cycles, both by gender and by overwintering sites differences in mass were not determined. Individuals at the beginning of aestivation were heavier than individuals in other critical periods. This is because the new generation adults are fed enough for aestivation, hibernation, and one year later for survival and reproduction before migrating to the overwintering area, and they stored maximum levels of fat, protein, and carbohydrates in their bodies.

Our results are similar to other studies. Karel (1958) and Paulian and Popov (1980) report that new generation adult of sunn pest (*E. integriceps*) feed intensively before migrating to the overwintering site, storing large amounts of fat and increasing their body mass.

By different researchers that the sunn pest having finished feeding that migrated to the overwintering site for aestivation had the highest body mass, during the period from aestivation site to hibernation site migration, there was a large decrease in average body mass, and a similar situation was detected in the period from hibernation to the time of migration to the plain is the emphasized (İslamoğlu et al. 2013, Kılınçer et al. 1987, Lazarov et al. 1969, Memişoğlu 1985, Radjabi 1995, Yüksel 1968).

When we consider in terms of life cycles, although it varied according to the critical period and gender, the highest values were found in the second and fifth life cycles. Although there was no difference at the beginning of hibernation except for one period, the differences were generally detected at the onset of aestivation and active period (Table 8). Body mass also differed according to years due to changes in nutritional performance depending on the climatic conditions and feeding time in the period up to the exit of the sunn pest to the overwintering site for aestivation after nymph become to the new generation adult. Many studies have also demonstrated that the body mass of the new generation adult vary by years (Areshnikov et al. 1977, Doronina and Makarova 1973, Karaca et al. 2003, Lazarov et al. 1969, Popov 1977, Strogaia 1955, Taranukha et al. 1967, Yüksel 1968).

**Table 7.** Fat consumption rates (%) of different generations during aestivation and hibernation

Critical periods	Fat consumption rates (%)					
	mean $\pm$ standard error of mean					
	Life cycle 1 2013-2014	Life cycle 2 2014-2015	Life cycle 3 2015-2016	Life cycle 4 2016-2017	Life cycle 5 2017-2018	Life cycle 6 2018-2019
Aestivation	25.83 $\pm$ 1.51 a <sup>1</sup>	22.99 $\pm$ 2.19 a	23.43 $\pm$ 2.95 a	26.34 $\pm$ 1.02 a	22.81 $\pm$ 3.70 a	21.41 $\pm$ 1.70 a
Hibernation	12.98 $\pm$ 2.30 ab	18.76 $\pm$ 3.08 a	14.82 $\pm$ 1.55 ab	8.77 $\pm$ 1.20 b	12.93 $\pm$ 2.83 ab	7.67 $\pm$ 1.57 b

<sup>1</sup>Values with different minuscule in the same row are statistically different from each other.

**Table 8.** Mass of male and female individuals beginning of aestivation, hibernation and active life periods collected from Ekecik and TV tower overwintering sites in 2013-2019

Overwintering site	Gender	Critical period	Body weight (mg)					
			mean ± standard error of mean					
			(min. – max.)					
		Life cycle 1 2013-2014	Life cycle 2 2014-2015	Life cycle 3 2015-2016	Life cycle 4 2016-2017	Life cycle 5 2017-2018	Life cycle 6 2018-2019	
Ekecik (Aksaray province)	♀ <sup>1</sup>	Beginning of aestivation	113.42±1.30) b (87.3-154.6) A	116.79±0.83 ab (88.0-152.3) A	115.11±1.65 b (87.9-151.6) A	114.67±1.41 b (85.7-158.2) A	119.63±1.09 a (77.6-159.8) A	112.60±0.08 b (83.7-149.3) A
		Beginning of hibernation	104.46±1.39 a (87.1-120.1)B	108.42±0.78 a (82.8-139.5) B	104.78±1.54 a (67.5-152.5) B	107.55±1.49 a (70.7-150.8) B	107.87±1.37 a (74.0-154.1) B	106.39±1.29 a (64.6-166.0) B
		Beginning of active life periods	105.79±1.23 b (75.6-128.8) B	98.83±1.70 c (61.7-125.9) C	*	106.29±1.27 b (70.3-145.0) B	111.99±1.68 a (64.0-142.0) B	103.96±0.68 b (63.1-142.8) C
	♂	Beginning of aestivation	114.71±1.38 ab (88.0-154.8) A	114.60±0.99 ab (84.7-153.9) A	111.45±1.50 b (87.8-156.8) A	114.08±1.32 ab (87.5-151.4) A	117.65±1.78 a (82.8-163.7) A	109.80±0.80 b (82.6-143.5) A
		Beginning of hibernation	106.21±1.31 a (81.5-133.8) B	107.00±0.89 a (68.9-143.1) B	103.15±1.27 a (66.7-124.0) B	103.13±1.45 a (78.6-142.1) B	105.40±1.57 (67.2-139.7) B	103.14±1.38 a (51.0-152.3) B
		Beginning of active life periods	102.07±1.16 a (75.6-131.2) C	102.15±1.56 a (68.1-143.2) C	*	102.22±1.35 a (61.9-155.8) B	103.79±1.92 a (61.7-128.0) B	97.63±0.72 b (64.5-129.4) C
TV tower (Kırşehir province)	♀	Beginning of aestivation	112.90±1.53 bc (72.7-136.8) A	117.69±0.87 a (91.8-141.4) A	115.25±1.57ab (67.3-148.7) A	110.93±1.22 c (63.4-137.7) A	117.72±1.12 a (85.2-153.0) A	106.60±1.40 c (104.0-140.9)A
		Beginning of hibernation	107.17±1.20 a (70.6-135.8) B	107.06±1.16 a (71.8-139.0) B	106.28±1.58 a (68.4-135.8) B	106.57±1.40 a (77.3-142.7) B	108.69±1.01 a (64.2-129.4) B	103.39±1.25 a (62.7-134.0) B
		Beginning of active life periods	105.54±1.20 b (78.1-139.2) B	101.22±1.44 b (74.9-130.8) C	*	104.31±1.19 b (61.2-139.4) B	109.44±1.89 a (69.0-134.0) B	100.15±0.94 b (60.2-147.6) C
	♂	Beginning of aestivation	112.34±0.96 b (79.2-134.3)A	116.23±0.90 a (82.3-149.3) A	104.85±1.54 c (71.1-138.7) A	109.68±1.50 b (63.6-139.7) A	116.30±0.98 a (89.4-139.6) A	105.30±0.80 c (72.1-133.1) A
		Beginning of hibernation	103.97±1.26 b (67.8-133.9)B	102.21±1.23 b (67.2-132.3) B	102.60±1.40 b (70.5-131.6) A	99.34±1.67 b (76.5-125.4) B	107.77±0.94 a (80.4-139.8) B	100.56±1.09 b (62.4-132.2) B
		Beginning of active life periods	101.67±1.16 a (73.5-149.6)B	95.11±1.65 b (53.2-121.8) C	*	98.55±1.16 b (69.6-131.1) B	104.46±2.05 a (72.0-134.2) B	96.16±1.03 b (51.8-132.1) C

<sup>1</sup>Each gender has been evaluated within itself.

<sup>a</sup>Values with different minuscule in the same row are statistically different from each other.

<sup>A</sup>Values with different majuscule in the same column are statistically different from each other.

<sup>1</sup>Individuals were taken for fat analysis without weighing.

The differences in the mass in the life cycles at the beginning of aestivation have also been stated above, depending on the climatic conditions from the age of the new generation to the exit to overwintering for aestivation. Temperature (mean; min.; max.), relative humidity, and wind speed in the period until the exit of new generation adults to the

overwintering site affect the nutritional performance of the pest (Table 9). As shown in Table 9, the increase in mass was high in parallel with the temperature, whereas the increase in mass was also low as high relative humidity and wind speed reduce the nutritional performance of the pest.

**Table 9.** Relationship between body fat mass and some climatic data

Variables	Statistical data			
	r	r <sup>2</sup>	Sign.	
Body weight (mg)	Mean	0.748	0.560	0.000**
	Minimum	0.707	0.499	0.000**
	Maximum	0.753	0.567	0.000**
Relative humidity (%)	-0.682	0.465	0.000**	
Average wind speed (km/h)	-0.459	0.211	0.021*	

\*The correlation is significant at the 0.05 level.

\*\*The correlation is significant at the 0.01 level.



At the beginning of hibernation, only the weights obtained from male individuals in the TV tower overwintering site differed according to cycles. These differences were parallel to the difference at the beginning of aestivation (Table 8). As a result of the correlation analysis, it is revealed that a significant part of the difference at the beginning of hibernation was due to the mass at the beginning of aestivation ( $r = 0.612$ ;  $r^2 = 0.374$ ;  $P = 0.004$ ). At the beginning of the active period, we believe that the differences in mass of males and females in both overwintering sites according to their life cycle change depending on the nutritional performance of the new generation adult and the conditions during aestivation and hibernation periods.

When evaluated fresh body mass according to gender; although it varies according to overwintering site, critical period and life cycle, there was a in favor of the female individual in the differences detected (Table 10). Similarly, Strogaia (1955), Vinogradova (1969),

Taranukha et al. (1967), Yüksel (1968), Lazarov et al. (1969), Gospodinov (1973), Özkan and Kansu (1987), Javahery (1993), Popov (1977 2002), Karaca et al. (2003) and Amiri et al. (2010) report that females were generally heavier than males.

Although there was usually no difference in body mass of adult according to overwintering site in which they were collected, it has been revealed that individuals collected from Ekecik overwintering site have higher body mass according to gender, critical period, and life cycle (Table 11). Lazarov et al. (1969) reported, as a result of their work in Bulgaria in 1964-1967, adults of *E. integriceps* in the 150-200 meters deep from the edge of the forest heavier than individuals at 50 meters deep from the edge of the forest. Likewise, Areshnikov et al. (1977) stated that the body mass of individuals collected from overwintering site in different regions in Ukraine varied according to the regions.

**Table 10.** Male and female body mass (mg) collected from Ekecik and TV tower overwintering sites in the beginning of aestivation, hibernation and active life periods in 2013-2018

Overwintering site	Critical period	Gender	Body weight (mg)					
			mean $\pm$ standard error of mean					
			(min. - max.)					
			Life cycle 1 2013-2014	Life cycle 2 2014-2015	Life cycle 3 2015-2016	Life cycle 4 2016-2017	Life cycle 5 2017-2018	Life cycle 6 2018-2019
Ekecik (Aksaray province)	Beginning of aestivation <sup>1</sup>	♀	113.42 $\pm$ 1.30 a (87.3-154.6)	116.79 $\pm$ 0.83 a (88.0-152.3)	115.11 $\pm$ 1.65 a (87.9-151.6)	114.67 $\pm$ 1.41 a (85.7-158.2)	119.63 $\pm$ 1.09 a (77.6-159.8)	112.60 $\pm$ 0.08 a (83.7-149.3)
		♂	114.71 $\pm$ 1.38 a (88.0-154.8)	114.60 $\pm$ 0.99 a (84.7-153.9)	111.45 $\pm$ 1.50 a (87.8-156.8)	114.08 $\pm$ 1.32 a (87.5-151.4)	117.65 $\pm$ 1.78 a (82.8-163.7)	109.80 $\pm$ 0.80 a (82.6-143.5)
	Beginning of hibernation	♀	104.46 $\pm$ 1.39 a (87.1-120.1)	108.42 $\pm$ 0.78 a (82.8-139.5)	104.78 $\pm$ 1.54 a (67.5-152.5)	107.55 $\pm$ 1.49 a (70.7-150.8)	107.87 $\pm$ 1.37 a (74.0-154.1)	106.39 $\pm$ 1.29 a (64.6-166.0)
		♂	106.21 $\pm$ 1.31 a (81.5-133.8)	107.00 $\pm$ 0.89 a (68.9-143.1)	103.15 $\pm$ 1.27 a (66.7-124.0)	103.13 $\pm$ 1.45 b (78.6-142.1)	105.40 $\pm$ 1.57 a (67.2-139.7)	103.14 $\pm$ 1.38 a (51.0-152.3)
	Beginning of active period	♀	105.79 $\pm$ 1.23 a (75.6-128.8)	98.83 $\pm$ 1.70 a (61.7-125.9)	*	106.29 $\pm$ 1.27 a (70.3-145.0)	111.99 $\pm$ 1.68 a (64.0-142.0)	103.96 $\pm$ 0.68 a (63.1-142.8)
		♂	102.07 $\pm$ 1.16 b (75.6-131.2)	102.15 $\pm$ 1.56 a (68.1-143.2)	*	102.22 $\pm$ 1.35 b (61.9-155.8)	103.77 $\pm$ 1.92 b (61.7-128.0)	97.63 $\pm$ 0.72 b (64.5-129.4)
TV tower (Kırşehir province)	Beginning of aestivation	♀	112.90 $\pm$ 1.53 a (72.7-136.8)	117.69 $\pm$ 0.87 a (91.8-141.4)	115.25 $\pm$ 1.57 a (67.3-148.7)	110.93 $\pm$ 1.22 a (63.4-137.7)	117.72 $\pm$ 1.12 a (85.2-153.0)	106.60 $\pm$ 1.40 a (104.0-140.9)
		♂	112.34 $\pm$ 0.96 a (79.2-134.3)	116.23 $\pm$ 0.90 a (82.3-149.3)	104.85 $\pm$ 1.54 b (71.1-138.7)	109.68 $\pm$ 1.50 a (63.6-139.7)	116.30 $\pm$ 0.98 a (89.4-139.6)	105.30 $\pm$ 0.80 a (72.1-133.1)
	Beginning of hibernation	♀	107.17 $\pm$ 1.20 a (70.6-135.8)	107.06 $\pm$ 1.16 a (71.8-139.0)	106.28 $\pm$ 1.58 a (68.4-135.8)	106.57 $\pm$ 1.40 a (77.3-142.7)	108.69 $\pm$ 1.01 a (64.2-129.4)	103.39 $\pm$ 1.25 a (62.7-134.0)
		♂	103.97 $\pm$ 1.26 b (67.8-133.9)	102.21 $\pm$ 1.23 b (67.2-132.3)	102.60 $\pm$ 1.40 b (70.5-131.6)	99.34 $\pm$ 1.67 b (76.5-125.4)	107.77 $\pm$ 0.94 a (80.4-139.8)	100.56 $\pm$ 1.09 a (62.4-132.2)
	Beginning of active period	♀	105.54 $\pm$ 1.20 a (78.1-139.2)	101.22 $\pm$ 1.44 a (74.9-130.8)	*	104.31 $\pm$ 1.19 a (61.2-139.4)	109.44 $\pm$ 1.89a (69.0-13.4)	100.15 $\pm$ 0.94 a (60.2-147.6)
		♂	101.67 $\pm$ 1.16 b (73.5-149.6)	95.11 $\pm$ 1.65 b (53.2-121.8)	*	98.55 $\pm$ 1.16 b (69.6-131.1)	104.46 $\pm$ 2.05 b (72.0-134.2)	96.16 $\pm$ 1.03 b (51.8-132.1)

<sup>a</sup>Values with different minuscule in the same column are statistically different from each other.

<sup>1</sup>Each critical period has been evaluated within itself.

Individuals were taken for fat analysis without weighing.

**Table 11.** Body weight (mg) of male and female individuals collected from Ekecik and TV tower overwintering sites in the beginning of aestivation, hibernation and active life periods in 2013-2019

Gender	Critical period	Overwintering site	Body weight (mg) mean ± standard error of mean (min. – max.)					
			Life cycle 1 2013-2014	Life cycle 2 2014-2015	Life cycle 3 2015-2016	Life cycle 4 2016-2017	Life cycle 5 2017-2018	Life cycle 6 2018-2019
♀	Beginning of aestivation <sup>1</sup>	Ekecik	113.42±1.30 a (87.3-154.6)	116.79±0.83 a (88.0-152.3)	115.11±1.65 a (87.9-151.6)	114.67±1.41 a (85.7-158.2)	119.63±1.09 a (77.6-159.8)	112.60±0.08 a (83.7-149.3)
		TV tower	112.90±1.53 a (72.7-136.8)	117.69±0.87 a (91.8-141.4)	115.25±1.57 a (67.3-148.7)	110.93±1.22 b (63.4-137.7)	117.72±1.12 a (85.2-153.0)	106.60±1.40 b (104.0-140.9)
	Beginning of hibernation	Ekecik	104.46±1.39 a (87.1-120.1)	108.42±0.78 a (82.8-139.5)	104.78±1.54 a (67.5-152.5)	107.55±1.49 a (70.7-150.8)	107.87±1.37 a (74.0-154.1)	106.39±1.29 a (64.6-166.0)
		TV tower	107.17±1.20 a (70.6-135.8)	107.06±1.16 a (71.8-139.0)	106.28±1.58 a (68.4-135.8)	106.57±1.40 a (77.3-142.7)	108.69±1.01 a (64.2-129.4)	103.39±1.25 a (62.7-134.0)
	Beginning of active period	Ekecik	105.79±1.23 a (75.6-128.8)	98.83±1.70 a (61.7-125.9)	*	106.29±1.27 a (70.3-145.0)	111.99±1.68 a (64.0-142.0)	103.96±0.68 a (63.1-142.8)
		TV tower	105.54±1.20 a (78.1-139.2)	101.22±1.44 a (74.9-130.8)	*	104.31±1.19 a (61.2-139.4)	109.44±1.89 a (69.0-134.0)	100.15±0.94 a (60.2-147.6)
♂	Beginning of aestivation <sup>1</sup>	Ekecik	114.71±1.38 a (88.0-154.8)	114.60±0.99 a (84.7-153.9)	111.45±1.50 a (87.8-156.8)	114.08±1.32 a (87.5-151.4)	117.65±1.78 a (82.8-163.7)	109.80±0.80 a (82.6-143.5)
		TV tower	112.34±0.96 a (79.2-134.3)	116.23±0.90 a (82.3-149.3)	104.85±1.54 b (71.1-138.7)	109.68±1.50 b (63.6-139.7)	116.30±0.98 a (89.4-139.6)	105.30±0.80 a (72.1-133.1)
	Beginning of hibernation	Ekecik	106.21±1.31 a (81.5-133.8)	107.00±0.89 a (68.9-143.1)	103.15±1.27 a (66.7-124.0)	103.13±1.45 a (78.6-142.1)	105.40±1.57 a (67.2-139.7)	103.14±1.38 a (51.0-152.3)
		TV tower	103.97±1.26 a (67.8-133.9)	102.21±1.23 b (67.2-132.3)	102.60±1.40 a (70.5-131.6)	99.34±1.67 a (76.5-125.4)	107.77±0.94 a (80.4-139.8)	100.56±1.09 a (62.4-132.2)
	Beginning of active period	Ekecik	102.07±1.16 a (75.6-131.2)	102.15±1.56 a (68.1-143.2)	*	102.22±1.35 a (61.9-155.8)	103.79±1.92 a (61.7-128.0)	97.63±0.72 a (64.5-129.4)
		TV tower	101.67±1.16 a (73.5-149.6)	95.11±1.65 b (53.2-121.8)	*	98.55±1.16 b (69.6-131.1)	104.46±2.05 a (72.0-134.2)	96.16±1.03 a (51.8-132.1)

<sup>1</sup>Each critical period was evaluated within itself.

<sup>a</sup>Values with different minuscule in the same column are statistically different from each other.

<sup>\*</sup>Individuals were taken for fat analysis without weighing.

*Relationship body mass and fat ratios*

It was determined that there was a significant positive correlation between body mass and fat ratios, increase in fat ratio in direct proportion to the increase in weight ( $r= 0.862$ ;  $r^2= 0.744$ ;  $P=0.000$ ). The similar results were obtained when have evaluated according to gender (in female individuals,  $r= 0.823$ ;  $r^2= 0.678$ ;  $P= 0.000$ ; in male individuals,  $r= 0.883$ ;  $r^2= 0.779$ ;  $P= 0.000$ ).

It is also stated by different researchers that there was a positive relationship between body mass and nutrient reserves and that individuals with large bodies generally have more fat reserves than small ones (Sinclair and Marshall 2018, Williams et. al. 2015). Popov (1977, 2002) reported that there was positive linear relationship ( $r= 0,885$ ) between the body mass and fat ratio of the sunn pest, and that as the mass increases in both genders, there was a similar increase in the fat ratio. Areshnikov et al. (1977) stated that, there was a positive relationship between body mass and fat ratios of adults in the overwintering site.

High fat ratio increases the survival rate during diapause as well as increases reproductive. High fat reserves, along with favorable climatic conditions, affect the size of the adult population in the overwintering site. As a result of our studies (Table 12), especially individuals of females have a fat content of over 27% at the beginning of the active life period, high temperature, low rainfall, and wind speed conditions during the active life period increase the reproductive power of the pest. Consequently, there was an increase in the overwintering population at the beginning of aestivation. If the situation in question continues for two years in a row, sunn pest outbreaks begin.

Similarly, Zwölfer (1942), Yüksel (1968), Racz (1975) reported that the sunn pest may cause an outbreak if suitable climatic conditions occur for two consecutive years with the presence of physiologically strong individuals in the population. Forecast and warning systems are decision support systems, by anticipating pest outbreaks that help to optimizing pest control, reduce costs, and ultimately

**Table 12.** Hibernation period of densities (adult m<sup>-2</sup>), female fat ratio (%)

Overwintering site	Critical periods	Gender	Lifecyle (Years)						
			Life cycle 1 2013-2014	Life cycle 2 2014-2015	Life cycle 3 2015-2016	Life cycle 4 2016-2017	Life cycle 5 2017-2018	Life cycle 6 2018-2019	Life cycle 6 2019-2020
Ekecik (Aksaray province)	Beginning of aestivation	Adult density	12,00*	53.50	56.00	41.50	61.00	<b>298.00</b>	<b>324.50</b>
		Fat ratio (%)	39.57	40.36	39.00	37.21	40.18	39.00	37.16
	Beginning of hibernation	Adult density	11.00*	48.00	38.50	29.14	49.00	282.00	147.00
		Fat ratio (%)	28.60	29.98	27.75	26.86	32.08	30.54	26.57
	Beginning of active period	Adult density	10.00*	45.50	22.54	21.67	39.00	231.00	
		Fat ratio (%)	26.66	23.57	24.75	24.67	27.50	29.02	
TV tower (Kırşehir province)	Beginning of aestivation	Adult density	50.00	51.33	50.00	51.00	78.00	<b>273.00</b>	<b>266.50</b>
		Fat ratio (%)	38.94	38.92	37.18	38.26	39.93	39.07	37.43
	Beginning of hibernation	Adult density	46.00	53.33	32.44	27.00	47.00	293.00	174.00
		Fat ratio (%)	27.96	31.02	31.42	28.52	30.68	32.61	28.19
	Beginning of active period	Adult density	42.00	39.00	21.37	24.96	38.00	250.00	
		Fat ratio (%)	23.47	26.87	26.18	26.11	<b>27.19</b>	<b>28.84</b>	

\* Survey area has been changed. In the following years, compared to the new survey area, approximately 30 more adults per square meter were recorded.

minimize product loss, by predicting pest outbreaks and providing time to manage outbreaks to occur.

One of the important indicators that we can use in the forecast of the sunn pest outbreak is that the pest is physiologically strong, together with suitable climatic conditions and nutrient abundance. Especially fat is (fats are) of vital importance in pests such as sunn pest that spend a large part of their life cycle in diapause. High fat ratio not only increases the survival rate during diapause but also affects the spread of the pest to wider areas by increasing the migration performance from overwintering site after diapause, also increases the reproductive potency. There is a positive relationship between body mass and fat ratios. Since individuals with large bodies generally have a higher percentage of fat reserves, they show higher performance than small ones in the period after diapause and during diapause.

As a result of our studies (Table 12), especially individuals of females have a fat content of over 27% at the beginning of the active life period, high temperature, low rainfall, and wind speed conditions during active life period increase the reproductive power of the pest. Consequently, there is an increase in the overwintering population at the beginning of aestivation. If the situation in question continues for two years in a row, sunn pest outbreaks begin.

Another important result obtained as a result of the study was that the population of sunn pest has a very heterogeneous structure in overwintering sites. The population varies in terms of both body mass and fat ratio depending on individuals, gender, years, and critical

periods. It has been demonstrated that the results that have been obtained are important indicators to be used in conjunction with climate and nutrient in the forecast and warning system to be created to forecast sunn pest outbreaks.

## ÖZET

Süne (*Eurygaster maura* L. (Hem: Scutelleridae)) salgınlarının tahmin edilmesini amaçlayan bu çalışma; iki kışakta 2013-2019 yıllarında 6 yaşam döngüsü süresince yürütülmüştür. Çalışma süresince her yaşam döngüsünde 3 kritik periyod; yazlama, kışlama ve aktif yaşam dönemleri başlangıçlarında kışlak yoğunlukları ile sünenin yağ oranları ve ağırlıkları belirlenmiş, aralarındaki ilişkiler saptanmıştır. Süne kışlak popülasyonu hem ağırlık, hem de yağ oranları açısından cinsiyetlere, yaşam döngülerine, kritik dönemlere bağlı olarak farklılıklar gösterdiği, vücut kitlesi ile yağ oranları arasında pozitif bir ilişkinin bulunduğu belirlenmiştir. Yağ oranının uygun iklim koşulları ve besin bolluğu ile birlikte süne salgın tahminlerinde kullanabileceğimiz önemli göstergelerden olduğu, hibernasyon sonrası yağ oranının yüksek; özellikle dişi bireylerde %27 ve üzerinde, aktif yaşam süresince iklim koşullarının uygun; yüksek sıcaklık, düşük orantılı nem, rüzgar hız ve yağış ile uygun besin bolluğunun olduğu koşulların üst üste iki yıl süreyle gerçekleşmesi durumunda popülasyon büyüklüğünde önemli ölçüde artışların olduğu ve zararlının salgın oluşturabildiği ortaya konulmuştur.

Anahtar kelimeler: besin, fizyolojik durum, iklim, tahmin ve uyarı

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Original article

## Determination of thrips (Thysanoptera) fauna on stone and pome fruit trees in Balıkesir province, Türkiye

Balıkesir ilinde taş ve yumuşak çekirdekli meyve ağaçlarındaki thrips (Thysanoptera) türlerinin saptanması

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### ABSTRACT

This study was carried out to identify species belonging to Thysanoptera in stone and pome fruit orchards in the districts (Balya, Bandırma, Bigadiç, Burhaniye, Dursunbey, Erdek, Gönen, Havran, Kepsut and Manyas) of Balıkesir Province, Turkey in 2018 and 2019. The survey revealed 32 Thysanoptera (thrips) species. Thrips were examined in 9900 flowers, leaves, and fruits from 198 orchards during the research. The most widespread (total number of samples) and abundant (total number of individuals) species were: *Taeniothrips inconsequens* (Uzel) (Thripidae: Thysanoptera) (158 specimens- 2922 individuals), *Haplothrips reuteri* (Karny) (Phlaeothripidae: Thysanoptera) (76- 529), *Thrips meridionalis* (Priesner) (55- 359) and *Thrips minutissimus* Linnaeus (21- 209) (Thripidae: Thysanoptera). The highest number of thrips specimens were detected from cherry (*Prunus avium* L.), followed by apple (*Malus domestica* L.), peach (*Prunus persica* Batsch), and plum (*Prunus domestica* L.) trees. *Ta. inconsequens* predominated the thrips fauna on peach and cherry. *H. reuteri* was the most abundant species on pear. Thrips were found in 41.94% of flowers, while they were detected in 1.83% and 1.16% of fruit and leaves. This study was designed to fill the gaps in the data such as diversity in plant species associated, spatial distribution on plant parts, and temporal distribution in relation to phenological periods.

### INTRODUCTION

Turkey is ranking as the first country in the world in the production of cherry and sour cherry. Turkey produces 19.2% of the world's total production of cherries, 13% of sour cherries, 8% of apricots, 3% of apples and peaches, and 2% of pears and plums. Turkey's production most of which are stone and pome fruit production (tons) are apple (4.300.486), apricot (833.398), peach (729.804), cherry

(724.944), pear (545.569), plum (329.056), and sour cherry (189.184) (TUIK 2020).

Many studies have been conducted on the pests and beneficial species of stone and pome fruit trees in Turkey (Atakan 2008a, 2008b, 2008c, Bolu et al. 2007, Çınar et al. 2004, Ertop and Özpinar 2011, Hazır et al. 2011, Güven 2013, Kaplan and Tezcan 2011, Özder 1998, Öztürk and

Atakan 2008, Tezcan 1995, Tezcan and Önder 1999, Ulusoy et al. 1999,2009).

Approximately 6.288 species of thrips are known in the world (Thrips Wiki 2018). From Turkey, 194 species belonging to the order of Thysanoptera are registered (Başar and Yaşar 2018, Tunç and Hastenpflug-Vesmanis 2016) and Thysanoptera species constitute 0.57% of the species in the Turkish insect fauna (Tezcan 2020).

Bagnall (1934) provided the first faunistic report on the Thysanoptera fauna of Turkey. Lodos (1993) was the first to compile a list of species belonging to the order Thysanoptera in Turkey (Lodos 1993). Tunç and Hastenpflug-Vesmanis (2016) provided the species list of Turkey's Thysanoptera fauna, which contains new records for Turkey. Other researchers have contributed significantly to this issue (Atakan 2008a, 2008b, 2008c, 2009, Barış et al. 2019, Hazır et al. 2011, Kaplan et al. 2016, Maya and Tezcan 2018, Ölçülü and Atakan 2013, Özsemceri et al. 2006, Öztürk and Atakan 2008, Şahin and Tezcan 2014, Uzun et al. 2015).

Although some published studies related to insect species found in stone and pome fruit orchards in Balıkesir have been reported (Erözmen and Yaşar 2017, Giray 1969), there was no record of species belonging to the Thysanoptera order in these studies.

Gargani (1996) stated that *Thrips tabaci* Lindeman, 1889, *Th. major* Uzel, 1895, *Th. angusticeps* Uzel, 1895 and *Th. minutissimus* Linnaeus, 1758 (Thripidae: Thysanoptera) were commonly seen in nectarine, peach, and plum in Tuscany (Italy). He reported that other thrips species, except *Th. major*, were harmful during the development and ripening stages of fruits, and this damage reached 40-60% in nectarines. Thrips cause different types of damage depending on where they are found. In California and New Zealand, the damage is caused both by larval feeding on immature fruit at fruit petal fall, which results in severe reddish-brown of the fruit surface, as well as by silvery injury (Bournier 1970). It has been reported that *Th. major* causes severe spots and deformities in citrus fruits in several North African countries, with adults and larvae feeding on blooms and fruits (Bournier 1963).

Atakan (2008a), in the study on apple, plum, and nectarine trees in Adana, determined that *Frankliniella occidentalis* (Pergande, 1895) is an economically important thrips species. In a previous study to determine Thysanoptera species in nectarines and their fruit damage, it was discovered that thrips (primarily larvae) feeding on the ovary causes brown scar tissue formation in the fruit, as well as discoloration called silvery on the fruit surface during the pre-harvest period (Hazır et al. 2011). The major species

in nectarine blossoms was *F. occidentalis*, according to a study conducted in Balcalı (Adana), and this species caused spotting in fruits at rates ranging from 5-30 percent (Atakan 2008a). The percentage of fruits with thrips injury was highest on nectarines, 29, followed by plums, 17, according to a study conducted in orchards in the Çukurova Region (Atakan 2008d).

In this study, we aimed to determine the species of Thysanoptera, their local distribution, frequency, and abundance; to ascertain species composition, spatial distribution on plant parts, and temporal distribution in relation to phenological periods on stone and pome fruit orchards in the districts of Balıkesir province (which is an important production area of stone and pome fruits in the western part of Turkey).

The data collected contribute to the knowledge of Thysanoptera fauna of one of the least touched parts of Turkey in this regard. The gaps in the data such as diversity in plant species associated, spatial distribution on plant parts, and temporal distribution in relation to phenological periods are filled.

## MATERIALS AND METHODS

The material collected from ten districts in Balya, Bandırma, Bigadiç, Burhaniye, Dursunbey, Erdek, Gönen, Havran, Kepsut, and Manyas districts of Balıkesir Province from March to August in 2018 and 2019. The field surveys were conducted in 198 orchards in the districts studied, and included apple (*Malus domestica* L.), cherry (*Prunus avium* L.), peach (*Prunus persica* B.), pear (*Pyrus communis* L.), plum (*Prunus domestica* L.), and sour cherry (*Prunus cerasus* L.) trees. The pesticide was not used to control thrips in the orchards sampled for this study.

The number of orchards sampled was determined by taking into account the actual fruit production potential of the districts. The number of orchards investigated in each district were Balya (8), Bandırma (25), Bigadiç (22), Burhaniye (15), Dursunbey (26), Erdek (9), Gönen (33), Havran (35), Kepsut (15), Manyas (10).

A total of 50 flowers and 50 leaf samples were taken from 13 randomly selected trees in each grove, by walking diagonally. The collected samples were placed in paper bags, after that, the samples were placed in a plastic bag with the district, date, and garden number written on them and brought to the laboratory in an icebox. For fruit sampling, the beating method was applied for a total of 50 fruits, from four sides of 13 trees. Thrips on fruits were collected by beating them on a 19 × 31 cm white cloth stretched over a frame in the field. And, this was repeated three times for the early, mid-season, and late-maturing varieties. Thus, 9.900 flowers, 9.900



leaves, and 9.900 fruits were sampled. Flowers were sampled in March and April, while leaves and fruits were sampled in March, April, May, June, July, and August, depending on the fruit variety. The flower and leaf samples brought to the laboratory were brushed separately into white dishes using sable brushes. Each sample was individually shaken into a white tray with a sable brush, and the thrips that fell into the tray were collected using the brush. The flower petals and sepals were dissected and examined for the separation of the remaining thrips if any. The specimens were placed into Eppendorf tubes containing preservation fluid, AGA, (9 parts 60% ethyl alcohol, 1-part glycerine, and 1 part acetic acid). After a preliminary inspection and identification with a stereo microscope, the collected thrips were preserved in 60% ethyl alcohol. Samples whose diagnosis has been completed are labeled and kept in the collection of Balıkesir University, Faculty of Arts and Sciences, Department of Biology, Zoology Museum.

The pre-diagnosis process of thrips was made by comparing with previously identified specimens and using Zur Strassen (2003). Pre-diagnosed specimens were identified/confirmed by Prof. Dr. Ekrem ATAĞAN.

#### Data analysis and evaluation

A simple district or host index was used to analyze the distribution of a certain thrips species in the districts or on different plants. The following formulas were used to determine the district and host indices: the total number of individuals of a given thrips species in the district divided by the total number of samples in the district gives the district index.

The total number of individuals of a certain thrips species on the plant species divided by the total number of samples from the host species is the host index.

## RESULTS AND DISCUSSION

A total of 32 species belonging to four families of Thysanoptera were identified as a result of this research, as shown in Table 1. The species found are listed with their frequency (number of samples in which the species was found) and abundance (total number of individuals of the species collected).

Most of the common species were from Thripidae; representatives of Phlaeothripidae, Aeolothripidae, and Melanthripidae were relatively less common.

The most common and abundant species, with the number of samples-specimens they were represented by, were *Taeniothrips inconsequens* (Uzel, 1895) (158 specimens-2922 individuals), *Haplothrips reuteri* (Karny, 1907) (76-529), *Thrips meridionalis* (Priesner, 1926) (55- 359) and *Th.*

*minutissimus* Linnaeus, 1758 (21- 209).

The number of the specimens found was 2481 and 1967 in 2018 and 2019, respectively. The abundance of the thrips species was highest in April in both years regarding the seasonal distribution of the species by months. Plums and peaches were at the pink bud stage in March 2019, daily minimum temperatures (°C) dropped below zero, causing frost damage to fruit trees in the winter and late spring. While the number of thrips collected in March 2018 was 644, the number of thrips collected in March 2019 was 143. Lower temperatures in 2019 resulted in a fall in the number of thrips collected, according to research. In the previous study carried out in fruit orchards in Antalya, it was stated that *Th. meridionalis*, which was the most common species, could not be observed in June-October, but continued to exist in winter and spring (Tunç 1989b). Consistent with the results obtained from this study conducted in Antalya, it was observed in this study that *Th. meridionalis* could not be sampled in the summer months (from June to October), but could be sampled in the spring months.

The species reported from Balıkesir for the first time; *Haplothrips andresi* Priesner, *H. arenarius* Priesner, *H. distinguendus* (Uzel), *H. globiceps* (Bagnall) and *H. minutus* (Uzel) (Phlaeothripidae), *Th. angusticeps* Uzel, *Th. australis* (Bagnall), *Th. euphorbiae* Knechtel, *Th. dubius* Priesner, *Th. mareoticus* (Priesner), *Th. pillichi* Priesner and *Th. simplex* (Morison) (Thripidae); *Aeolothrips ericae* Bagnall, *A. fasciatus* (Linnaeus), *A. gloriosus* Bagnall, *A. priesneri* Knechtel, *A. versicolor* Knechtel and *Orothrips priesneri* (Titschack) (Aeolothripidae); *Melanthrips pallidior* Priesner and *M. rivnayi* Priesner, (Melanthripidae).

*Aeolothrips collaris*, *A. gloriosus*, *A. intermedius*, *M. fuscus*, *M. pallidor*, *Or. priesneri*, *F. occidentalis*, *Ox. ajugae*, *T. inconsequens*, *T. frici*, *T. angusticeps*, *T. major*, *T. meridionalis*, *T. minutissimus*, *T. tabaci*, *Haplothrips aculeatus*, *H. globiceps*, *H. reuteri* have been recorded previously in the fruit production areas in different regions of Turkey (Maya and Tezcan 2018, Şahin and Tezcan 2014, Tunç 1989a, 1989b, Uzun et al. 2015).

#### Distribution of thrips species in districts

Out of 32 species 22 (68.75%) were found in Havran, 20 (62.50%) in Dursunbey, 16 (50.00%) in Bigadiç, 13 (40.62%) in Kepsut, 10 (32.25%) in Gönen, 9 (28.12%) in Burhaniye and Bandırma, 8 (25.00%) in Manyas, 6 (18.00%) in Balya, and 2 (2%) in Erdek.

*Ta. inconsequens* was found in all areas, but *H. reuteri*, *Th. meridionalis* and *Th. minutissimus* were less common and tended to be confined locally (Table 2). The majority (44.07%) of the specimens were collected in Bigadiç. The

**Table 1.** List of identified thrips species with their overall frequency and overall abundance on stone and pome fruit trees in Balıkesir region of Turkey during 2018-2019

Family/ Species	Yearly frequency		Yearly abundance		Overall frequency	%	Overall abundance	
	2018	2019	2018	2019				%
<i>Aeolothripidae</i>								
<i>Aeolothrips ericae</i>	2	0	3	0	2	0.40	3	0.07
<i>Aeolothrips fasciatus</i>	3	2	4	6	5	1.01	10	0.22
<i>Aeolothrips gloriosus</i>	5	2	6	6	7	1.41	12	0.27
<i>Aeolothrips intermedius</i>	10	0	11	0	10	2.02	11	0.25
<i>Aeolothrips priesneri</i>	1	2	1	2	3	0.61	3	0.07
<i>Aeolothrips versicolor</i>	1	0	1	0	1	0.20	1	0.02
<i>Orothrips priesneri</i>	2	0	2	0	2	0.40	2	0.04
<i>Melanthripidae</i>								
<i>Melanthrips fuscus</i>	0	1	0	1	1	0.20	1	0.02
<i>Melanthrips pallidior</i>	3	0	3	0	3	0.61	3	0.07
<i>Melanthrips rivnayi</i>	1	0	1	0	1	0.20	1	0.02
<i>Thripidae</i>								
<i>Frankliniella occidentalis</i>	16	9	37	34	25	5.05	71	1.60
<i>Oxythrips ajugae</i>	2	10	3	35	12	2.42	38	0.85
<i>Taeniothrips inconsequens</i>	99	59	1558	1364	158	31.92	2922	65.69
<i>Tenotherips frici</i>	0	1	0	1	1	0.20	1	0.02
<i>Thrips angusticeps</i>	16	10	48	30	26	5.25	78	1.75
<i>Thrips australis</i>	2	3	14	7	5	1.01	21	0.47
<i>Thrips dubius</i>	4	0	9	0	4	0.81	9	0.20
<i>Thrips euphorbia</i>	1	0	1	0	1	0.20	1	0.02
<i>Thrips major</i>	7	5	18	10	12	2.42	28	0.63
<i>Thrips mareoticus</i>	1	0	6	0	1	0.20	6	0.13
<i>Thrips meridionalis</i>	39	16	225	134	55	11.11	359	8.07
<i>Thrips minutissimus</i>	12	9	146	63	21	4.24	209	4.70
<i>Thrips pillichi</i>	9	3	20	9	12	2.42	29	0.65
<i>Thrips simplex</i>	2	0	2	0	2	0.40	2	0.04
<i>Thrips tabaci</i>	12	9	15	41	21	4.24	56	1.26
<i>Phlaeothripidae</i>								
<i>Haplothrips aculeatus</i>	1	1	1	1	2	0.40	2	0.04
<i>Haplothrips andresi</i>	15	0	24	0	15	3.03	24	0.54
<i>Haplothrips arenarius</i>	0	1	0	1	1	0.20	1	0.02
<i>Haplothrips distinguendus</i>	1	0	1	0	1	0.20	1	0.02
<i>Haplothrips globiceps</i>	0	1	0	1	1	0.20	1	0.02
<i>Haplothrips minutus</i>	3	5	3	10	8	1.62	13	0.29
<i>Haplothrips reuteri</i>	55	21	318	211	76	15.35	529	11.89
<i>Total</i>			495		4.448			

district index for Bigadiç was 13.85. Based on the district index and the percentage of specimens in a total number of specimens collected in a given district *Ta. inconsequens* was the dominant species in Bigadiç and Kepsut districts.

#### Distribution of thrips in fruit orchards

As a result of the field studies carried out in 2018-2019, the highest number of thrips specimens were collected from cherry which is 2.168, followed by apple with 1.227 and peach with 733, 205 pieces of plum, 101 pears, and 14 sour cherries.

*Ta. inconsequens* was present on all stone and pome fruit species. But the other major thrips species, *H. reuteri*, *Th. meridionalis* and *Th. minutissimus* were not found in some fruit species and found very low numbers on other fruit species (Table 3).

Based on the percentage of specimens in a total number of specimens collected on all fruit species and values of the host index, *Ta. inconsequens* predominated thrips fauna on peach and cherry whereas *H. reuteri* was the most abundant species on pear.

**Table 2.** List of identified thrips species with their overall frequency and overall abundance on stone and pome fruit trees in Balıkesir region of Turkey during 2018-2019

District	Number of individuals and district index <sup>a</sup> of the species										
	Total number of			<i>Taeniothrips inconsequens</i>		<i>Haplothrips reuteri</i>		<i>Thrips meridionalis</i>		<i>Thrips minutissimus</i>	
	Individuals	Samples	General index	No.	Index	No.	Index	No.	Index	No.	Index
Balya	16	8	2.0	2	0.3	1	0.1	0	0	2	0.3
Bandırma	129	24	5.4	70	2.9	12	0.5	37	1.5	1	0
Bigadiç	1422	93	15.3	1288	13.8	42	0.5	0	0	10	0.1
Burhaniye	67	15	4.5	9	0.6	22	1.5	15	1.0	0	0
Dursunbey	942	120	7.9	486	4.1	209	1.7	133	1.1	18	0.2
Erdek	32	5	6.4	24	4.8	0	0	0	0	0	0
Gönen	616	77	8.0	304	3.9	170	2.2	77	1.0	0	0
Havran	611	89	6.9	265	3.0	40	0.4	36	0.4	173	1.9
Kepsut	501	36	13.9	428	11.9	9	0.3	45	1.3	3	0.1
Manyas	112	28	4.0	46	1.6	24	0.9	16	0.6	2	0.1
Total	4.448	495		2.922		529		359		225	

<sup>a</sup>The total number of individuals of a given thrips species in the district divided by the total number of samples in the district yields the district index.

*Th. australis*, popularly known as eucalyptus thrips (Mound 2010), was initially recorded in plum and cherry flowers at three separate places (Burhaniye, Dursunbey, and Havran) in Balıkesir.

*Ta. inconsequens*, *H. reuteri*, *Th. meridionalis* and *Th. minutissimus* are noteworthy by being phytophagous. Additionally, the presence of species like *M. fuscus*, *M. pallidior*, and *M. rivnayi* which feed on pollen, and species like *A. collaris*, *A. fasciatus*, *A. intermedius*, *A. versicolor*, and *Or. priesneri* which are known as predators of small arthropods in orchards are important in terms of biological diversity and natural balance (Tunç et al. 2012a, 2012b, Zur Strassen 2003).

#### Distribution of plant parts

Thrips were detected in a total of 9.900 flowers, leaves, and fruits from 198 orchards during the research. Thrips were

found in 41.94% of flowers, while they were detected in 1.83% and 1.16% of fruit and leaves.

When the distribution of Thysanoptera species was analyzed based on the plant parts it was determined that the thrips density was higher in flowers than in other plant sections. *A. ericae*, *A. fasciatus*, *A. gloriosus*, *A. intermedius*, *A. priesneri*, *A. versicolor*, *H. distinguendus*, *H. globiceps*, *H. arenarius*, *M. pallidior*, *M. rivnayi*, *M. fuscus*, *Or. priesneri*, *Te. frici*, *Th. australis*, *T. euphorbiae*, *T. major*, *T. mareoticus*, *T. simplex*, and *T. tabaci* were found in only flowers. *Ox. ajugae*, *Ta. inconsequens*, *Th. meridionalis*, *H. reuteri* were found in leaves. *Ta. inconsequens*, *Th. meridionalis*, *Th. angusticeps*, *Th. minutissimus*, *Th. pillichii*, *H. aculeatus*, *H. andresi*, *H. minutus*, and *H. reuteri* were found in fruits.

Thrips were found in high numbers during the blooming stage of fruit trees, but there were no or only a few thrips

**Table 3.** Distribution of major thrips species on stone and pome fruits species in Balıkesir in 2018-2019

Fruit species	Number of individuals and plant index <sup>a</sup> of the species										
	Total number of			<i>Taeniothrips inconsequens</i>		<i>Haplothrips reuteri</i>		<i>Thrips meridionalis</i>		<i>Thrips minutissimus</i>	
	Individuals	Samples	General index	No.	Index	No.	Index	No.	Index	No.	Index
Apple	1.227	199	6.17	559	2.81	287	1.44	173	0.87	36	0.18
Cherry	2.168	187	11.59	1.572	8.41	164	0.88	97	0.52	154	0.82
Peach	733	56	13.09	612	10.93	33	0.59	55	0.98	3	0.05
Pear	101	16	6.31	43	2.69	42	2.63	0	0	0	0
Plum	205	28	7.32	130	4.64	3	0.11	31	1.11	16	0.57
Sour cherry	14	9	1.56	6	0.67	0	0	3	0.33	0	0
Total	4.448	495		2.922		529		259		209	

<sup>a</sup>The total number of individuals of a certain thrips species on the plant species divided by the total number of samples taken from the plant species yields the host index

in the fruits after flowering, according to earlier research (Maya and Tezcan 2018, Ölçülü and Atakan 2013, Şahin and Tezcan 2014, Uzun et al. 2015).

Different kinds of fruits and vegetables are produced in Balıkesir Province because of favorable climate conditions. While peach cultivation is focused on in Kepsut district, apple cultivation is prominent in Dursunbey. Barley, safflower, wheat, rye, corn, chickpea, sunflower, forage crops are grown in Dursunbey district. In recent years, walnut orchards have increased considerably throughout the province. Dursunbey district is a mountainous region due to its high altitude (672 m) and is closed to sea breezes, and 65% of the total area of the district consists of forests. 51.61% of the species found at the end of the study were collected from this region. Especially Edremit gulf region (Havran, Burhaniye) is located in the coastal zone in terms of climate, this region has allowed the formation of fruit gardens consisting of different fruit species. Olive, mandarin, plum, and fig cultivation is carried out intensively in Havran district and covers large areas. Twenty-two (70.97%) of the species found as a result of this study carried out in the fruit production areas of Balıkesir Province were found in Havran. The reason for the high diversity of species is due to the cultivation of different cultural plants in the district. The presence of forest areas around the area where *Th. minutissimus*, which is known to be typically found in the flowers of species belonging to the genus *Quercus*, was sampled in Havran is thought to be the reason for the high number of this species.

Systematic sampling, investigations, experiments, and other work that would yield data required to define the fruit orchard ecosystems with an emphasis on their thrips associates should be carried out in different locations in a limited number of orchards throughout the year and multiple years rather than surveying orchards in different parts of the country for a limited period during the season. Such surveys should be capable to identify ecosystem variables on which the integrated pest management would be implemented.

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#### ÖZET

Taş ve yumuşak çekirdekli meyve bahçelerinde bulunan Thysanoptera takımına bağlı türleri saptamak amacıyla yürütülen bu çalışma, 2018 ve 2019 yıllarında Balıkesir

ilinin Balya, Bandırma, Bigadiç, Burhaniye, Dursunbey, Erdek, Gönen, Havran, Kepsut ve Manyas ilçelerinde gerçekleştirilmiştir. Çalışma süresince, 198 bahçedeki taş ve yumuşak çekirdekli meyve ağaçlarından alınan toplam 9900 çiçek, meyve ve yaprakta bulunan thripsler sayılmıştır. Çalışma sonunda 32 thrips (Thysanoptera) türü saptanmıştır. En yaygın ve en yüksek sayıda bireyle temsil edilen türler, bulunduğu örnek sayısı-birey sayısı ile birlikte *Taeniothrips inconsequens* (Uzel) (Thripidae: Thysanoptera) (158 örnek- 2922 birey), *Haplothrips reuteri* (Karny) (Phlaeothripidae: Thysanoptera) (76-529), *Thrips meridionalis* (Priesner) (55- 359) ve *Thrips minutissimus* Linnaeus (21- 209)'dur. En fazla sayıda thrips bireyi kiraz (*Prunus avium* L.) bahçelerinden toplanırken, bunu elma (*Malus domestica* L.), şeftali (*Prunus persica* Batsch) ve erik (*Prunus domestica* L.) izlemektedir. Şeftali ve kiraz bahçelerinde baskın tür, *Ta. inconsequens* olarak saptanmıştır. *H. reuteri* armut bahçelerinde en yaygın bulunan türdür. Buna göre, Balıkesir'de çiçeklerde thrips oranı %41.94 iken, meyvelerde %1.83 ve yapraklarda %1.16 olarak bulunmuştur. Bu çalışmada bulunan thripsler farklı bitki türleri, bitki kısımları ve farklı fenolojik dönemler hakkında bilgi eksikliğini giderecek nitelikte olup, daha önceki yıllarda yapılan çalışmalarla bütünleşecek şekilde tamamlanmıştır.

Anahtar kelimeler: kiraz, elma, şeftali, *Taeniothrips inconsequens*, *Haplothrips reuteri*, çiçek

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# Bitki Koruma Bülteni / Plant Protection Bulletin

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Original article

## The effect of hoeing time for weed management on yield and yield criteria of sunflowers (*Helianthus annuus* L.)

Ayçiçeğinde (*Helianthus annuus* L.) yabancı ot mücadelesinde çapalama süresinin verim ve verim kriterlerine olan etkisi

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### ABSTRACT

Hoeing time is important in weed management and is effective to reduce weed populations in sunflower cultivation. The study was conducted between 2018-2019 to determine the hoeing times for mechanical weed control in sunflower in Adana province of Turkey. To determine weed control time in experimental fields, weeds were allowed to germinate in natural conditions at intervals between 15 days for mechanical hoeing at the emergence of sunflower to the harvest time. The interactions between weeds and sunflower yield criteria were observed by periodic hoeing treatments. At the end of the experiments, the criteria for sunflower yield, seed yield, oil content, oil quality, head diameter, plant height, weed biomass, and coverages of hoeing time effects were determined. Combining the two-year data, it was observed that the sunflower yield and seed yield were the highest in plots of 75 and 90 days weed-free, while the lowest yield and seed yield were observed in 60, 75, and 90 days weedy. It was determined that the sunflower oil yield was higher in plots of long hoeing period time, but the oil quality did not change. It was found that sunflower height were statistically similar for each year in different hoeing period times, moreover, sunflower head diameter was not affected. In weedy plots with shorter hoeing times, higher weed biomass due to increased weed coverage was noted. As a result, it was determined that long-term hoeing in sunflower weed management increases the yield, and hoeing time is significant in sunflower weed management.

### INTRODUCTION

Sunflower (*Helianthus annuus* L.) is one of the significant crops used for oil production in Turkey similar to the world. It has an important place in terms of human health due to its ingredients such as protein, fat, and carbohydrates, and a large part of Turkey's vegetable oil production is provided from sunflower (Arioğlu 2007, Gül et al. 2016). Moreover, as

a good alternation crop, it leaves a clean and ventilated soil for the next rotation crop (Arioğlu 2007).

According to FAO data for 2018, Turkey was listed among the top 10 countries in the world in terms of sunflower harvest areas, with 2.67% of the total cultivation area (734.190 ha),

and 1.86% (26549 kg ha<sup>-1</sup>) of the world production (FAO 2018). The Marmara region had the highest production in Turkey in 2019 by providing 51.30% (381.881 ha) of sunflower cultivation. This was followed by Central Anatolia 20.05% (149.287 ha) and Mediterranean regions 13.93% (103.695 ha), respectively (TUIK 2019).

There are various pests that reduce sunflower yield and quality, and the most important of them are weeds, which cause major yield losses. Weeds compete with crops, increasing the production costs as well as decreasing the yield and quality of the crops (Oerke et al. 1994, Tepe 2014). It was reported that yield losses in sunflower, without weed management, varied between 25-75% (D'Alessandro et al. 1992, Dharam et al. 1993, Fleck et al. 1991, Heidarian et al. 2012, Kaya et al. 2020, Onofri and Tei 1994). As in the world, some important weed species in sunflower were found in Turkey such as *Acroptilon repens* (L.) DC., *Amaranthus retroflexus* L., *Chenopodium album* L., *C. vulvaria* L., *Convolvulus arvensis* L., *Cuscuta campestris* Yuncker, *Cynodon dactylon* (L.) Pers., *Cyperus rotundus* L., *Daucus carota* L., *Datura stramonium* L., *Echinochloa colonum* (L.) Link., *Euphorbia prostrata* Aiton, *Heliotropium europaeum* L., *Orobancha* spp., *Polygonum aviculare* L., *Portulaca oleracea* L., *Prosopis farcta* (Banks & Sol.) Macbride, *Sinapis arvensis* L., *Solanum nigrum* L., *Tribulus terrestris* L. and *Xanthium strumarium* L. (Asav and Serim 2019, Başaran et al. 2017, Erol 2010, Karabacak and Uygur 2017, Özkil et al. 2019, Tepe 2014, Tursun et al. 2017, Yay 2015 ).

In order to minimize the weed damage on crops, there is an increase in labor and other aspects of input in controlling the weeds, and therefore, serious economic losses are experienced. In this respect, it is necessary to determine the weed control time correctly and to integrate the appropriate weed control methods in integrated weed management concept (Swanton and Weise 1991). As a matter of fact, it has been reported that weed control is required to reduce weed density in the early growing period of sunflower and

inclusion of different treatment methods in integrated weed management systems to reduce herbicide use (D'Alessandro et al. 1992). In the case of high weed density in sunflowers, and without managing it, significant yield losses occur on sunflowers (Hossein et al. 2010, Kaya 2016).

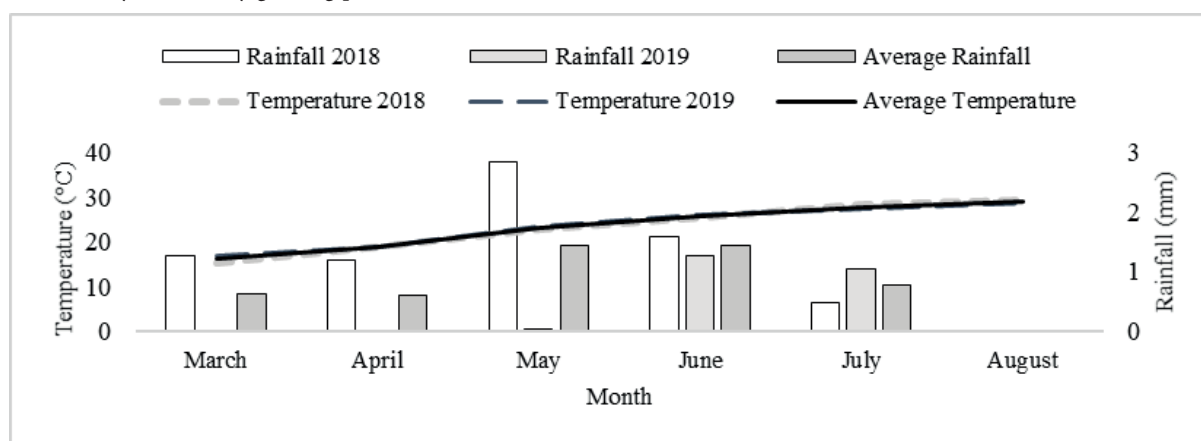
Today, weed control is mostly provided by herbicides. Intensive use of herbicides may cause resistance to weeds and leads to environmental pollution. Hence, excessive use of herbicides and failure of the integrated weed management control strategies also causes economic losses. This study was aimed to determine the effects of selected hoeing treatments and different hoeing periods for weed control on sunflower yield and yield criteria.

## MATERIALS AND METHODS

This study was conducted in 2018 and 2019 (37.10°N, 35.41°E) in a sunflower field in Ceyhan (Sağkaya) district of Adana, Turkey. In the first year of the experiment, the field trial was started on March 10, 2018 and finished on July 10, 2018. In the second year, the experiment was established on May 02, 2019 and finished on August 15, 2019. The climatic data obtained in the experiment are given in Figure 1, and soil characteristics are given in Table 1.

**Table 1.** Soil characteristics of the experiment field (0–30 cm soil depth)

Soil characteristics	
Values	2018-2019
Saturation (%)	65.78
P <sub>2</sub> O <sub>5</sub> (mg kg <sup>-1</sup> )	3.37
Organic matter (%)	2.15
K <sub>2</sub> O (mg kg <sup>-1</sup> )	126.00
Total soluble salt (%)	0.025
Iron-Fe (mg kg <sup>-1</sup> )	5.20
Manganese-Mn (mg kg <sup>-1</sup> )	0.45
Lime-CaCO <sub>3</sub> (mg kg <sup>-1</sup> )	6960.00
Magnesium (mg kg)	1333.20
pH (1 : 2.5)	7.83



**Figure 1.** Climatic conditions for experiment area in 2018 and 2019

**Table 2.** Determined hoeing treatments for sunflower

Weed-free treatments	Weedy treatments
1) 15 days weed-free after emergence	8) 15 days weedy after emergence
2) 30 days weed-free after emergence	9) 30 days weedy after emergence
3) 45 days weed-free after emergence	10) 45 days weedy after emergence
4) 60 days weed-free after emergence	11) 60 days weedy after emergence
5) 75 days weed-free after emergence	12) 75 days weedy after emergence
6) 90 days weed-free after emergence	13) 90 days weedy after emergence
7) Weed-free	14) Weedy

Sunflower seeds were sown with a seed drill set for 70 cm inter-row and 30 cm intra-row spacing. The experiment design was established with 3 replications according to the randomized complete block design with a plot size of 8.40 m<sup>2</sup> (2.80 m x 3.00 m). In experimental area, safety strips between the blocks (1.0 m) and the plots (0.5 m) were left. There were four sunflower rows in each plot, and the weeds in the plots were removed by hand hoe or pulling. Weeds' removal was started after sunflowers emerged. Hoeing was carried out every 15 days on the experimental plots. In the study, 14 different hoeing times were implemented (Table 2).

#### *Determination of weed species, weed coverages, and fresh weed biomass*

In the experimental field, 3 points of 1 m<sup>2</sup> were fixed in each plot and periodic observations of weed species and densities were regularly observed. Accordingly, weed coverages (%) of hoeing treatments were determined, then weeds in these points were cut from the soil surface and the fresh weed biomasses (g m<sup>-2</sup>) were weighed on an assay balance (Odum 1971, Uygur et al. 1984). Then, the effects of hoeing time treatments were evaluated.

#### *Data collection of sunflower yield and yield criteria*

In the study, weeds were removed from the plots by hand hoeing or pulling, depending on the hoeing times in the experiment. The yield (kg ha<sup>-1</sup>) was calculated by harvesting the middle two sunflower rows for each plot out of four sunflower rows (Erol 2010, Kaya et al. 2020). At the end of the experiment, the yield was obtained from the plots where the treatments were carried out and were compared with the yield data from weed-free and weedy plots during the season. Sunflower seeds were counted and kernel weight (g) was calculated by taking four sunflower heads from rows of each plot. Moreover, 10 random sunflower heads (cm) and sunflower heights (m) from the soil surface were measured before harvesting. In each treatment plot, 10 sunflower plant samples were harvested in the middle of 2 sunflower rows.

At the last, sunflower oil was obtained from the seed samples taken from the plots by using the Soxhlet Device with petroleum ether or hexane solvents, as in the extraction

method. The values were calculated as a percentage (%), thus, the seed yield values per hectare determined for each plot were multiplied by the oil ratio values (%) determined for the plots (TS EN ISO 659, 2009).

#### *Statistical analysis*

SPSS package software was used to analyze the comparison of obtained data. In the Multiple Comparison Tests, the data between selected characters depending on the hoeing times were grouped at 0.05 significant levels statically using the Duncan test (SPSS, 2015).

## RESULTS AND DISCUSSION

#### *Treatment observation dates, weed species and density of the experimental area*

In Adana, a total of 17 weed species belonging to 11 families were identified in the experimental area in 2018 and 2019. Major weed species such as *C. album*, *C. vulvaria*, *H. europaeum*, *C. arvensis*, *C. rotundus* and *P. farcta* have been observed. Poaceae family was significant, with 4 weed species, followed by 2 weed species each belonging to Amaranthaceae, Euphorbiaceae, and Polygonaceae families, respectively. In previous studies conducted in Turkey, 52 weed species belonging to 23 families in Çukurova region (Adana, Mersin, and Osmaniye provinces) (Karabacak and Uygur 2017), 67 weed species belonging to 30 families in Adana province (Özgil et al. 2019) were identified in the sunflower surveys. Accordingly, in Çukurova region, it has been noted that the highest weed densities were *A. retroflexus*, *C. album*, *C. arvensis*, *C. vulvaria*, *C. rotundus* and *H. europaeum* (Karabacak and Uygur 2017, Özgil et al. 2019). Between 2014 and 2015, 58 weed species belonging to 24 families (Asav and Serim 2019) in Ankara province, 36 weed species belonging to 17 families in Edirne in 2013 (Yay 2015) were also detected. In all these studies, the most prominent weed species were of Poaceae and Asteraceae families, and similar weed species were seen in the experimental field of the current study. 17 different weed species, especially Poaceae and Amaranthaceae families, were identified in the experimental area, and important similar weed species were found in sunflowers, such as *C. album*, *C. vulvaria*, *H.*

**Table 3.** Hoeing treatments, data observation dates, and weed species in sunflower experimental field in 2018 and 2019, Adana

Treatments	Observation dates			Major weed species 2018-2019		
	Observation time	2018	2019			
Weed-free	15 days weed-free	Before harvest (7 <sup>th</sup> )			Amaranthaceae	Chenopodium album L.
	30 days weed-free		10 July	15 August	Amaranthaceae	Chenopodium vulvaria L.
	45 days weed-free				Boraginaceae	Heliotropium europaeum L.
	60 days weed-free				Convolvulaceae	Convolvulus arvensis L.
	75 days weed-free				Cucurbitaceae	Cucumis melo L. var. agrestis Naudin
	90 days weed-free				Cyperaceae	Cyperus rotundus L.
	Weed-free		-	-	-	Euphorbiaceae
Weedy	15 days weedy	15 days (1 <sup>st</sup> )	18 April	30 May	Euphorbiaceae	Euphorbia prostrata Aiton
	30 days weedy	30 days (2 <sup>nd</sup> )	2 May	14 June	Fabaceae	Prosopis farcta (Banks and Sol.) Mac.
	45 days weedy	45 days (3 <sup>th</sup> )	14 May	28 June	Papaveraceae	Fumaria officinalis L.
	60 days weedy	60 days (4 <sup>th</sup> )	30 May	8 July	Poaceae	Cynodon dactylon (L.) Pers.
	75 days weedy	75 days (5 <sup>th</sup> )	12 June	19 July	Poaceae	Echinochloa colonum (L.) Link.
	90 days weedy	90 days (6 <sup>th</sup> )	26 June	1 August	Poaceae	Echinochloa crus-galli (L.) P. Beauv.
	Weedy	Before harvest (7 <sup>th</sup> )	10 July	15 August	Poaceae	Sorghum halepense (L.) Pers.
					Polygonaceae	Polygonum aviculare L.
					Polygonaceae	Rumex spp.
					Solanaceae	Physalis spp.

europaeum, *C. arvensis*, *C. rotundus* and *P. farcta* (Table 3). In a study conducted in US, morphologies and development biologies of *Abutilon theophrastii* Medik., *A. retroflexus*, *C. album*, and *X. strumarium* species were examined and the small seed weeds were found more competitive than large seed weeds in sunflower (Selbert and Pearce 1993). In experimental areas of sunflower, Salera (1991) found that *A. retroflexus*, *C. album*, *E. crus-galli*, *Lolium multiflorum* Lam., *Polygonum aviculare* L., *P. persicaria*, *S. arvensis*, *S. nigrum* and *Sonchus arvensis* L. species were the most common weeds in Italy. Fleck et al. (1991) in Brazil determined that different weed species (*Digitaria ciliaris* (Retz.) Koeler, *Echinochloa* spp., *Amaranthus* spp., *Ambrosia artemisiifolia* L., *Bidens pilosa* L. *Fallopia convolvulus* (L.) Á.Löve, *Richardia brasiliensis* Gomes and *Silene gallica* L.) may also cause yield losses in sunflower.

Statistical analysis was performed in the fresh weed biomass and weed coverages ( $P \leq 0.05$ ) in both years. With the increase of hoeing time in plots that are constantly infested by weeds, a decrease in fresh weed biomass and weed coverages has been observed. In 2018 and 2019, 0.51 g m<sup>-2</sup> and 40.41 g m<sup>-2</sup> fresh weed biomass were obtained in 90 days weed-free, 10.16 g m<sup>-2</sup> and 121.96 g m<sup>-2</sup> in 15 days weed-free, respectively. Weed coverages were 2.00% and 14.67% in 90 days weed-free, 28.67%, and 50.33% in 15 days weed-free. It was determined that the fresh weed biomass in the plots of 75 and 90 days weedy in both years varied between 48.81 and 276.67 g m<sup>-2</sup> (Table 4). It has been reported in a previous study that in sunflower cultivation hoeing should be done at least 2-3 times following by the sunflower emergence to control weeds and also to aerate the soil (Atakişi and Turan 1989). Çoruh and Zengin (2009) suggested that in Erzurum,

**Table 4.** The effects of the treatments determined in the experiment between 2018-2019 on fresh weed biomass (g m<sup>-2</sup>) and weed coverages (%) in Adana province (+SD)

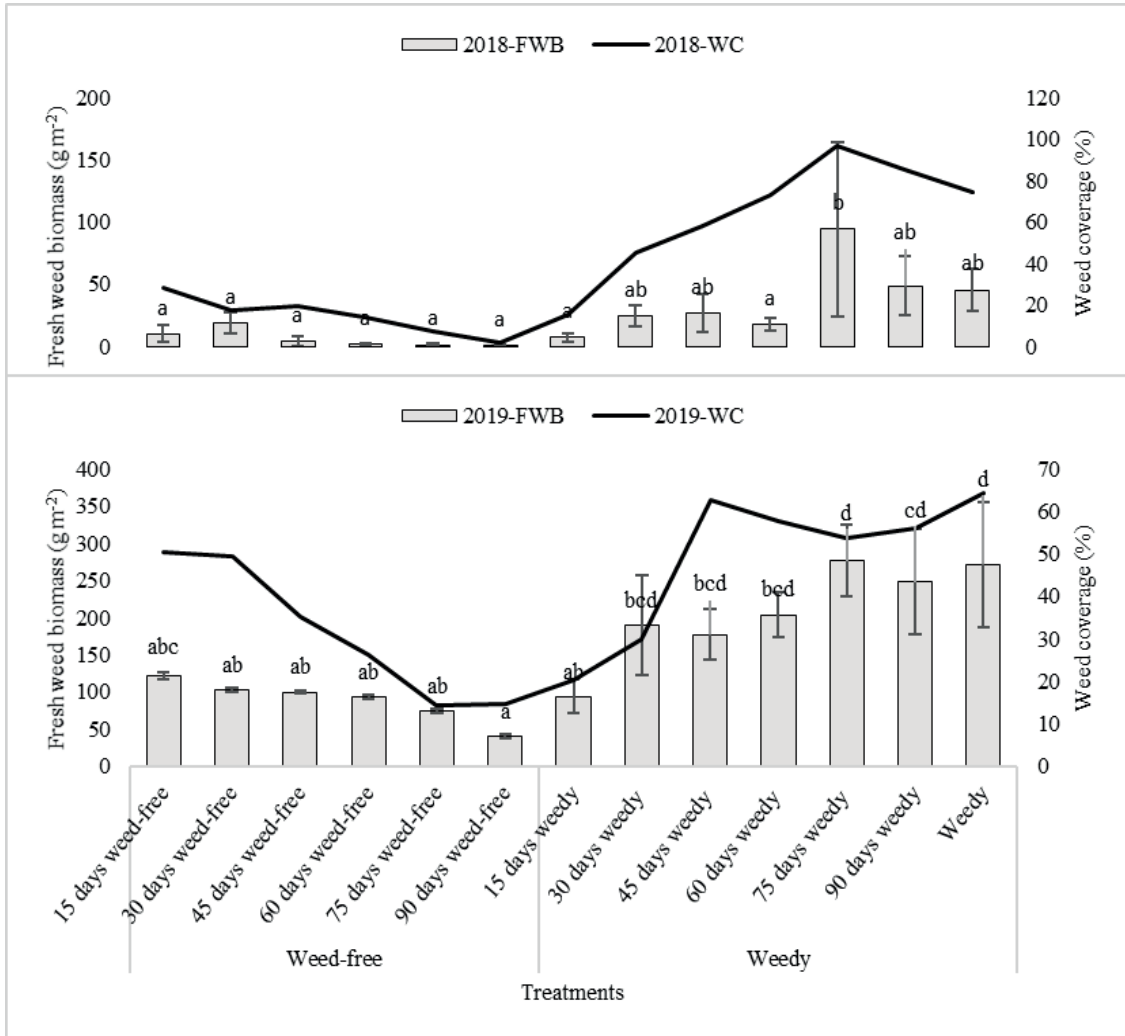
Treatments	Weed biomass (g m <sup>-2</sup> ) (+SD)		Weed coverage (%) (+SD)		
	2018	2019	2018	2019	
Weed-free	15 days weed-free	10.16 a (11.98)	121.96 abc (8.31)	28.67 cd (1.92)	50.33 bcde (4.35)
	30 days weed-free	18.69 a (14.99)	102.08 ab (5.05)	17.33 bc (4.19)	49.33 bcde (2.17)
	45 days weed-free	4.43 a (6.35)	99.95 ab (4.02)	19.67 c (3.67)	35.33 abcd (3.44)
	60 days weed-free	1.53 a (1.12)	93.08 ab (5.89)	14.33 bc (1.24)	26.33 ab (7.36)
	75 days weed-free	1.39 a (1.93)	74.25 ab (5.84)	7.00 ab (11.26)	14.33 a (6.52)
	90 days weed-free	0.51 a (0.85)	40.41 a (4.38)	2.00 a (2.12)	14.67 a (1.91)
Weedy	15 days weedy	7.26 a (6.39)	94.17 ab (40.64)	15.33 bc (11.73)	20.33 a (3.21)
	30 days weedy	24.41 ab (15.34)	190.00 bcd (68.02)	45.00 de (8.48)	30.00 abc (9.00)
	45 days weedy	26.50 ab (26.26)	177.50 bcd (60.15)	58.33 ef (14.05)	62.67 e (16.57)
	60 days weedy	18.14 a (8.54)	204.17 bcd (51.37)	73.33 fg (7.06)	57.67 de (11.56)
	75 days weedy	94.37 b (69.95)	276.67 d (84.31)	96.67 h (7.45)	53.67 cde (7.14)
	90 days weedy	48.81 ab (41.36)	248.33 cd (71.55)	85.33 g (1.25)	56.00 de (9.13)
	Weedy	45.36 ab (29.06)	271.67 d (84.12)	74.33 fg (3.94)	64.33 e (10.26)

\*Statistically significant at  $P \leq 0.05$  level.



weed management should be done between the 3rd and 6th weeks in sunflower fields with the weed emergence.

sunflower height (1.49 m), kernel weight (46.83 g), and yield (25.92 kg ha<sup>-1</sup>) were higher than the other



**Figure 2.** The interactions between the average fresh weed biomass (g m<sup>-2</sup>) and average weed coverages (%) obtained from the plots in the experimental field

In another study conducted in Tokat, it was reported that the most suitable period for controlling weeds in sunflower was the period covered between the 4th and the 6th weeks in Turkey (Kaya et al. 2020).

It was found that the fresh weed biomass was higher in weedy plots with 75 and 90 days weedy. In Figure 2, it is seen that there is a decrease in weed coverages increasing the hoeing times. However, these parameters may differ according to climatic conditions and the characteristics of the weed species (Table 4). Sağlam (1992) compared two different harrowing methods and five different hoeing methods in sunflower and discovered that the best method for weed control is a milling hoe machine. As a matter of fact, he revealed that, in the plots allocated to the milling hoe machine, the sunflower heads (19.88 cm), sunflower stem,

plots of hand hoeing. In a study conducted in 2008, it was observed that, with the increasing density of *X. strumarium*, decreasing in the seed yield per sunflower plant was 27.00% (65.87-90.28 g), in the oil quality was 16.00%, in kernel weight was 22.00% (60.54-47.08 g), in oil content was 51.00% (20.03- 9.72 kg ha<sup>-1</sup>) and in the yield was 42.00% (43.07-24.88 kg ha<sup>-1</sup>) (Erol 2010) in Tekirdağ. Also, it has been stated that *A. retroflexus* resulted in approximately a 50.00% decrease in sunflower yield depending on the density of the weed (Heidarian et al. 2012).

Natural weed emergence occurred in the experimental area. The density distribution of *C. dactylon*, *E. colonum*, *E. crus-galli*, *E. prostrata*, *F. officinalis*, *Physalis* spp., *P. aviculare*, *Rumex* spp. and *S. halepense* were found lowest. Statistically significant differences

**Table 5.** Weed species and numbers (weed m<sup>-2</sup>) in treatments on sunflower in the experiment of Adana province in 2018-2019 (+SD)

Weed species (weed m <sup>-2</sup> ) (+SD)	Full season weedy						Full season weed-free						Weedy	Average weed number
	15 days weed- free	30 days weed- free	45 days weed- free	60 days weed- free	75 days weed- free	90 days weed- free	15 days weedy	30 days weedy	45 days weedy	60 days weedy	75 days weedy	90 days weedy		
<i>Chenopodium album L.</i>	0.97 ab (0.93) A	0.56 a (0.88) A	0.22 a (0.54) A	0.78 ab (1.04) A	0.17 a (0.40) A	0.00 a (0.00) A	2.36 bc (3.45) AB	3.03 a (3.65) AB	6.14 b (3.38) B	3.06 a (1.92) AB	3.72 a (3.48) AB	5.64 b (4.91) A	3.86 a (3.35) AB	2.35 bc (2.86)
<i>Chenopodium vulvaria L.</i>	1.56 ab (1.56) AB	0.22 a (0.54) A	2.22 bc (2.48) AB	0.44 ab (0.68) A	0.00 a (0.00) A	0.67 ab (1.11) A	0.67 ab (1.11) A	1.56 a (2.00) AB	0.89 a (2.18) A	1.78 a (2.00) AB	4.22 a (2.19) AB	3.11 ab (1.45) AB	5.78 a (3.09) B	1.78 abc (2.97)
<i>Chrozophora tinctoria (L.) Rafin.</i>	1.50 ab (0.98) BC	0.81 a (0.96) AB	0.83 ab (0.98) AB	1.11 ab (1.10) ABC	0.00 a (0.00) A	0.00 a (0.00) A	0.17 a (0.25) AB	0.92 a (1.06) AB	0.25 a (0.41) AB	0.17 a (0.40) AB	0.42 a (0.66) AB	1.56 ab (1.46) BC	2.33 a (2.34) C	0.77 ab (0.81)
<i>Convolvulus arvensis L.</i>	2.45 ab (1.35) A	3.75 bc (2.06) A	2.44 bcd (2.72) A	4.78 cd (2.34) A	0.89 a (1.37) A	0.44 ab (0.68) A	1.11 abc (0.72) A	1.56 a (1.31) A	1.39 a (1.23) A	2.22 a (1.22) A	2.45 a (1.21) A	2.44 a (1.39) A	2.67 a (1.69) A	2.20 bc (2.57)
<i>Cucumis melo L. var agrestis Naudin</i>	0.17 a (0.40) AB	0.67 a (0.81) B	0.08 a (0.20) AB	0.17 a (0.40) AB	0.00 a (0.00) A	0.25 a (0.41) AB	0.33 a (0.60) AB	0.25 a (0.41) AB	0.17 a (0.25) AB	0.17 a (0.40) AB	0.33 a (0.60) AB	0.17 a (0.25) AB	0.58 a (0.73) AB	0.26 a (0.36)
<i>Cynodon dactylon (L.) Pers.</i>	0.45 a (0.45) B	0.00 a (0.00) A	0.00 a (0.00) A	0.00 a (0.00) A	0.00 a (0.00) A	0.00 a (0.00) A	0.00 a (0.00) A	0.00 a (0.00) A	0.00 a (0.00) A	0.00 a (0.00) A	0.00 a (0.00) A	0.00 a (0.00) A	0.00 a (0.00) A	0.03 a (0.17)
<i>Cyperus rotundus L.</i>	7.39 c (2.06) ABC	4.56 c (1.86) AB	4.22 d (1.74) AB	6.97 d (3.23) ABC	3.50 b (1.82) A	2.95 c (1.62) A	2.86 c (1.37) A	15.31 b (6.04) ABC	12.61 c (2.67) ABC	13.33 b (4.59) ABC	16.84 b (5.63) BC	18.06 c (5.54) C	17.08 b (6.63) BC	9.67 d (1.84)
<i>Echinochloa colonom (L.) Link.</i>	0.75 a (1.25) AB	1.17 a (1.80) B	0.00 (0.00) A	0.00 a (0.00) A	0.00 a (0.00) A	0.00 a (0.00) A	0.00 a (0.00) A	0.00 a (0.00) A	0.00 a (0.00) A	0.00 a (0.00) A	0.00 a (0.00) A	0.00 a (0.00) A	0.00 a (0.00) A	0.15 a (0.57)
<i>Echinochloa crus-galli (L.) P. Beauv.</i>	0.67 a (1.63) A	0.00 a (0.00) A	0.00 a (0.00) A	0.25 ab (0.61) A	0.25 a(0.61) A	0.00 a (0.00) A	0.00 a (0.00) A	0.17 a (0.40) A	0.00 a (0.00) A	0.00 a (0.00) A	0.00 a (0.00) A	0.00 a (0.00) A	0.00 a (0.00) A	0.10 a (0.29)
<i>Euphorbia prostrata Aiton</i>	0.78 a (0.88) AB	1.44 a (0.95) B	0.00 a (0.00) A	0.70 ab (1.13) AB	0.67 a (1.63) AB	0.00 a (0.00) A	0.08 a (0.20) A	0.08 a (0.20) A	0.00 a (0.00) A	0.00 a (0.00) A	0.00 a (0.00) A	0.17 a (0.25) A	0.25 a (0.61) A	0.32 a (0.51)
<i>Fumaria officinalis L.</i>	0.00 a (0.00) A	0.44 a (0.68) A	0.00 a (0.00) A	0.00 a (0.00) A	0.00 a (0.00) A	0.00 a (0.00) A	0.22 a (0.54) A	0.00 a (0.00) A	0.00 a (0.00) A	0.00 a (0.00) A	0.67 a (1.11) A	0.22 a (0.54) A	0.45 a (1.09) A	0.15 a (0.35)
<i>Heliotropium europaeum L.</i>	1.72 ab (1.51) A	1.75 ab (1.02) AB	0.58 ab (1.02) AB	0.50 ab (0.77) ABC	0.42 a (0.49) ABC	0.86 ab (0.99) ABC	1.22 abc (1.47) ABC	1.22 a (1.24) ABC	1.22 a (1.50) ABC	1.56 a (0.72) ABC	1.86 a (0.89) ABC	2.06 ab (1.54) BC	2.20 a (1.30) C	1.32 ab (0.85)
<i>Physalis spp.</i>	0.00 a (0.00) A	0.00 a (0.00) A	0.00 a (0.00) A	0.00 a (0.00) A	0.00 a (0.00) A	0.00 a (0.00) A	0.00 a (0.00) A	0.08 a (0.08) A	0.08 a (0.08) A	0.08 a (0.08) A	0.33 a (0.17) B	0.00 a (0.00) A	0.00 a (0.00) A	0.05 a (0.13)
<i>Polygonum aviculare L.</i>	0.00 a (0.00) A	0.00 a (0.00) A	0.00 a (0.00) A	0.00 a (0.00) A	0.00 a (0.00) A	0.00 a (0.00) A	0.00 a (0.00) A	0.00 a (0.00) A	0.00 a (0.00) A	0.00 a (0.00) A	0.00 a (0.00) A	0.22 a (0.22) B	0.00 a (0.00) A	0.02 a (0.08)
<i>Prosopis farcta (Banks. and Sol.) Mac.</i>	3.53 b (2.19) A	4.78 c (2.15) A	3.67 cd (1.83) A	3.45 bc (1.56) A	1.67 a (0.85) A	1.75 bc (1.94) A	1.08 abc (1.62) A	1.92 a (1.08) A	2.17 a (2.38) A	3.08 a (1.39) A	4.92 a (2.49) A	3.33 ab (1.63) A	3.42 a (1.58) A	2.98 c (2.70)
<i>Rumex spp.</i>	0.00 a (0.00) A	0.22 a (0.22) A	0.22 a (0.22) A	0.00 a (0.00) A	0.00 a (0.00) A	0.00 a (0.00) A	0.22 a (0.22) A	0.67 a (0.67) A	1.33 a (0.91) A	0.00 a (0.00) A	1.56 a (1.31) A	1.33 ab (1.09) A	0.67 a (0.46) A	0.48 a (0.94)
<i>Sorghum halepense (L.) Pers.</i>	0.92 ab (0.61) B	0.00 a (0.00) A	0.83 ab (0.53) B	0.00 a (0.00) A	0.00 a (0.00) A	0.00 a (0.00) A	0.00 a (0.00) A	0.00 a (0.00) A	0.00 a (0.00) A	0.00 a (0.00) A	0.00 a (0.00) A	0.00 a (0.00) A	0.00 a (0.00) A	0.13 a (0.47)
<b>Total</b>	22.83 C (5.12)	20.36 BC (7.03)	15.33 ABC (6.03)	19.14 ABC (8.34)	7.56 A (5.78)	6.92 A (4.32)	10.33 AB (7.88)	26.75 CDE (13.19)	26.25 CD (8.45)	25.44 CD (10.65)	37.31 DEF (10.79)	38.30 EF (12.28)	39.28 F (16.70)	

\* The average means have shown with different small letters in the same column and different capital letters in the same row are different from each other at the P≤0.05 significant level statically according to the Duncan Multiple Comparison Test.

**Table 6.** Sunflower kernel weight (g) and yield (kg ha<sup>-1</sup>) parameters from the plots in the experimental field (+SD)

Treatments	Sunflower yield (kg ha <sup>-1</sup> ) (+SD)		Kernel weight (g) (+SD)		
	2018	2019	2018	2019	
Weed-free	15 days weed-free	35.91 a (3.84)	29.89 bc (7.11)	78.43 ab (6.17)	82.06 cdef (3.55)
	30 days weed-free	34.60 a (2.41)	35.45 abc (4.16)	71.38 ab (8.35)	83.60 cde (2.57)
	45 days weed-free	28.97 abc (0.88)	35.98 abc (4.16)	69.96 b (7.04)	84.96 cde (0.97)
	60 days weed-free	34.47 a (0.87)	36.11 abc (5.20)	72.93 ab (1.34)	88.08 cd (1.96)
	75 days weed-free	34.95 a (7.16)	37.56 ab (5.04)	67.67 b (5.70)	93.55 bc (6.80)
	90 days weed-free	31.39 abc (4.71)	39.28 ab (5.36)	70.56 b (8.32)	103.91 ab (13.93)
	Weed-free	32.20 ab (0.85)	42.06 a (7.27)	86.67 a (12.60)	110.96 a (13.11)
Weedy	15 days weedy	28.96 abc (3.99)	33.33 abc (2.86)	69.43 b (8.23)	79.21 defg (1.41)
	30 days weedy	26.43 abcd (1.64)	32.41 abc (3.68)	68.63 b (13.26)	76.78 defg (1.55)
	45 days weedy	24.63 bcd (4.14)	32.67 abc (3.77)	50.35 c (2.74)	75.23 efgh (3.18)
	60 days weedy	28.85 abc (5.52)	30.95 bc (2.09)	66.61 b (11.45)	73.41 efgh (5.86)
	75 days weedy	22.08 cd (6.11)	30.82 bc (4.20)	69.08 b (8.33)	70.41 fgh (3.30)
	90 days weedy	28.65 abc (6.99)	30.29 bc (4.65)	62.57 bc (1.11)	69.10 gh (3.26)
	Weedy	18.99 d (2.96)	26.19 c (6.55)	63.95 bc (11.48)	64.05 h (7.69)

\* Statistically significant at  $P \leq 0.05$  level.

were determined between the numbers of weed species in treatment plots ( $P \leq 0.05$ ). Considering the weedy and weed-free treatment plots, the most prominent species were *C. rotundus* 9.67 weed m<sup>-2</sup>, *P. farcta* 2.98 weed m<sup>-2</sup>, *C. album* 2.35 weed m<sup>-2</sup>, and *C. arvensis* 2.20 weed m<sup>-2</sup> respectively (Table 5).

In the experiment, the lowest weed density was seen in *C. dactylon*, *E. crus-galli*, *Physalis* spp., *P. aviculare* and *S. halepense* (Table 5). It was observed that weed species of this experiment found similar to other studies in sunflower (Arslan and Kara 1997, Başaran et al. 2017, Çoruh and Zengin 2009, İyigün et al. 1997, Tursun et al. 2017).

#### *The effect of treatments on sunflower yield and yield criteria*

Since sunflower was sown in March-April in the Çukurova region, harvested towards summer, and due to the climate-dependent abiotic stress factors as well as being affected by weeds, changes were observed in the oil content of sunflower in 2018-2019. It was determined that there were no statistical differences in the obtained values of the sunflower head diameter and sunflower oil content ( $P \geq 0.05$ ) (Figure 3). However, İyigün et al. (1997) compared 10 and 40 days weedy plots with weed-free plots (sunflower head weight 168.63 kg ha<sup>-1</sup>, seed weight 74.17 kg ha<sup>-1</sup>) in their experiments in Tokat (Kazova) between 1995-1996, and found that the yield loss in average sunflower head weights varied between 7.95-26.39% (124.13-155.23 kg ha<sup>-1</sup>) and the yield loss in seed weights varied between 3.15-25.69% (71.83-55.12 kg ha<sup>-1</sup>). In Erzurum, Kara (1986) reported that the loss in oil content of sunflower varieties varied between 31.10-50.50%, depending on the climate and environmental factors, and the average oil contents were changed between 8.08-38.05%. Cardinali et al. (1986) reported that the oil

quality rate in sunflower may vary between 31.30-50.00% depending on environmental factors. We can say that different data obtained in this study are due to the ecological locations where the experiments were established, and the sunflower varieties were cultivated.

In the first year of the experiment, there was close difference in sunflower height between weed-free and weedy plots during the season. In the second year, statistically significant differences were detected after it was determined that the highest plant height in the 75 and 90 days weed-free applications and in the weed-free control (Figure 3). Similar to this study, Erol (2010) reported that the increasing *X. strumarium* numbers in plots were affected the sunflower height, head, oil yield, and protein ratio. However, Johnson (1973) stated that in the first 4 weeks of sunflower development, weeds compete with sunflower and affect the sunflower head and height criteria. Likewise, Vasilev et al. (1991) reported in their studies conducted in Russia, weed populations affected sunflower yield between 2.09-2.39 t ha<sup>-1</sup>, continued to increase densities for 3 months after sunflower emergence (>5-11 g m<sup>-2</sup>), and the seed yield was negatively affected. Fleck et al. (1991) noted that continuous mechanical weed control with row hoeing in sunflower varied sunflower seed yield, seed weight, sunflower head-seed weight ratio, sunflower head, and height in Brazil. Similar to other studies, different sunflower varieties, ecological and climatic conditions can also affected the sunflower growth parameters except weed treatments. The values of the yield (kg ha<sup>-1</sup>) and kernel weights (g) were obtained at sunflower harvest are given in Table 6 ( $P \leq 0.05$ ).

In the field studies were carried out during the sunflower growing season, the plots yield of 15 days to 90 days

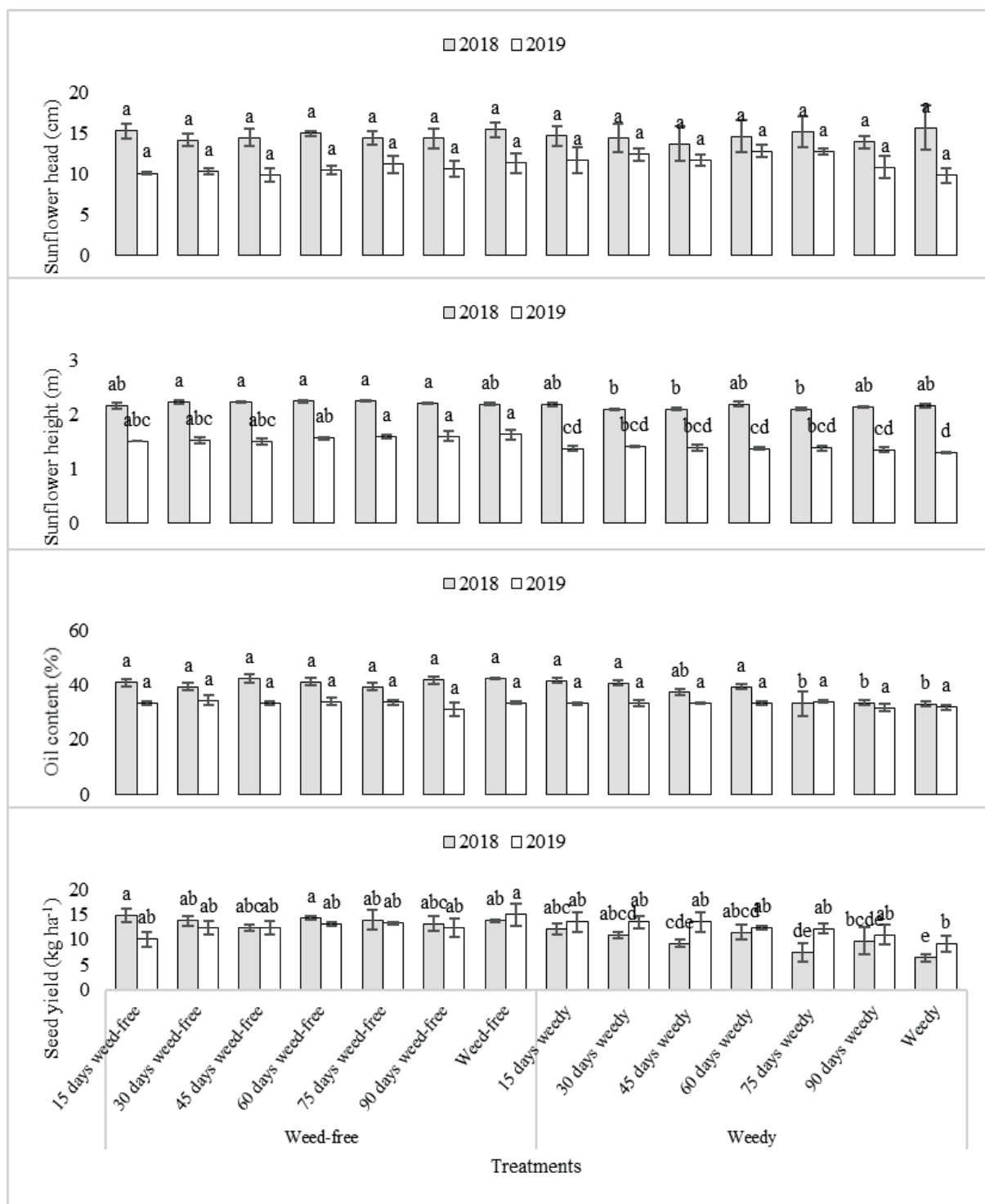


Figure 3. Sunflower head (cm), height (m), oil content (%), and seed yield (kg ha<sup>-1</sup>) parameters from the plots in the experimental field

weed-free in 2018 were obtained as close to each other as 28.97-35.91 kg ha<sup>-1</sup>, and the weed-free plot was 32.30 kg ha<sup>-1</sup>. Similar to 2018, parameters between 35.45-39.28 kg ha<sup>-1</sup> were observed in 30 days to 90 days weed-free (except for 15 days weed-free), and weed-free was found to be 42.06 kg ha<sup>-1</sup> which was the highest value in 2019. Thus, as hoeing time increased in both years, rising sunflower

yields were observed in plots (Table 6). In Iran in 2007, it was determined that season-long weedy treatments, according to weed density, resulted in yield losses up to 27.50% in seed yield and 43.00% in oil content compared to weed-free treatments throughout the season in sunflower (Hossein et al. 2010).

When the kernel weights were examined, different effects were observed in weed-free and weedy treatment plots. In plots where 15-90 days weed-free was carried out in the first and second years, variations between 69.96-78.43 g (excluding 75 days weed-free) and 82.06-103.91 g were determined respectively. Moreover, kernel weights in weed-free plots were found to be the highest in both years (Table 6). In many studies, changes were observed in yield and kernel weights depending on weeds (Çoruh and Zengin 2009, Fleck et al. 1991, İyigün et al. 1997, Kara 1986, Vasilev et al. 1991). It has been reported that early weed management is important in early development periods of sunflower, and it is necessary to control weeds in the first 4-5 weeks. Because it has been revealed that weeds cause yield losses up to 60% in the sunflower yield approximately if it is late to manage weeds (D'Alessandro et al. 1992).

In both years, it was observed that the fresh weed biomass and weed coverages were the highest between 30 and 90 days weedy plots during the season and the fresh weed biomass and weed coverages decreased as the time of the hoeing was extended. Duration of weed hoeing did not change the sunflower head and oil content. Depending on climatic conditions and environmental factors, the highest parameters of sunflower height and oil content were obtained between 30 and 90 days in weed-free plots during the season. Similarly, it is observed that the yield obtained from sunflower yield and kernel weight were the highest as the hoeing time is extended.

As a result; it was determined that weed management must be performed in the development period of sunflower, which is one of the important oil cultivated crops. In addition to this, as the hoeing time increases, the values of the sunflower yield and yield criteria increase. With the study carried out, it has been revealed that it is important to implement the appropriate long-term hoeing times for managing weeds after the sunflower growing period.

#### ACKNOWLEDGEMENTS

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#### ÖZET

Ayçiçeği yetiştiriciliğinde yabancı otlarla mücadelede çapalama süresi önemli olup, yabancı ot yoğunluklarının

azaltılmasında etkilidir. Bu çalışmada, Adana ili ayçiçeğinde yabancı ot mücadelesi için mekanik çapalama sürelerine olan etkilerinin belirlenmesi amacıyla 2018-2019 yılları arasında gerçekleştirilmiştir. Deneme kurulan tarlalarda yabancı ot kontrol zamanlarını belirlemek için ayçiçeğinin çıkışından hasada kadar geçen sürede mekanik çapalamanın 15 gün ara ile etkileri belirlenmiş, yabancı otların doğal koşullarda oluşması sağlanmıştır. Parseller kurularak, ayçiçeği yetiştirme döneminde periyodik olarak yapılan mekanik çapalamanın yabancı ot-ayçiçeği verim kriterlerine olan etkileşimleri gözlemlenmiştir. Deneme sonunda yabancı otların yaş ağırlığı ve kaplama alanı ile çapalama süresi uzunluğunun ayçiçeği verimi, dane verimi, yağ verimi, yağ kalitesi, tabla çapı ve bitki boyuna olan kriterler belirlenmiştir. İki yıllık çalışma sonucuna göre ayçiçeği verimi ile dane verimi en yüksek 75 ve 90 gün boyunca çapa yapılmış parsellerde olduğu, en düşük ise 60, 75 ve 90 gün boyunca yabancı otlu bırakılan çapa yapılmayan parsellerde olduğu görülmüştür. Yağ veriminin çapa süresi daha uzun bırakılan parsellerde yüksek olduğu, ancak yağ kalitesinin değişmediği saptanmıştır. Çapa süresine bağlı olarak ayçiçeği bitki boy gelişimlerinin birbirine yakın olduğu, tabla çaplarının ise etkilenmediği ortaya çıkarılmıştır. Çapa süresi daha az olan yabancı otlu parsellerde yabancı ot kaplama alanlarının artışına bağlı olarak yabancı ot yaş ağırlıklarında da artışların olduğu kaydedilmiştir. Sonuç olarak ayçiçeğinde yabancı ot yönetiminde uzun süre çapa yapılmasının verimi arttırdığı ve ayçiçeğinde yabancı otlarla mücadelede çapalama süresinin önemli olduğu belirlenmiştir.

Anahtar kelimeler: çapalama süresi, ayçiçeği, yabancı ot yönetimi, verim ve verim unsurları

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