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Original article (Orijinal araştırma)

Spinosad resistance in a population of *Frankliniella occidentalis* (Pergande, 1895) from Antalya and its cross resistance to acrinathrin and formetanate[1]

Antalya'dan alınan bir *Frankliniella occidentalis* (Pergande, 1895) popülasyonunda spinosad direnci ve bu popülasyonun acrinathrin ve formetanate'a karşı çapraz direnci

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Abstract

Frankliniella occidentalis (Pergande, 1895) (Thysanoptera: Thripidae) is a serious agricultural pest worldwide. Spinosad is used against many major pest species in Turkey, including *F. occidentalis*. However, resistance to spinosad was detected in *F. occidentalis* population collected from a greenhouse-grown peppers in Kumluca, Antalya, Turkey in 2015. This population (Antalya-2015) had been exposed to intensive, long-term spinosad application in this greenhouse. Cross resistance to acrinathrin and formetanate in the Antalya-2015 population was investigated and the stability of its resistance to spinosad was monitored over 1 year. LC values of susceptible and Antalya-2015 populations were determined using a leaf-dip bioassay and resistance ratios calculated. The resistance ratio of the Antalya-2015 population to spinosad was extremely high (up to 235-fold) and showed a 15-fold cross resistance to acrinathrin. Cross resistance to formetanate was not detected. In addition, spinetoram and spinosad were tested on the Antalya-2015 population using the recommended rates; mortalities were 88 and 38%, respectively. In the assay for stability of resistance to spinosad, mortality in Antalya-2015 population did not significantly change even after the population had been maintained in an insecticide-free environment for 1 year. Thus, resistance to spinosad in the Antalya-2015 population was stable.

Keywords: Antalya, cross resistance, *Frankliniella occidentalis*, spinosad resistance, stability

Öz

Frankliniella occidentalis (Pergande, 1895) (Thysanoptera: Thripidae) tarımda dünya çapında yaygın olan önemli bir zararlıdır. Spinosad, *F. occidentalis* türü de dahil olmak üzere Türkiye'de başlıca zararlılara karşı kullanılmaktadır. Fakat 2015 yılında Kumluca, Antalya, Türkiye'den bir biber serasından alınan *F. occidentalis* popülasyonunda spinosad'a direnç belirlenmiştir. Bu popülasyon (Antalya-2015) alındığı serada uzun süre yoğun spinosad uygulamasına maruz kalmıştır. Aynı popülasyonun acrinathrin ve formetanate'a karşı çapraz direnci araştırılmıştır ve bunun spinosad'a karşı direncinin kalıcılığı 12 aydan daha fazla bir süre boyunca izlenmiştir. Yaprak-daldırma biyoassayi ile Hassas ve Antalya-2015 popülasyonlarının LC değerleri tespit edilmiştir ve direnç oranları hesaplanmıştır. Antalya-2015 popülasyonu spinosad'a 235-kat kadar oldukça yüksek düzeyde dirençlidir ve bu popülasyon acrinathrin'e 15-kat çapraz-direnç göstermiştir. Formetanate'a çapraz direnç tespit edilmemiştir. Ek olarak, spinetoram ve spinosad etiket dozlarında Antalya-2015 popülasyonu üzerinde test edilmiştir. Bu aktif maddelerle elde edilen ölüm oranları sırasıyla %88 ve %38'dir. Spinosad direncinin kalıcılık testinde, Antalya-2015 popülasyonu insektisitlere maruz kalmaksızın 1 yıllık bir süre bekletildiğinde bile ölüm oranında 12 ay öncesine göre önemli bir değişiklik bulunmamıştır. Bu yüzden Antalya-2015 popülasyonunda spinosad direnci kalıcı haldedir.

Anahtar sözcükler: Antalya, çapraz-direnç, *Frankliniella occidentalis*, spinosad-direnci, kalıcılık

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Introduction

The western flower thrips, *Frankliniella occidentalis* (Pergande, 1895) (Thysanoptera: Thripidae), is a serious (polyphagous) pest of numerous agricultural crops worldwide, including vegetables, ornamentals, fruits, and industrial crops (Kirk & Terry, 2003; Mouden et al., 2017). *Frankliniella occidentalis* causes significant economic loss to many agricultural crops, both through its feeding and by transmission of several destructive plant viruses, including tomato spotted wilt virus (TSWV) (EPPO, 1999; Kirk & Terry, 2003).

This invasive thrips was first detected in Antalya, Turkey in 1993 (Tunç & Göçmen, 1995). Currently, crops in many regions of Turkey are infested with *F. occidentalis* (Ulubilir & Yabaş, 1996; Bulut & Göçmen, 2000; Atakan, 2003, 2008a, b; Kılıç & Yoldaş, 2004; Özsemerci et al., 2006; Sertkaya et al., 2006; Nas et al., 2007; Doğanlar & Aydın, 2009; Tekşam & Tunç, 2009; Hazır et al., 2011; Yıldırım & Başpınar, 2013). Furthermore, TSWV is still expanding its range across Turkey (Şevik, 2011; Şevik & Arlı-Sökmen, 2012; Fidan, 2016). Greenhouse production amounts to about 3.2 Mt in Antalya, making Antalya the most important center for greenhouse production in Turkey (GTHB, 2017). *Frankliniella occidentalis* is a particularly serious pest in greenhouses across coastal Antalya at almost all times of the year. *Frankliniella occidentalis* is also an important quarantine pest (EPPO, 2018). Much of the produce from greenhouses in Antalya is exported. Therefore, successful management of this pest species is critical for preventing large economic losses for agricultural exports.

Recently, the management of *F. occidentalis* and transmission of TSWV have met several obstacles. First, the number of registered insecticides for use in rotational applications against *F. occidentalis* is limited and some insecticides have become ineffective due to development of resistance in many thrips populations. Second, some crop species that were once resistant to TSWV strains have lost their resistance (Fidan, 2016). Insecticide resistance in *F. occidentalis* has become a common, worldwide problem over the last two decades, including in various parts of the USA, several European countries, Israel, Turkey, and Australia (Immaraju et al., 1992; Brodsgaard, 1994; Jensen, 1998; Kontsedalov et al., 1998; Espinosa et al., 2002; Herron & James 2005; Bielza et al., 2007; Dağlı & Tunç, 2007; Thalavaisundaram et al., 2008; Zhang et al., 2008; Gao et al., 2012). Currently, resistance against 30 active ingredients has been recorded in *F. occidentalis* populations (APRD, 2018).

Spinosad is a critically important bioinsecticide commonly used worldwide against many greenhouse insect pests, including *F. occidentalis*, *Helicoverpa armigera* (Hübner, 1808) (Lepidoptera: Noctuidae), *Spodoptera littoralis* (Boisduval, 1833) (Lepidoptera: Noctuidae) and *Tuta absoluta* (Meyrick, 1917) (Lepidoptera: Gelechiidae). Insecticide resistance management (IRM) is essential for delaying or preventing pest populations from becoming resistant to the active ingredients in insecticides (such as those in spinosad) (Croft, 1990; Soderlund & Bloomquist, 1990). The first step in developing an efficient IRM strategy is to determine the susceptibilities of pest populations to various insecticides. Furthermore, insecticide mode of action, cross-resistance spectrum and resistance mechanisms in insect populations should be investigated (Croft, 1990). Rotational use of insecticides is a major strategy to delay the resistance development in pest populations. However, to inhibit insect resistance to any insecticide, insecticide use should be rotated with other types of insecticides. However, this strategy is most-effectively implemented after determining if there are any cross resistance occurring among insecticides. The most-effective insecticides are those for which the pest population does not exhibit any cross resistance (or multiple resistance).

In a previous study (2007-2009) investigating the susceptibility of *F. occidentalis* populations in Antalya to several insecticides, it was found that only two of 10 *F. occidentalis* populations were resistance to spinosad (Dağlı et al., 2010). However, more recently, growers from many different parts of Antalya have been reporting that spinosad is becoming less effective against *F. occidentalis.* This reported increase in resistance was the impetus for this study.

In the present study, the level of resistance of an *F. occidentalis* population (Antalya-2015) to spinosad was measured. This population, collected from greenhouse-grown peppers in Kumluca (Antalya), Turkey in 2015, had been exposed to intensive, long-term spinosad. Cross resistance of the Antalya-2015 population to acrinathrin and formetanate, two other widely-used insecticides, were investigated. In addition to acrinathrin and formetanate, the efficacy of spinetoram on the Antalya-2015 population was determined at the recommended rate. Furthermore, the stability of resistance to spinosad in the same population was monitored for 1 year at two diagnostic doses. The main objective was to obtain information that could be used to develop rotational insecticide programs to better control *F. occidentalis* outbreaks.

Material and Methods

Frankliniella occidentalis populations

A population of *F. occidentalis* determined to be susceptible to spinosad was obtained in 2014. This population was collected from a bean plant in a home garden in Şuhut, Afyonkarahisar Province, Turkey (38°31'43.79"N, 30°32'42.97"E). The garden was far from fields or greenhouses, and located at an elevation of about 1000 m above sea level. In 2015, a population of *F. occidentalis* (Antalya-2015) from peppers grown in a greenhouse in Kumluca, Antalya Province, Turkey (36°21'51.95"N, 30°14'19.16"E) was collected. This population had been exposed to intensive, long-term spinosad applications. However, other active ingredients (acrinathrin, formetanate and spinetoram) had not been used in this greenhouse before collecting samples.

Insecticides

Active ingredients, commercial names and modes of action of insecticides used in this investigation are detailed in Table 1.

Table1. Active ingredients and commercial names for insecticides and their mode of action

Active ingredient	Commercial name	IRAC mode of action*
Spinosad	Laser, SC 480, Dow Agro Sciences	Nerve action, Nicotinic acetylcholine receptor (nAChR) allosteric modulators, (5).
Acrinathrin	Rufast, 75 EW, AgriNova	Nerve action, Sodium channel modulators, (3 A).
Formetanate	Dicarzol, 50 SP, AMC TARIM	Nerve action, Acetylcholinesterase (AChE) inhibitors, (1A).
Spinetoram	Radiant, 120 SC, Dow Agro Sciences	Nerve action, Nicotinic acetylcholine receptor (nAChR) allosteric modulators, (5).

* (IRAC, 2018).

Rearing of *Frankliniella occidentalis*

Frankliniella occidentalis was reared on green bean pods in a laboratory. Rearing followed the methods used in several other studies (Steiner & Goodwin, 1998; Murai & Loomans, 2001; Espinosa et al., 2002), but with some minor modifications. Transparent plastic cups (18 x 11 cm diameter) were used as thrips rearing cages (Figure 1). The lids of these cups were screened with filter paper to provide ventilation. Several layers of paper towels were placed at the base of the rearing cups to provide shelter for the thrips during their pupal stage. Green bean pods were prepared as follows before releasing thrips into the rearing cages. The pods were (1) soaked in sodium hypochlorite solution (about 6 g/L) for 2-3 min and then thoroughly rinsed with water, (2) immersed in sugar solution (about 5 g/L) for 2-3 min, and (3) dried before placing them into rearing cages. These pretreated pods were put into rearing cages before adult *F. occidentalis* were released into the cages (Figure 1). Generally, the pods in rearing cages were replaced by a fresh pod every 3-4 days. All *F. occidentalis* populations in cages were maintained in a climate-controlled room maintained at 23±1ºC and a 16:8 h L:D photoperiod.

Figure 1. Cages used to rear *Frankliniella occidentalis.*

Bioassay

A leaf-dip method was used for determining population LC values (Zhang et al., 2008). A series of serially-diluted concentrations (5-6) of spinosad, acrinathrin and formetanate were prepared in distilled water with TritonX-100 solution. These concentrations of each insecticide caused 0 to 100% mortality in the tested populations. Additionally, spinetoram was tested, but only at its recommended rate (60 mg a.i./L). Both susceptible and Antalya-2015 populations had been reared in an insecticide-free environment for 1 year. Two diagnostic doses of spinosad, the recommended rate and one tenth of that rate, were used to determine the stability of spinosad resistance in the Antalya-2015 population. Discs (3 cm diam.) were excised from leaves of potted bean plants and dipped (5 s) in one of the various insecticide concentrations or in water (control treatment) mixed with TritonX-100 solution. After drying, the treated leaf discs were placed in Petri dishes that were prepared as follows. (1) Agar (1.5%) was prepared in distilled water and boiled in a microwave, (2) after cooling for 30 min, the agar was poured into 3-cm diam. plastic Petri dishes, and (3) the treated leaf discs were embedded in that agar. Adult female thrips were then collected from rearing cages with a mouth aspirator, anesthetized with CO_2 and transferred to the leaf discs. The Petri dishes were covered with perforated plastic wrap to prevent the thrips from escaping but allow ventilation (Figure 2). Three replicates of each insecticide concentration was prepared. Fifteen to 30 thrips were used in each replicate. Mortality was recorded after 48 h with the aid of a microscope. Thrips were recorded as dead if they did not respond when prodded with a pin or brush.

Figure 2. The insecticide bioassay test cells for *Frankliniella occidentalis* (top view).

Data analysis

Probit analysis was applied to the number of alive and dead thrips (PoloPlus, 2002-2009 LeOra Software, Petaluma, CA, USA) to determine the LC values for the populations. Resistance ratios for spinosad, acrinathrin and formetanate were calculated as LC_{50} values of the Antalya-2015 population divided by the LC_{50} values for the susceptible population. Mortality data obtained from spinetoram bioassays were corrected using the Abbott formula (Abbott, 1925). Mortalities related to stability of spinosad resistance were likewise corrected. Mortalities from the first and the second measurement in resistance stability bioassays were analyzed using Pearson's chi-squared test.

Results

Mortalities of thrips in control treatments were less than 6.5% in all bioassays. LC_{50} values of susceptible and Antalya-2015 populations for spinosad, acrinathrin and formetanate are given in Tables 2, 3 and 4, respectively. Resistance ratios for thrips in the Antalya-2015 population to these insecticides (at LC_{50}) are also presented in these tables. Resistance of the Antalya-2015 population to spinosad, acrinathrin were 235- and 15-fold higher, respectively, than that of the susceptible population. No resistance to formetanate was recorded. LC_{90} values of the Antalya-2015 population for spinosad and acrinathrin were also higher than at the recommended rate for these insecticides. However, LC_{90} values for the Antalya-2015 population for formetanate were lower than at its recommended rate.

Table 2. The resistance ratio (at LC_{50}) to spinosad in susceptible and spinosad-resistant (Antalya-2015) populations of *Frankliniella occidentalis*

Populations	n*	Slope±S.E	LC_{50} mg (a.i.)/L (95% CL)	Resistance Ratio**	LC_{90} mg (a.i.)/L (95% CL)	***Registered rate mg(a.i.)/L
Susceptible	497	1.4±0.2	0.6 0.4-1.0	-	5.4 3.2-10.6	96
Antalya-2015	658	1.4±0.2	141.0 96.0-238.0	235	1216.5 555.9-6503.4	96

* n: number adult female thrips used in bioassay;
** Resistance ratio: LC_{50} of the Antalya-2015 population / LC_{50} of the susceptible population;
*** (GTHB,2018);
Highest mortality in control treatments was 4.8%.

Table 3. The resistance ratio (at LC_{50}) to acrinathrin in susceptible and spinosad-resistant (Antalya-2015) populations of *Frankliniella occidentalis*

Populations	n*	Slope±S.E	LC_{50} mg (a.i.)/L (95% CL)	Resistance Ratio	LC_{90} mg (a.i.)/L (%95% CL)	***Registered rate mg (a.i.)/L
Susceptible	484	1.2±0.1	1.8 0.7-3.5	-	19.5 10.9-38.9	60
Antalya-2015	278	1.5±0.2	26.5 11.6-46.8	14.7	191.5 101.3-614.9	60

* n: number adult female thrips used in bioassay;
** Resistance ratio: LC_{50} of the Antalya-2015 population / LC_{50} of the susceptible population;
*** (GTHB,2018);
Highest mortality in control treatments was 4.7%.

Table 4. The resistance ratio (at LC_{50}) to formetanate in susceptible and spinosad-resistant (Antalya-2015) populations of *Frankliniella occidentalis*

Populations	n*	Slope±S.E	LC_{50} mg (a.i.)/L (95% CL)	Resistance Ratio	LC_{90} mg (a.i.)/L (95% CL)	***Registered rate mg(a.i.)/L
Susceptible	436	2.0±0.3	14.2 6.5-32.7	-	62.0 28.3-612.5	500
Antalya-2015	608	1.3±0.1	16.0 8.5-26.6	1.1	147.3 85.6-305.7	500

* n: number adult female thrips used in bioassay;
** Resistance ratio: LC_{50} of the Antalya-2015 population / LC_{50} of the susceptible population;
*** (GTHB,2018);
Highest mortality in control treatments was 4.2%.

Mortality ratios from with spinetoram and spinosad bioassays are shown in Table 5. The efficacy of spinetoram on the Antalya-2015 population was determined only at its recommended rate. Both insecticides caused 100% mortality to the susceptible population of *F. occidentalis* at their recommended rates. However, mortality in the Antalya-2015 population caused by spinetoram and spinosad were lower: 88 and 38%, respectively (Table 5). Mortality-related stability of spinosad resistance in the Antalya-2015 population is provided in Table 6.

Two diagnostic doses of spinosad, the recommended rate and on tenth of that rate, were used to determine the stability of spinosad resistance in the Antalya-2015 population. In this population, the recommended rate of spinosad caused 30% mortality, whereas the tenth rate caused only 2% mortality. For the Antalya-2015 population that had been reared in an insecticide-free environment for 1 year, mortalities at these two diagnostic doses were 39% (recommended rate) and 5% (one tenth rate), not much different than when the population had not been reared in an insecticide-free environment (Table 6). Therefore, the reversion (i.e., dilution) rate of spinosad resistance in the Antalya-2015 population was quite low (2-9%).

Table 5. Mortality ratios for susceptible and spinosad-resistant (Antalya-2015) populations of *Frankliniella occidentalis*in bioassays with spinosad and spinetoram

Populations	Spinosad recommended rate: 96 mg a.i./L		Spinetoram recommended rate: 60 mg a.i./L	
	n*	Mortality (%)	n*	Mortality (%)
Susceptible	74	100	66	100
Antalya-2015	63	38	69	88

* n: number adult female thrips used in bioassay;
Highest mortality in control treatments was 5.8%.

Table 6. Stability of spinosad resistance in susceptible and spinosad-resistant (Antalya-2015) populations of *Frankliniella occidentalis*

Spinosad rates	Populations	First measurement (first generation)		Second measurement (12 months later)	
		n*	Mortality (%)	n*	Mortality (%)
9.6 mg a.i./L	Susceptible	52	100.0	80	94.4
	Antalya-2015	154	1.6	121	5.1
96 mg a.i./L	Susceptible	55	100.0	109	100.0
	Antalya-2015	138	29.6	214	39.2

* n: number of adult female thrips used in bioassay;
Mortality ratios obtained from second measurements were not significantly different from first measurement, (Pearson's chi-squared test, P>0.05);
Highest mortality in control treatments 6.5%.

Discussion

The susceptible *F. occidentalis* population used in this study was highly susceptible to the insecticides tested. The LC_{90} values of susceptible population for spinosad, acrinathrin and formetanate (5.4, 19.5 and 62 mg a.i./L) were much lower than the recommended rates of those insecticides (96, 60 and 500 mg a.i./L). Furthermore, this susceptible population was also sensitive to spinetoram. The recommended rates of spinetoram (60 mg a.i./L) caused 100% mortality in this susceptible population. The existence of such susceptible populations in nature is needed to provide dilution in resistance and is critically important for monitoring changes in insecticide resistance in greenhouse or field populations.

The *F. occidentalis* population Antalya-2015 was collected from a greenhouse that had been treated with frequent spinosad applications over a long period of time. As expected, a high level of spinosad resistance was detected in this population, which was up to 235 times more resistant to spinosad than the susceptible population. Dağlı et al. (2010) had previously documented resistance of *F. occidentalis* populations in Antalya to spinosad; however, the 235-fold resistance detected in present study was much higher than the previous finding. The LC_{90} value of spinosad for the Antalya-2015 population (1217 mg a.i./L) was much higher than the recommended rate (96 mg a.i./L). The recommended rate of spinosad caused 100% mortality in the susceptible population, whereas mortality was only 30% in the Antalya-2015 population. Thus, spinosad would clearly be ineffective in controlling the Antalya-2015 population. This level of resistance is likely to be the main reason spinosad could not control *F. occidentalis* infestations under field conditions.

Resistance of *F. occidentalis* to spinosad has also been reported in other countries, including Spain, Australia, Japan and China (Herron & James 2005; Bielza et al., 2007; Zhang et al., 2008; Gao et al., 2012). In the case of the population in Murcia, Spain, resistance to spinosad was low in 2001-2002 because it had not been used previously in this region, however, after intensive application of spinosad, resistance had become 3682-fold higher by 2004 (Bielza et al., 2007).

Insecticide resistance in *F. occidentalis* is not limited to spinosad. In fact, Gao et al. (2012) has reported that *F. occidentalis* has significant potential to develop resistance to many other active ingredients used in insecticides. Resistance in *F. occidentalis* has been recorded worldwide against 30 different active ingredients used in insecticides, including a variety of well-known and often-used insecticides, including spinosad, spinetoram, cypermethrin, deltamethrin, acrinathrin, abamectin, chlorpyrifos, malathion, methiocarb and formetanate (Gao et al., 2012; ARDB, 2018).

Spinosad is a valuable bioinsecticide for pest management because it is highly effective in controlling many important pest species and is not very toxic to mammals in low doses. Currently, the most-widely recommended registered insecticides against *F. occidentalis* in Antalya greenhouses are spinosad (spinosyn), acrinathrin (pyrethroid), formetanate (carbamate) and spinetoram (spinosyn). It was shown that the Antalya-2015 population is highly resistant to spinosad, and has moderate cross

resistance to acrinathrin (15 times higher than the susceptible population) but has no cross resistance to formetanate. Therefore, acrinathrin would not be a suitable alternative for insect infestations that have already acquired some cross resistance (such as the spinosad-resistant *F. occidentalis* population used in this study). In contrast, formetanate would likely provide an effective alternative for resistant insect populations because in the Antalya-2015 population it had high efficacy and no cross resistance. Probably, the mechanisms of resistance to spinosad and formetanate were not the same in the spinosad-resistant *F. occidentalis* population. In two different studies, resistance to spinosad in *F. occidentalis* population was detected as non-metabolic (Bielza et al., 2007; Zhang et al., 2008). Similarly, Shono & Scott (2003) reported that metabolic detoxification enzymes (monooxygenases, hydrolases or glutathioneS-transferases) were not involved in spinosad resistance in house fly, *Musca domestica* L., 1758 (Diptera: Muscidae). In contrast, metabolic enzymes such as esterase and GST were determined to be a major mechanism of resistance to carbamate and organophosphate insecticides in *F. occidentalis* populations (Jensen, 1998; Jensen, 2000; Maymo et al., 2002). Therefore, the efficacy of formetanate on the spinosad-resistant population possibly had not been affected by the spinosad resistance mechanism. Similarly, spinosad resistance was not associated with cross resistance to formetanate in *F. occidentalis* populations in Spain (Bielza et al., 2007).

In present study, the efficacy of spinetoram on the Antalya-2015 population was evaluated on the basis of its recommended rate. Spinetoram has the same mode of action as spinosad (IRAC, 2018). However, spinosad was registered in 2003 in Turkey, whereas the registration date for spinetoram was 2014 (Tosun & Onan, 2014). In present study, both spinetoram and spinosad caused 100% mortality in the susceptible population of *F. occidentalis*, but spinetoram showed higher efficacy than spinosad in the Antalya-2015 population (88% mortality for spinetoram vs. 38% mortality for spinosad). Considering these differences in mortalities, cross resistance to spinetoram in the spinosad-resistant population was low. However, more detailed data are needed on LC values to determine degree of cross resistance between spinetoram and spinosad in the Antalya-2015 population.

In the assay of the stability of spinosad resistance, mortality rate in Antalya-2015 population did not significantly change after it had been maintained in an insecticide-free environment for 1year. Therefore, its resistance to spinosad was stable (i.e., it is unlikely to revert to a spinosad-susceptible population). Several factors might help maintain stability of spinosad resistance in this population. Initial gene frequencies, mode of resistance inheritance, the fitness cost and immigration of susceptible insect are the major effective factors in resistance stability (Bielza et al., 2008). Based on the results from the present study, the effect of two factors on the resistance stability could be possible. (1) Initial resistant gene frequency in the Antalya-2015 population was quite high with almost the entire population being resistant individuals because a diagnostic dose of spinosad (9.6 mg a.i./L) causes 100% mortality in the susceptible population. However, the same dose caused only 1.6% mortality in spinosad-resistant population. (2) Dilution of resistance might not be possible in Antalya-2015 population because it was maintained in rearing cages. Therefore, immigration of susceptible thrips from the outside was not possible. Additional genetic investigation of the susceptible and resistant populations, such as reciprocal crosses and backcross studies, will be necessary to determine the mode of inheritance and the number of effective genes conferring resistance to these insecticides. Similar results for resistance stability of *F. occidentalis* have been reported from Spain, where resistance remained high and stable for 8 months, until 2 months after susceptible individuals migrated into the resistant population, after which resistance declined (Bielza et al., 2008). This suggests that spinosad resistance may be diluted in greenhouses by *F. occidentalis*-susceptible individuals that migrate from a nearby environment. Therefore, the frequency of insecticide application in any given greenhouse should be reduced to lower the risk of pest populations developing resistance to pesticides. Otherwise, when pesticide resistance develops in a greenhouse population, reduction of resistance will be extremely difficult without purposively introducing susceptible populations to dilute the gene pool of pest species. Therefore, all resistance management strategies should be implemented before resistance in pest populations becomes evident. Otherwise, many active ingredients suitable for integrated pest management, such as those in spinosad, will become useless when pests become resistant to a large variety of pesticides.

Given the high level of resistance to spinosad found in the investigated population of *F. occidentalis*, the prevalence of spinosad resistance in *F. occidentalis* populations should be determined for more populations in greenhouses in Antalya and across Turkey. In addition to spinosad, other registered, commonly used active ingredients should be investigated for pest resistance and insecticide rotational strategies should be updated. However, relying only insecticides may not be adequate to efficiently control *F. occidentalis* and the TSWV it transmits. The frequency of insecticide application of spinosad should be reduced to provide for a more sustainable management of pests using more efficient insecticides. Even more importantly, cultural, biological and other alternatives should be integrated into pest management strategies.

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Original article (Orijinal araştırma)

Reproduction of *Meloidogyne chitwoodi* Golden et al., 1980 (Tylenchida: Meloidogynidae) on different potato cultivars and its effect on plant growth[1]

Meloidogyne chitwoodi Golden et al.,1980 (Tylenchida: Meloidogynidae)' nin farklı patates çeşitleri üzerindeki üremesi ve bunun bitki gelişimine etkisi

Emre EVLİCE[2*] **Şerife BAYRAM[3*]**

Abstract

The Columbia root-knot nematode, *Meloidogyne chitwoodi* Golden et al., 1980 (Tylenchida: Meloidogynidae), is one of the most damaging nematode parasites of potato. It can cause economic damage in many cultivated plants and infest weeds. In this study, the reproduction of *M. chitwoodi* was assessed in 19 potato cultivars and one candidate cultivar under climate chamber conditions, and the effects of *M. chitwoodi* on plant growth were assessed in a greenhouse in 2014. *Meloidogyne chitwoodi* reproduced well on the potato cultivars tested based on numbers of egg and egg mass, reproduction factor and egg mass index. There were approximately 2-fold differences between some cultivars according to minimum and maximum values of the number of egg masses (205 in Challenger and 423 in Adora) and reproduction factor (28.2 in Marabel and 58.6 in Adora). Some plant growth parameters were significantly different between nematode inoculated and uninoculated plants for each cultivar according to t-test. Additionally, significant percentage decreases for some plant growth parameters were recorded, including plant height (7.6-13.4%), fresh (14.6-24.5%) and dry (14.2-26.7%) weights of shoots, fresh (15.0-25.1%) and dry (16.2-26.2%) root. It was found that all potato cultivars tested were susceptible to *M. chitwoodi*, and the degree of adverse effect on their growth varied between cultivar.

Keywords: Host, *Meloidogyne chitwoodi*, plant growth, potato, resistance

Öz

Kolombiya kök-ur nematodu, *Meloidogyne chitwoodi* Golden et al., 1980 (Tylenchida: Meloidogynidae), patateste zarar yapan en önemli türlerden biridir. Birçok kültür bitkisinde ekonomik kayıplara neden olabilmekte ve yabancı otları enfekte edebilmektedir. Bu çalışmada, *M. chitwoodi*'nin Türkiye'de yaygın olarak yetiştirilen 19 patates çeşidi ve 1 aday çeşit açısından iklim odası şartlarında üreme durumu ve sera şartlarında bitki gelişimine etkisi 2014 yılında belirlenmiştir. *Meloidogyne chitwoodi*, elde edilen yumurta paketi sayısı, yumurta sayısı, üreme katsayısı ve yumurta paketi indeksine göre patates çeşitlerinin tamamında iyi derecede çoğalmıştır. En çok ve en az üreme görülen çeşitler arasında yumurta paketi sayısı (205, Challenger; 423, Adora) ve üreme katsayısı (28.2, Marabel; 58.6, Adora) açısından yaklaşık 2 kat fark olduğu belirlenmiştir. Bazı bitki büyüme parametrelerinde t-testine göre her çeşidin nematod inokule edilmiş ve edilmemiş bitkileri arasında önemli ölçüde farklılık tespit edilmiştir. Ayrıca, bitki boyu (%7.6-13.4), üst aksam yaş (%14.6-24.5) ve kuru (%14.2-26.7) ağırlığı, kök yaş (15.0-25.1) ve kuru (16.2-26.2) ağırlığını içeren bitki büyüme parametrelerinde önemli bir azalış tespit edilmiştir. Çalışmaya dahil edilen tüm patates çeşitlerinin *M. chitwoodi*'ye duyarlı olduğu ve bitki büyümesine olumsuz etkisinin çeşitlere bağlı olarak değiştiği belirlenmiştir.

Anahtar sözcükler: Konukçu, *Meloidogyne chitwoodi*, bitki gelişimi, patates, dayanıklılık

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Introduction

Potato is one of the most important agricultural products all over the world and is one of the major food sources in many countries. Unlike other important food sources; potato is a key food as it produces more dry-matter and calories per unit area, and has well-balanced protein (Hasan et al., 2014). Turkey has highly suitable conditions for the cultivation of potato, and potatoes are produced almost everywhere in Turkey, with the country ranked nineteenth in global production (Çalışkan et al., 2010; FAO, 2016). In the majority of potato-producing areas of the world, plant-parasitic nematodes, especially potato cyst nematodes and root-knot nematodes, are among the most important pests of the potato. Root-knot nematodes, *Meloidogyne* spp. are obligate parasites and responsible for limiting agricultural productivity. Columbia root-knot nematode *Meloidogyne chitwoodi* Golden, O'Bannon, Santo & Finley, 1980 (Tylenchida: Meloidogynidae) was first described from potatoes (*Solanum tuberosum* L.) in the Pacific Northwest (USA) and has been reported in Africa (South Africa, Mozambique), Europe (Belgium, France, Germany, Netherlands, Portugal, Sweden, Turkey), and North and South America (Mexico, Argentina) (Golden et al., 1980; EPPO, 2018a). In Turkey, *M. chitwoodi* was detected for the first time in potato tubers collected from Niğde Province in 2006 (Özarslandan et al., 2009). It has since been reported in Nevşehir, Konya, Kayseri, Aksaray, Balıkesir, Bitlis, Isparta, İzmir, Kütahya and Manisa Provinces (Yıldız et al., 2009; Ulutaş, 2010; Özarslandan et al., 2013; Evlice & Bayram, 2016).

Meloidogyne chitwoodi has a broad host range comprising several plant families (including Solanaceae, Umbelliferae, Gramineae, Leguminosae, Brassicaceae and Cucurbitaceae) and can cause severe damage to many economically important crops, such as potato, carrot, tomato, carrot, maize, wheat and bean (Santo et al., 1980; O'Bannon et al., 1982; Brinkman et al., 1996; den Nijs et al., 2004). In potato, it causes blisters on the tuber surface and brownish spots in the tuber tissue, as well as infecting the roots, and tubers with these internal and external symptoms are unmarketable for fresh consumption or processing. The economic loss caused by *M. chitwoodi* can reach $9900/ha (Ingham et al., 2007). The economic damage threshold for quality loss of potatoes has been established at 1 J2 (second-stage juvenile)/250 ml of soil in the USA and 10 J2s/100 ml of soil in the Netherlands (Santo et al., 1981; Norshie et al., 2011). However, due to the rapid multiplication of the *M. chitwoodi*, the environmental factors such as temperature, growing season, soil structure are much more important determinants of damage. Spread and damage were observed in the areas with sandy soil with over 1500 degree-days (>5°C) (Griffin, 1985; Pinkerton et al., 1991). *Meloidogyne chitwoodi* is generally regarded as a quality pest (Suffert & Giltrap, 2012), but it also reduced plant development and tuber yield (Santo & O'Bannon, 1981; Pinkerton et al., 1986; Scholte, 1990; Hafez & Sundararaj, 2009). This paper reports reproduction of *M. chitwoodi* on some potato cultivars and its effect on their growth.

Material and Methods

Nematode inoculum

A population of *M. chitwoodi* (NEV-10) was used in this study. Pure stock culture of NEV-10 (Suvermez Kasabası/Nevşehir, 38°21'48.60" N, 34°40'16.20" E) was identified by morphological and molecular methods (Evlice & Bayram, 2016). Nematode cultures were maintained on tomato plants (*Solanum lycopersicum* cv. Tueza) in a climate chamber. Four leaf stage tomato seedlings, established in pots (760 ml, 10x10x11 cm), were inoculated with 10-15 egg masses and allowed to multiply for 8 weeks (Mistanoğlu et al., 2016). Egg masses were collected from infected roots and J2s were obtained using a Petri dish method at 23ºC. J2s that hatched in the first 24 h were discarded, and thereafter J2s were collected and stored at 4ºC. J2s were used within 72 h for inoculation (Nyczepir et al., 1999).

Plant material

Nineteen potato cultivars, the most commonly cultivated in Turkey and one candidate cultivar from Turkey were used in this study (Table 1). Except the candidate cultivar, only certified potato tubers were tested. Of the tested cultivars, eight were grown from mini tubers. For the others tuber were cut into 25-28 mm pieces. Tubers were sprouted to about 1 cm long before planting, with excess sprouts removed to obtain tubers with single sprout for planting.

Reproduction of *Meloidogyne chitwoodi* on potato cultivars

All experiments were conducted at Plant Protection Central Research Institute (Ankara, Turkey) in 2014. The experiment was conducted in a climate chamber at 23±2ºC with a 14:8 h L:D photoperiod (3000 lux). The plants were established in pots (550 ml, 9x9x10 cm) filled with autoclaved (Smith & Onions, 1994) soil mixture (85% silver sand, 15% soil). Osmocote® (Scotts, Marysville, OH, USA) (18-6-12), a slow release fertilizer, was applied at a rate of 1 g/kg soil mixture. Five pots for each cultivar were inoculated with 750 J2 (the initial population density, Pi) at planting. The inoculum was delivered into 3-4 cm deep holes and covered with soil. Tomato plants (Tueza F1) served as a susceptible control. The pots were arranged in a completely randomized design. Plants were watered and fertilized regularly. Sixty days after inoculation, plants were removed from the pots, the root systems were gently washed with tap water and then soaked in a Phloxine B solution (0.15-0.20 g/L; Sigma-Aldrich, St. Louis, MO, USA) to stain nematode egg masses. Gall and egg masses per root system were counted and ranked on a scale of 0 to 10, where 0 = no galls or no egg masses, 1 = 1-2, 2 = 3-10, 3 = 11-30, 4 = 31-100, 5= more than 100 galls or egg masses per root system, and the cultivars assigned according to egg mass index (EI) (Hartman & Sasser, 1985). Eggs were extracted from roots (Hartman & Sasser, 1985), the final population (Pf) and reproduction factor (Rf = Pf/Pi) was determined. Cultivars were classified as non-host (immune) (EI = 0 and Rf = 0), poor host (resistant) (EI = 1-3 and Rf < 1) and a suitable host (susceptible) (EI = 4-5 and Rf > 1) (Oostenbrink, 1966).

Effect of *Meloidogyne chitwoodi* on plant growth

An experiment was conducted in a greenhouse and uninoculated plants were included for comparing some vegetative parameters. The experiment was designed as previously indicated. The pots were arranged randomly on a bench in greenhouse (24.4±4.7ºC) with a 16:8 h L:D photoperiod. Plants were watered and fertilized regularly. The experiment was terminated 60 d after inoculation. The roots were stained and the egg masses counted. Plant shoots and roots were dried at 70ºC in an oven for 48 h and dry weights determined (Mohammad et al., 2007). Plant growth was assessed as plant height (cm), and fresh and dry weights (g) of shoots and roots.

Statistical analysis

The mean numbers of egg masses and reproduction factor were square root transformed. Variance analysis was performed with transformed data and the differences between treatments were analyzed by means of Duncan's multiple range test ($P < 0.05$). Plant growth parameters as plant height (cm), and fresh and dry weights (g) of shoots and roots were analyzed by paired t-test. All statistical analyses were performed using SPSS 20 software (IBM Corp., Armonk, NY, USA).

Results

Reproduction of *Meloidogyne chitwoodi* on potato cultivars

Egg masses of *M. chitwoodi* were observed on all plants tested (Table 1). The number of egg masses on each root system ranged from 205 to 423 and showed significant variation ($F = 3.24$ $P < 0.05$) between the cultivars. Challenger (205), Van Gogh (216), Marabel (229), Granola (249) and Orchestra (251.00) had the lowest mean number of egg masses. However, the highest number of egg masses were in Adora (423), Hermes (374), Lady Rosetta (358), 614002 (353) and Melody (324). *Meloidogyne chitwoodi* reproduced on all the cultivars with significant differences ($F = 3.15$, $P < 0.05$) between the cultivars. The highest Rf of *M. chitwoodi* was in Adora (58.6) followed by 614002 (58.0), Hermes (56.2), Lady Rosetta (56.2) and Agria (44.8). Whereas, the lowest Rf values were in Marabel (28.2), Challenger (29.0), Orchestra (30.0), Pomquen (31.0) and Van Gogh (32.8). Therefore, all the potato cultivars were classified as susceptible, having a ranking of 5 and $R > 1$. Few or no galls were detected on the potato roots, but were observed on the tomato roots (Figure 1). However, these galls are not considered to be as large a problem as those induced by other *Meloidogyne* species, such as *M. incognita* and *M. javanica*. Santo et al. (1980) and Golden et al. (1980) also reported that *M. chitwoodi* causes little or no galling on roots of potato and tomato cv. Rutgers.

Table 1. Number of egg masses, reproduction factor (Rf: Pf/Pi) and egg mass index of *Meloidogyne chitwoodi* on different potato cultivars 60 d after inoculation with 750 second-stage juveniles

Cultivar	Number of egg masses[1]		Rf[1]		Egg-mass index
Adora	423±37	a[2]	58.6±5.8	ab	5
Agria	273±25	abcd	44.8±5.2	abc	5
Alegria	285±31	abcd	39.4±4.4	abc	5
Borwina	281±18	abcd	37.0±2.1	abc	5
Challenger	205±9	d	29.0±6.1	bc	5
Granola	249±21	abcd	41.2±3.4	abc	5
Hermes	374±34	abc	56.2±8.2	abc	5
İnnovator	291±16	abcd	37.4±2.3	abc	5
Jearla	312±18	abcd	42.8±6.5	abc	5
Lady Olimpia	263±40	abcd	44.6±7.4	abc	5
Lady Rosetta	358±30	abcd	56.2±5.2	abc	5
Marabel	229±27	bcd	28.2±3.1	c	5
Melody	324±38	abcd	38.2±7.3	abc	5
Orchestra	251±33	abcd	30.0±1.3	bc	5
Pomqueen	310±42	abcd	31.0±6.8	bc	5
Russet	263±23	abcd	42.8±6.8	abc	5
Spunta	314±64	abcd	37.2±7.2	abc	5
Toscana	260±26	abcd	36.8±5.5	abc	5
Van Gogh	216±26	cd	32.8±3.1	abc	5
614002	353 ±10	abcd	58.0±4.3	abc	5
Tomato	391±48	ab	64.2±11.7	a	5

[1] Data are means±SE;
[2] Different letters following means in the same column indicate statistical significance from each other (ANOVA $P < 0.05$, Duncan's multiple range test).

Figure 1. Symptoms caused by *Meloidogyne chitwoodi* on roots of potato plant stained with Phloxine B.

Effect of *Meloidogyne chitwoodi* on plant growth

The mean plant growth parameters with and with nematode inoculation are shown in Table 2 along with the number of egg mass on the inoculated plants. Egg masses were detected on all cultivars, with mean number differing significantly ($F = 2.09$, $P < 0.05$) between cultivars. The highest number of egg masses were on Adora (389), Lady Rosetta (363), Hermes (360), Jearla (359) and Alegria (337), while Toscana (233), Agria (241), Challenger (243), Lady Olympia (254) and Marabel (268) had the lowest number.

Table 2. Effect of *Meloidogyne chitwoodi* on the plant growth of different potato cultivars 60 days after inoculation with 750 second-stage juveniles[1]

Cultivar	Nematode[2]	Plant height (cm)	Shoot fresh weight (g)	Shoot dry weight (g)	Root fresh weight (g)	Root dry weight (g)	Egg mass number**
Adora	+	94±2.4*	25.5±1.3*	5.1±0.1*	13.8±0.9*	1.7±0.2	389±37 a
	-	106±2.3	34.2±1.8	5.9±0.2	18.1±0.9	2.3±0.2	
Agria	+	106±1.4	19.3±0.7*	4.5±0.1	15.1±0.9*	1.7±0.1	241±15 d
	-	114±5.7	22.9±0.8	4.8±0.1	18.1±1.8	2.1±0.1	
Alegria	+	86±4.5	26.6±1.3*	5.2±0.1	12.9±0.8*	1.5±0.2	337±60 abcd
	-	93±3.6	31.6±1.5	5.7±0.1	15.9±1.2	1.8±0.2	
Borwina	+	90±3.8	18.7±0.6*	4.4±0.1*	17.3±0.7*	1.7±0.2	301±31 abcd
	-	102±4.8	23.5±1.2	4.9±0.1	20.6±0.7	2.1±0.3	
Challenger	+	93±4.0	27.9±1.1*	5.3±0.1*	18.2±1.0*	2.0±0.3	243±42 d
	-	101±3.5	32.9±1.2	5.8±0.1	22.1±1.4	2.7±0.3	
Granola	+	86±1.8	21.6±1.1*	4.7±0.1*	14.5±0.9*	1.6±0.1	299±36 abcd
	-	96±5.8	26.8±1.1	5.2±0.1	19.2±1.2	1.9±0.2	
Hermes	+	92±2.9*	19.9±1.3*	4.5±0.1	13.8±0.7*	1.8±0.1	360±20 abc
	-	107±2.8	24.5±0.9	5.0±0.1	17.3±1.7	2.4±0.2	
İnnovator	+	89±.3.2	23.5±1.6*	4.9±0.2	17.6±1.6*	1.7±0.2	279±35 abcd
	-	98±4.7	27.9±2.9	5.3±0.3	21.1±1.2	2.1±0.2	
Jearla	+	108±2.9	24.9±0.9*	5.0±0.1	15.2±1.5*	1.9±0.2	359±32 abc
	-	117±5.7	29.2±1.3	5.5±0.1	18.3±1.5	2.4±0.2	
Lady Olympia	+	102±3.8	23.2±1.4*	4.9±0.1*	16.2±0.8*	1.6±0.2	254±21 cd
	-	117±5.5	27.1±2.1	5.2±0.2	19.6±0.8	2.1±0.2	
Lady Rosetta	+	73±3.0	22.8±1.5*	4.8±0.2*	17.7±1.0*	2.1±0.2	363±30 abc
	-	82±2.7	29.4±2.2	5.5±0.2	21.6±1.2	2.6±0.4	
Marabel	+	93±3.5*	21.6±1.7*	4.7±0.2*	15.6±0.7*	1.8±0.1	268±28 cd
	-	106±2.7	25.9±1.8	5.1±0.2	18.7±1.0	2.3±0.2	

Table 2. (Continued)

Cultivar	Nematode[2]	Plant height (cm)	Shoot fresh weight (g)	Shoot dry weight (g)	Root fresh weight (g)	Root dry weight (g)	Egg mass number**
Melody	+	94±3.3	23.3±1.9*	4.9±0.2	14.9±1.0*	1.9±0.3	279±33 abcd
	-	103±4.7	28.5±1.5	5.4±0.1	18.6±0.9	2.3±0.3	
Orchestra	+	109±1.9*	25.4±1.7*	5.1±0.2*	15.7±1.2*	1.6±0.1	277±43 bcd
	-	126±3.9	29.1±0.8	5.4±0.1	20.9±1.1	2.2±0.3	
Pomqueen	+	108±2.7*	22.8±1.4*	4.8±0.2*	15.4±0.7*	1.7±0.2	289±12 abcd
	-	120±1.9	27.5±1.3	5.3±0.1	18.7±1.1	2.1±0.3	
Russet	+	89±3.0	22.5±0.9*	4.8±0.1	17.2±0.9*	1.7±0.2	314±22 abcd
	-	100±4.7	26.3±1.8	5.2±0.2	20.3±1.1	2.1±0.2	
Spunta	+	99±4.6*	20.7±0.9*	4.6±0.1*	17.5±1.0*	1.8±0.2	293±21 abcd
	-	114±3.1	25.9±1.0	5.1±0.1	20.8±0.7	2.2±0.2	
Toscana	+	75±1.8*	17.5±0.9*	4.2±0.1*	14.3±0.8*	1.6±0.1	233±11 d
	-	84±2.9	21.6±0.9	4.7±0.1	17.5±0.9	1.9±0.1	
Van Gogh	+	90±.3.6*	23.8±0.8*	4.9±0.1*	17.6±0.8*	1.8±0.1	299±32 abcd
	-	100±1.2	28.9±1.1	5.4±0.1	20.8±1.1	2.2±0.2	
614002	+	73±2.9	19.0±1.3*	4.2±0.1	14.4±0.9*	1.7±0.2	297±36 abcd
	-	81±2.1	23.4±1.0	4.6±0.1	17.5±0.8	2.2±0.2	
Tomato	+	47±3.1	25.1±1.7*	5.1±0.2*	13.8±0.5*	2.1±0.2	380±18 ab
	-	52±3.6	29.1±0.7	5.4±0.1	17.9±1.7	2.8±0.2	

[1] Data is means±SE, n = 5;
[2] +: Nematode inoculated; -: Nematode uninoculated;
* Values between nematode inoculated and uninoculated plants of each cultivar are significantly different according to t-test ($P < 0.05$);
** Values indicated by the same letter are not significantly different according to Duncan's multiple range test at $P < 0.05$.

The growth parameters varied between cultivars, but there was no significant difference for some parameters between some cultivars. All cultivars showed significant decrease in shoot and root fresh weight, but no significant difference was found fin root dry weight. All parameters except dry root weight decreased significantly in Adora, Marabel, Orchestra, Pomqueen, Spunta, Toscana and Van Gogh. However, plant height, fresh and dry weights of shoots and roots were reduced by *M. chitwoodi* relative to uninoculated plants. For inoculated and uninoculated plants, the lowest plant heights (cm) were in Lady Rosetta (73 and 82, respectively) and 614002 (73 and 81), and the highest in Orchestra (109 and 126) and Pomqueen (108 and 120). The lowest shoot fresh weight was in Toscana (17.5, 21.6) and Borwina (18.7 and 23.5), the highest in Challenger (27.9 and 32.9) and Alegria (26.6, 31.6). The lowest and highest shoot dry weights were in 614002 (4.2 and 4.6) and Challenger (5.3 and 5.8), respectively. The lowest root fresh and dry weights were obtained in Alegria (12.9 and 15.9, and 1.5 and 1.8) while the highest root fresh and dry weights were in Challenger (18.2 and 22.1, and 2.0 and 2.7, respectively).

The percent effects on plant growth parameters due to the nematode inoculation are shown in Table 3. Plant height, fresh and dry weights of shoots and roots of the cultivars were reduced by inoculation with *M. chitwoodi* relative to uninoculated plants, but there were no significant differences between the cultivars ($P > 0.05$). The greatest decrease in plant height was in Hermes (13.4%) followed by Spunta (13.3%) and Orchestra (12.3%), while the least effects were in Alegria (7.6%), Agria (7.8%) and Melody (7.9%). The greatest effect on fresh and dry weight of shoots was in Adora (24.5 and 26.7%, respectively), followed by Lady Rosetta (21.8%) and Borwina (20.0%) for shoot fresh weight and Marabel (22.4%) and Hermes (21.98%) for shoot dry weight. The least effect on shoot fresh weight were in Orchestra (14.6%), Russet (14.6%) and Innovator (14.8%). For shoot dry weight, the least effect was in Jearla (14.2%), Spunta (14.3%) and Lady Olympia (15.6%). The greatest effects on root fresh and dry weights were in Orchestra (25.1%), Granola (24.4%), Adora (23.6%) and Challenger (26.2%), and in Adora (24.1%) and Innovator (23.8%), respectively. Whereas, the least effects were detected in Van Gogh (15.0%), Spunta (15.6%), Marabel (15.6%) and Pomqueen (16.2%), and Jearla (16.6%) and Alegria (17.2%), respectively.

Table 3. Percentage reduction in the plant growth of different potato cultivars caused by *Meloidogyne chitwoodi* 60 days after inoculation with 750 second-stage juveniles[1]

Cultivar	Plant height (%)	Shoot fresh weight (%)	Shoot dry weight (%)	Root fresh weight (%)	Root dry weight (%)
Adora	11.9±3.1	24.5±5.3	26.7±6.2	23.6±3.6	24.1±10.5
Agria	7.8±4.1	15.6±1.4	20.8±6.3	15.6±5.2	20.5±8.0
Alegria	7.6±1.9	15.7±3.2	17.2±5.9	17.6±7.1	17.2±6.5
Borwina	11.5±2.2	20.0±2.5	20.8±3.4	16.0±4.1	21.1±7.7
Challenger	8.5±1.8	15.4±1.8	16.8±2.8	17.4±4.3	26.2±11.5
Granola	9.4±3.5	19.1±2.8	21.1±5.1	24.4±6.9	19.6±3.7
Hermes	13.4±4.1	18.4±4.6	21.9±3.8	18.8±5.4	22.4±8.1
İnnovator	9.0±2.2	14.8±3.6	20.8±4.7	16.9±7.4	23.8±7.8
Jearla	9.7±3.6	15.8±4.6	14.2±5.1	17.3±6.4	16.6±10.0
Lady Olympia	12.1±5.5	15.7±5.9	15.6±5.6	17.3±4.7	23.7±4.3
Lady Rosetta	10.4±4.6	21.8±4.3	18.5±7.8	19.7±5.7	23.6±8.6
Marabel	11.9±1.8	17.9±4.9	22.4±8.3	15.6±5.1	21.9±6.2
Melody	7.9±2.3	18.5±3.5	19.9±5.4	18.2±5.6	19.1±6.7
Orchestra	12.3±4.0	14.6±5.3	17.5±7.7	25.1±4.0	22.9±11.1
Pomqueen	10.0±2.9	17.1±3.2	19.9±7.5	16.7±3.2	16.2±5.7
Russet	9.7±3.9	14.6±4.9	17.3±8.1	15.7±7.2	20.7±8.3
Spunta	13.3±3.4	19.5±5.8	14.3±5.5	15.6±4.9	22.4±9.8
Toscana	10.2±1.9	19.1±2.3	18.5±3.1	18.1±4.2	19.3±6.7
Van Gogh	9.8±3.8	17.1±3.8	19.6±6.9	15.0±2.8	18.3±5.4
614002*	8.9±3.3	18.9±2.9	21.1±11.4	17.3±6.3	22.7±5.7
Tomato	9.4±4.5	13.8±5.5	18.3±2.6	20.5±7.2	23.1±2.9

[1] Values are means±SE of five replicates. Percentage reduction in plant growth of cultivars do not differ significantly according to Duncan's multiple range test at $P < 0.05$;
* Candidate cultivar.

Discussion

Meloidogyne chitwoodi, a sedentary endoparasite nematode, causes damage to roots, stolons and tubers of potato. Tuber infection can result in both external symptoms, galling to the surface, and internal symptoms, brown spots surrounding adult females that are visible when the tuber is peeled. Infested tubers are not suitable for the fresh market or processing when these symptoms reach 5% or more (EPPO, 2018b). Potato is known as a good host for *M. chitwoodi* and it is generally considered quality pest for potato (Norshie et al., 2011). In addition to the quality damage caused by *M. chitwoodi*, the overall tuber yield can also be reduced (EPPO, 2018b). The results obtained from this experiment show that reproduction of *M. chitwoodi* and effect of *M. chitwoodi* infection on plant growth parameters as plant height, fresh and dry weights of shoots and roots vary between different cultivars. *Meloidogyne* species (*M. incognita*, *M. javanica*, *M. arenaria* and *M. mayaguensis*) having high reproduction potential in potato, but differences between the cultivars have been reported (Al-Hazmi et al., 1995; Ateka et al., 2001; Vovlas et al., 2005; Silva et al., 2010; Ibrahim et al., 2014).

Santo & O'Bannon (1981) found that *M. chitwoodi* and *M. hapla* reduced root growth and tuber yield of potato. *Meloidogyne chitwoodi* reduced tuber yield and yield loss reached 25% in the greenhouse and open field studies (Hafez & Sundararaj, 2002, 2006a, b, 2009). It has been found that the yield loss due to *M. chitwoodi* can be reduced by 17-40% as a result of the nematicide applications (Pinkerton et al., 1986; Scholte, 1990). *Meloidogyne chitwoodi* reduces both fresh and dry shoot and root weights of tomato up to 37 and 45%, respectively (Santo & O'Bannon, 1982; Hafez & Sundararaj, 1999). The damage caused by *M. chitwoodi* to potato tubers has been shown to vary between cultivars. Some potato cultivars have a lower tuber damage than others, for example Agria is reported to be much more tolerant than Hansa (Suffert & Giltrap, 2012). Under field conditions, it has been reported that the symptoms caused by *M. chitwoodi* in the tubers varied from 3 to 34%, and the maximum population density was 2778 and 4167 J2/100 cm^3 soil in the early and late cultivars, respectively (Van Riel, 1993, 1994). Separate resistance genes for root reproduction and tuber infection have been found against *M. chitwoodi* in potato (Brown et al., 1996, 2009). Additionally, some promising results have been reported for resistant commercial potato cultivar using these genes (Brown et al., 1991, 2004, 2006, 2009; Norshie et al. 2011; Dinh et al., 2014). Studies have also shown plant growth reduction due to *M. chitwoodi*, depending on temperature and population density, in host plants such as alfalfa, wheat, barley, carrot and some other legumes (Nyczepir et al., 1984; Griffin et al., 1986; Griffin, 1992, 1993; Griffin & Rumbaugh, 1996; Griffin & Jensen, 1997; Molendijk & Brommer, 1998).

Some studies reported that no yield loss was caused by *M. chitwoodi* in potatoes, but this is considered to be due to the specific environmental conditions, such as total grade-days, initial population and soil type (Mojtahedi et al., 1993; Umesh & Ferris 1994; Hafez & Sundararaj, 2003). However, total potato yield reduction due to *M. chitwoodi* in two out of five years was reported in a field experiment, therefore, the damage varies depending on the region (Griffin, 1985; Pinkerton et al., 1991). The damage threshold for *M. chitwoodi* was 1 J2/250 ml soil in the USA and 10 J2/100 ml soil in the Netherlands. Therefore, potatoes can lose market value when grown in the areas which have higher juvenile numbers than these thresholds (Santo et al., 1981; Norshie et al., 2011). However, for nematode damage, the environmental conditions can be more important than the initial populations of *M. chitwoodi*. Spread and damage of *M. chitwoodi* has been observed in the areas with coarse soil texture and an annual degree-day (>5°C) of at least 1500 (Griffin, 1985; Pinkerton et al., 1991). In infested areas, the level of damage was affected by temperature, the type of plant grown (sensitive, tolerant or durable), the duration and period of the production, soil structure, and rainfall and watering conditions.

In summary, this study showed that *M. chitwoodi* reproduced well in all potato cultivars tested and caused reduction in their growth. However, there was significantly difference between cultivars in nematode reproduction and effects on plant growth. Damage to tubers may be reduced by choice of less susceptible cultivars. Thus, correct diagnosis and estimation of soil population densities of *M. chitwoodi* should be performed before planting potato to facilitate effective integrated management. Furthermore, integrated management programs for *M. chitwoodi* should be established, and farmers should be informed about its importance and potential to cause damage.

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Original article (Orijinal araştırma)

Mite species of the vegetable crops in Ordu Province with first report of *Amblyseius rademacheri* Dosse, 1958 (Mesostigmata: Phytoseiidae) in Turkey[1]

Ordu ilinde sebzelerde bulunan akar türleri ile *Amblyseius rademacheri* Dosse, 1958 (Mesostigmata: Phytoseiidae)'nin Türkiye'de ilk kaydı

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Abstract

Turkey has suitable ecological conditions to grow a wide variety of vegetables. This research was conducted to investigate mite species on cultivated vegetables that include bean (*Phaseolus vulgaris* L., Fabaceae), corn (*Zea mays* L., Poaceae), cucumber (*Cucumis sativus* L., Cucurbitaceae), eggplant (*Solanum melongena* L., Solanaceae), leek (*Allium porrum* L., Alliaceae), lettuce (*Lactuca sativa* L., Asteraceae), onion (*Allium cepa* L., Alliaceae), pepper (*Capsicum annuum* L., Solanaceae), potato (*Solanum tuberosum* L., Solanaceae), radish (*Raphanus sativus* L., Brassicaceae), tomato (*Solanum lycopersicum* L., Solanaceae), watermelon (*Citrullus lanatus* (Thunb.) Matsum. & Nakai., Cucurbitaceae) and zucchini (*Cucurbita* sp., Cucurbitaceae) during 2013-2015 in Ordu Province in the Black Sea Region of Turkey. The samples were taken at weekly intervals from April to October each year. A total of 2030 mite specimens were collected and examined during the study. A total of 43 mite species belonging 15 families and 30 genera were identified. Among them, *Tetranychus urticae* Koch, 1836 (Prostigmata: Tetranychidae) was found to be the most common phytophagous mite, while *Amblyseius swirskii* Athias-Henriot, 1962 (Mesostigmata: Phytoseiidae) was the most abundant predator. In addition, *Amblyseius rademacheri* Dosse, 1958 (Mesostigmata: Pyhtoseiidae) was reported for the first time in Turkey. This new record redescribed and illustrated based on the specimens collected from *Solanum melongena* L. (Solanaceae). An identification key for the Turkish *Amblyseius* is also provided.

Keywords: Fauna, new record, phytophagous mites, predatory mites, survey, Turkey

Öz

Türkiye birçok sebze türünün yetişmesi için uygun ekolojik koşullara sahiptir. Bu çalışma 2013-2015 yılları arasında, Ordu ilinde yetiştirilen sebzelerden, fasulye (*Phaseolus vulgaris* L., Fabaceae), mısır (*Zea mays* L., Poaceae), hıyar (*Cucumis sativus* L., Cucurbitaceae), patlıcan (*Solanum melongena* L., Solanaceae), pırasa (*Allium porrum* L., Alliaceae), marul (*Lactuca sativa* L., Asteraceae), soğan (*Allium cepa* L., Alliaceae), biber (*Capsicum annuum* L., Solanaceae), patates (*Solanum tuberosum* L., Solanaceae), turp (*Raphanus sativus* L., Brassicaceae), domates (*Solanum lycopersicum* L., Solanaceae), karpuz (*Citrullus lanatus* (Thunb.) Matsum. & Nakai., Cucurbitaceae) ve kabak (*Cucurbita* sp., Cucurbitaceae) üzerinde bulunan akar türlerini belirlemek amacı ile yürütülmüştür. Örneklemeler her yılın nisan-kasım ayları arasında haftalık olarak yapılmıştır. Çalışma boyunca 2030 adet akar toplanmış ve incelenmiştir. Üç alt takıma bağlı 15 familya ve 30 cinsden toplam 43 akar türü saptanmıştır. Belirlenen akarlar arasında en yaygın bitki zararlısı tür, *Tetranychus urticae* Koch, 1836 (Prostigmata: Tetranychidae) iken en yaygın predator tür ise *Amblyseius swirskii* Athias-Henriot, 1962 (Mesostigmata: Phytoseiidae) olmuştur. Ayrıca çalışma da belirlenen *Amblyseius rademacheri* Dosse, 1958 (Mesostigmata: Pyhtoseiidae) türü, Türkiye predatör akar faunası için yeni kayıt niteliğindedir. *Solanum melongena* L. (Solanaceae) üzerinden toplanan bu tür tanımlanarak çizimleri sunulmuştur. Ayrıca Türkiye'de tespit edilmiş *Amblyseius* cinsi akarlar için teşhis anahtarı da verilmiştir.

Anahtar sözcükler: Fauna, yeni kayıt, fitofag akarlar, predator akarlar, survey, Türkiye

[1] This manuscript is a part of the master thesis of the first author's and a part of the study was presented as poster in Third International Persian Congress of Acarology (23-25 August 2017, Tehran, Iran) and oral in Central Anatolia Region First Agriculture and Food Congress (International Participated) (26-28 October 2017, Sivas, Turkey) and published as an abstract in the abstracts book.

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Introduction

Due to favorable ecological conditions, many vegetable species are grown in Turkey, with production totaling 30.8 Mt annually Turkey is the forth largest vegetable producer in the world. In Ordu Province, vegetables are produced on about 1300 ha in both open fields and greenhouses. In addition, attempts have been made in Ordu to encourage the production of vegetables especially in greenhouses in recent years. As a result, the total greenhouse vegetable production has increased from 974 t in 2011 to 2,569 t in 2017 (TÜİK, 2017).

Vegetables are indispensible component of daily diet due to their nutritional benefits. They contain many nutrients such as potassium, fiber, folate and vitamins (Abak et al., 2010). However, some mite species such as *Tetranychus urticae* Koch, 1836 (Prostigmata: Tetranychidae) and *Aculops lycopersici* (Tryon, 1917) (Trombidiformes: Eriophyidae) are of important pests of vegetable crops causing significant reduction in both yield and quality (Karagöz, 2010). Consequently, there are many predatory mites, especially belonging to family phytoseiidae [e.g. *Amblyseius swirskii* Athias-Henriot, 1962, *Neoseiulus californicus* (McGregor, 1954), *Phytoseiulus persimilis* Athias-Henriot, 1957 (Mesostigmata: Phytoseiidae)], found in association with these phytophagous mites (Al-Atawi, 2011; Kade et al., 2011; Radonjic & Hrncic, 2011; Binisha & Bhaskar, 2013; Şekeroğlu & Kazak, 1993).

A number of studies have been conducted on the mite species on vegetable crops in the different regions of Turkey. Soysal & Yayla (1988) identified *T. urticae* and *T. cinnabarinus* and their predator *Phytoseius finitimus* Ribaga, 1904 (Mesostigmata: Phytoseiidae) on eggplant in Antalya. In the same region, Çobanoğlu (1989a) determined seven phytoseiid species on vegetables. *Polyphagotarsonemus latus* (Banks) (Prostigmata: Tarsonemidae) has been reported on peppers in the Mediterranean Region (Yabaş & Ulubilir, 1995). Five phytophagous and three predatory mite species were determined in Şanlıurfa (Çıkman et al., 1996). Hıncal et al. (2002) identified *A. lycopersici* and its predator *Pronematus ubiquitis* (McGregor) (Trombidiformes: Tydeidae) on tomato in İzmir. *Phytoseiulus persimilis* was determined in many vegetables in southern Turkey (Şekeroğlu & Kazak, 1993) and cucumber greenhouses of the Samsun Province (Akyazı & Ecevit, 2008). Kılıç et al. (2012) found that the most common phytophagous species was *Rhizoglyphus robini* Claparede 1869 (Trombidiformes: Acaridae) and the most common predacious species was *Macrocheles merdarius* Berlese, 1889 (Trombidiformes: Macrochelidae) in fresh onion fields. Çobanoğlu & Kumral (2014) identified 34 mite species in tomato growing areas of Ankara, Bursa and Yalova Provinces.

However, so far, no studies have been conducted on the mite species on vegetable crops in Ordu Province. The aim of the study was to investigate the mite species on vegetables crops that are grown both in the open field and in greenhouses in Ordu Provinces between 2013 and 2015.

Material and Methods

Sampling

This study was conducted over the growing seasons in Ordu Province between 2013 and 2015. Samples were collected at 10-15 days intervals between April and November each year. Leaf samples were taken from 13 vegetable species, bean (*Phaseolus vulgaris* L., Fabaceae), corn (*Zea mays* L., Poaceae), cucumber (*Cucumis sativus* L., Cucurbitaceae), eggplant (*Solanum melongena* L., Solanaceae), leek (*Allium porrum* L., Alliaceae), lettuce (*Lactuca sativa* L., Asteraceae), onion (*Allium cepa* L., Alliaceae), pepper (*Capsicum annuum* L., Solanaceae), potato (*Solanum tuberosum* L., Solanaceae), radish (*Raphanus sativus* L., Brassicaceae), tomato (*Solanum lycopersicum* L., Solanaceae), watermelon (*Citrullus lanatus* (Thunb.) Matsum. & Nakai., Cucurbitaceae) and zucchini (*Cucurbita* sp., Cucurbitaceae). The samples were collected from 209 locations in 106 villages in 13 municipalities of Ordu Province (Figure 1). A total of 863 samples were collected; 794 in open fields and 69 in greenhouses (Table 1). The size of the sample varied according to the size of the sampling area and vegetable species; 25-30 leaf samples were taken from the large leafy vegetables such as *C. sativus* and *Cucurbita* sp., 10-20 leaf samples were collected from small leafy vegetables such as *P. vulgaris* and *S. lycopersicum* (Anonymous, 2008). Leaf samples were collected randomly from the lower, middle and upper parts of the plants. The samples were placed in paper bags. All bags were then packed in sealed plastic bags. The samples were kept in a refrigerator at 4ºC (Toros, 1974; Madanlar, 1991).

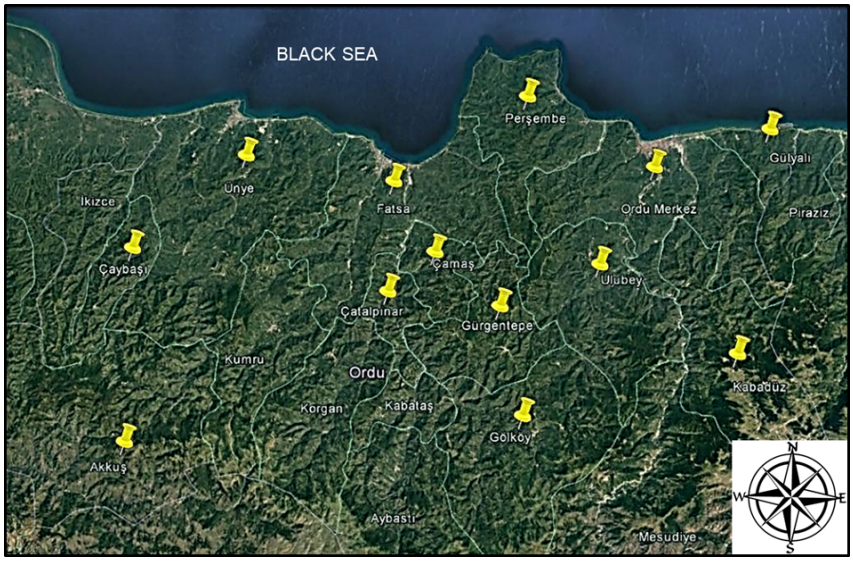

Figure 1. Municipalities sampled in Ordu Province in the Black Sea Region of Turkey (Adapted from www.google.com/maps).

Table 1. List of vegetable species sampled from open fields and greenhouses during growing season in 2013-2015 in 13 municipalities of Ordu Province, Turkey

Vegetable species sampled		Number of samples	
Family	Species	Open- field	Greenhouse
Fabaceae	*Phaseolus vulgaris* L.	175	10
Solanaceae	*Capsicum annuum* L.	150	12
Cucurbitaceae	*Cucurbita* sp.	99	1
Solanaceae	*Solanum melongena* L.	93	7
Solanaceae	*Solanum lycopersicum* L.	90	22
Poaceae	*Zea mays* L.	67	-
Cucurbitaceae	*Cucumis sativus* L.	62	17
Alliaceae	*Allium cepa* L.	23	-
Alliaceae	*Allium porrum* L.	16	-
Cucurbitaceae	*Citrullus lanatus* (Thunb.)	8	-
Asteraceae	*Lactuca sativa* L.	5	-
Brassicaceae	*Raphanus sativus* L.	3	-
Solanaceae	*Solanum tuberosum* L.	3	-
Total		794	69

Extraction, mounting and identification of mite specimens

Mites found on the adaxial and abaxial surfaces of the leaves were collected under a stereomicroscope (Leica S8 APO, Heerbrugg, Switzerland)) with a fine sable-hair brush. Subsequently, the leaf samples were placed in a Tullgren funnel to extract the mites. All mites collected were preserved in 70% ethyl alcohol (Ecevit, 1976; Krantz & Walter, 2009). Mites, except eriophyoids, were cleared in lactophenol solution and mounted in Hoyer's medium (Krantz & Walter, 2009). The eriophyoid specimens were cleared in Keifer's booster medium and slides were mounted using "F" medium as suggested by Amrine & Manson (1996). The slides were dried in an oven at 50ºC for 5-7 days.

Identification of mites at the species level was performed using the available taxonomic references such as Zhang (2000, 2003), Ueckermann (2013a) for Tarsonemidae; Pritchard & Baker (1955), Zhang et al. (2002), Zhang (2003), Seeman & Beard (2011), Ueckermann & Çobanoğlu (2012), Auger et al. (2013) for Tetranychidae; Edward & Donald (1987), Ueckermann & Çobanoğlu (2012), Çobanoğlu et al. (2016) for Tenuipalpidae; Muma & Denmark, (1970), Rowell et al. (1978), Çobanoğlu (1989a,b,c, 1993a,b,c,d), Faraji et al. (2007, 2011) for Phytoseiidae; Gonzalez-Rodrigeuz (1965), Fan & Zhang (2005) for Stigmaeidae; Fain et al. (1999), Yeşilayer & Çobanoğlu (2012) for Cheyletidae; Skvarla et al. (2014) for Cunaxidae; Solarz (2012) for Acaridae; Ripka et al. (2013), Ueckermann (2013b) for Iolinidae, Tydeidae and Triophtydeidae; Atyeo (1960) for Bdellidae. Mite species were identified under a light microscope (Leica DM 2500) equipped with phase contrast. The mite specimens were deposited in the Mite Collection at the Ordu University, Agricultural Faculty, Plant Protection Department, Ordu, Turkey.

Results and Discussion

A total of 43 mite species belonging to 15 families were identified (Table 2), with *Amblyseius rademacheri* Dosse, 1958 (Mesostigmata: Phytoseiidae) as a new record for Turkey. A total of 2030 mites were collected and identified during the study. Tetranychidae (1 species, 58%) is the most common family followed by Phytoseiidae (15 species 14.2%), Tarsonemidae (8 species, 11.3%), Iolinidae (2 species, 4.5%), Eriophyidae (1 species, 4.4%), Tydeidae (3 species, 0.9%), Tenuipalpidae (2 species, 1.2%), Stigmaeidae (1 species, 0.4%), Eupodidae (1 species, 0.3%), Acaridae (2 species, 0.2%), Erythraeidae (1 species, 0.1%), Trombidiidae (1 species, 0.1%), Triophtydeidae (2 species, 0.1%), Cheyletidae (2 species, 0.1%) and Bdellidae (1 species, 0.05%). The most common phytophagous species were *T. urticae* (58%), *P. latus* (9.4%) and *A. lycopersici* (4.4%). Among the predators, the most common species was *Homeopronematus staerki* (Schruft, 1972) (Trombidiformes: Iolinidae) (4.2%) followed by *A. swirskii* (3.6%) and *P. persimilis* (3.1%).

Table 2. Distribution of mite species on vegetable crops from the greenhouses and open fields in the Ordu Province between 2013 and 2015

Family	Species	Open field	Greenhouse	%	Pv	Ca	Cu	Sm	Sl	Zm	Cs	Ac	Ap	Cl	Ls	Rs	St
Phytoseiidae	*Amblyseius andersoni*	6	1	0.3		+		*			+						
	Amblyseius rademacheri	2	-	0.1				+									
	Amblyseius swirskii	73	-	3.6	+	+	+	+			+						
	Aristadromips masseei	9	6	0.7			+				+*						
	Euseius finlandicus	7	-	0.3	+	+					+						
	Euseius gallicus	20	-	1.0	+	+					+						
	Kampimodromus aberrans	2	-	0.1			+				+						
	Neoseiulus barkeri	7	1	0.4	+		+	+	+		*						
	Neoseiulus bicaudus	1	-	0.0								+					
	Neoseiulus californicus	2	15	0.8		*		+		+	*						
	Phytoseius finitimus	27	2	1.4	+	+	+	+	+		+*						
	Phytoseiulus persimilis	46	17	3.1	+	*	+	+	+		+*						
	Proprioseiopsis okanagensis	1	-	0.0			+										
	Transeius wainsteini	5	-	0.2		+	+				+						
	Typhlodromus athiasae	-	38	1.9		*					*						

Table 2. (Continued)

Family	Species	Open field	Greenhouse	%	Pv	Ca	Cu	Sm	Sl	Zm	Cs	Ac	Ap	Cl	Ls	Rs	St
Bdellidae	*Cyta* sp.	1	-	0.0				*									
Cheyletidae	*Cheletomimus berlesei*	1	-	0.0				+									
	Cheletogenes ornatus	1	-	0.0				+									
Eriophyidae	*Aculops lycopersici*	80	9	4.4					+*								
Erythraeidae	*Abrolophus* sp.	3	-	0.1							+*			+			
Eupodidae	*Eupodes* sp.	6	-	0.3			+										
Iolinidae	*Pronematus sextoni*	7	-	0.3	+	+		+			+						
	Homeopronematus staerki	78	7	4.2	+*	+*	+	+*	+*		+						
Stigmaeidae	*Zetzellia mali*	8	-	0.4	+		+	+	+								
Tarsonemidae	*Polyphagotarsonemus latus*	187	4	9.4	+*	+	+	+	+	+	+						
	Tarsonemus confusus	16	2	0.9				*	+*								
	Tarsonemus waitei	10	3	0.6			+		*		+	+					
	Daidalotarsonemus sp.	1	-	0.0			+										
	Tarsonemus sp. 1	2	-	0.1					+								
	Tarsonemus sp. 2	1	-	0.0				+									
	Tarsonemus sp. 3	1	-	0.0			+										
	Xenotarsonemus sp.	2	-	0.1		+											
Tetranychidae	*Tetranychus urticae* (GF)	938	239	58.	+*	+*	+	+*	+*	+	+*	+	+		+	+	
	Tetranychus urticae (RF)	69	12	4.0	+*			+*	*	+	+						
Tenuipalpidae	*Brevipalpus lewisi*	12	-	0.6	+	+		+									
	Brevipalpus obovatus	12	-	0.6	+			+	+	+	+						
Triophtydeidae	*Triophtydeus immanis*	2	-	0.1					+								
	Triophtydeus triophthalmus	1	-	0.0	+												
Trombidiidae	*Allothrombium pulvinum*	3	-	0.1	+	+		+									
Tydeidae	*Brachytydeus mali*	2	-	0.1	+	+											
	Tydeus californicus	2	-	0.1		+											
	Tydeus goetzi	15	-	0.7	+	+	+	+			+						
Acaridae	*Tyrophagus palmarum*	2	-	0.1						+	+						
	Tyrophagus putrescentiae	3	-	0.1	+			+									
	TOTAL	1674	356		18	19	17	22	13	5	21	3	1	1	1	1	-

%: percentage in total mites; GF: green form; RF: red form; +: open field, *: greenhouse; Pv: *Phaseolus vulgaris*, Ca: *Capsicum annuum*, Sm: *Solanum melongena*, Cu: *Cucurbita* sp., Sl: *Solanum lycopersicum*, Zm: *Zea mays*, Cs: *Cucumis sativus*, Ap: *Allium porrum*, Ac: *Allium cepa*, Ls: *Lactuca sativa*, Cl: *Citrullus lanatus*, Rs: *Raphanus sativus*, St: *Solanum tuberosum*

Tetranychidae

***Tetranychus urticae* Koch, 1836**

Open field green form:

Material examined: Akkuş, 15.09.2014, *P. vulgaris* (19♀♀, 12♂♂), *Cucurbita* sp. (11♀♀), *S. lycopersicum* (2♀♀); Çamaş, 21.08.2014, *Z. mays* (1♀), 05.08.2014, *P. vulgaris* (8♀♀), *S. melongena* (3♀♀), *C. sativus* (2♀♀),

Z. mays (4♀♀); Çatalpınar, 05.08.2014, *P. vulgaris* (5♀♀), 21.08.2014, *P. vulgaris* (24♀♀, 15♂♂), *Cucurbita* sp. (3♀♀,1♂), *S. melongena* (9♀♀, 3♂♂), *C. sativus* (3♀♀, 6♂♂), *C. annuum* (3♀♀), *Z. mays* (3♀♀, 3♂♂); Çaybaşı, 10.10.2104, *P. vulgaris* (6♀♀, 4♂♂), *S. melongena* (3♀♀), *C. sativus* (5♀♀), *C. annuum* (1♀); Fatsa, 13.09.2013, *P. vulgaris* (21♀♀,1♂), *Cucurbita* sp. (5♀♀), *S. melongena* (21♀♀, 1♂), *C. sativus* (2♀♀), *L. sativa* (1♀, 1♂), *R. sativus* (2♀♀), 28.08.2014, *P. vulgaris* (14♀♀, 2♂♂), *Cucurbita* sp. (8♀♀, 2♂♂), *S. lycopersicum* (7♀♀, 1♂), *C. sativus* (2♀♀), *S. melongena* (15♀♀, 2♂♂), *Z. mays* (7♀♀, 1♂); Gölköy, 14.07.2013, *P. vulgaris* (7♀♀), *Cucurbita* sp. (3♀♀), *L. sativa* (1♀); Gülyalı, 27.06.2013, *P. vulgaris* (4♀♀), *Cucurbita* sp. (1♂), *S. lycopersicum* (2♀♀, 1♂), *S. melongena* (16♀♀, 2♂♂), *C. sativus* (10♀♀), *C. annuum* (1♀), *Z. mays* (1♀); Gürgentepe, 02.10.2014, *P. vulgaris* (43♀♀, 22♂♂), *Cucurbita* sp. (15♀♀, 1♂), *S. melongena* (11♀♀, 1♂), *C. sativus* (3♀♀, 1♂), *C. annuum* (6♀♀, 3♂♂); Kabadüz, 26.09.2014, *P. vulgaris* (37♀♀, 17♂♂), *Cucurbita* sp. (12♀♀), *S. melongena* (13♀♀), *C. sativus* (18♀♀,2♂♂), *C. annuum* (1♀); Altınordu, 20.07.2013, *P. vulgaris* (17♀♀, 6♂♂), *Cucurbita* sp. (6♀♀, 2♂♂), *S. melongena* (15♀♀, 1♂), *C. sativus* (20♀♀, 1♂), *Z. mays* (2♀♀, 1♂), *A. porrum* (1♀), 23.08.2013, *P. vulgaris* (20♀♀), *Cucurbita* sp. (8♀♀), *S. melongena* (7♀♀), *C. sativus* (1♀), *C. annuum* (1♀), *Z. mays* (1♀); Perşembe, 23.07.2013, *C. sativus* (13♀♀), 27.09.2013, *P. vulgaris* (8♀♀), *Cucurbita* sp. (18♀♀, 1♂), *S. melongena* (7♀♀), *C. sativus* (10♀♀), *C. annuum* (1♀), 07.08.2014, *P. vulgaris* (6♀♀, 2♂♂), *Cucurbita* sp. (1♀, 1♂), *S. melongena* (6♀♀), *C. annuum* (6♀♀, 1♂), *Z. mays* (1♀); Ulubey, 14.07.2013, *P. vulgaris* (1♀), *Cucurbita* sp. (3♀♀), 06.09.2013, *P. vulgaris* (21♀♀, 5♂♂), *Cucurbita* sp. (17♀♀, 2♂♂), *S. lycopersicum* (1♀), *S. melongena* (11♀♀, 7♂♂), *C. sativus* (21♀♀), *C. annuum* (5♀♀), *Z. mays* (1♀), 11.09.2014, *P. vulgaris* (15♀♀, 4♂♂), *S. melongena* (8♀♀), *C. sativus* (9♀♀), *C. annuum* (1♀);, Ünye, 26.07.2013, *P. vulgaris* (11♀♀), *Cucurbita* sp. (8♀♀), *S. lycopersicum* (2♀♀), *S. melongena* (2♀♀), *C. sativus* (1♀), *C. annuum* (1♀), *A. porrum* (1♀), 07.08.2014, *P. vulgaris* (18♀♀, 3♂♂), *Cucurbita* sp. (8♀♀, 1♂), *S. lycopersicum* (2♀♀), *S. melongena* (7♀♀, 1♂), *C. sativus* (1♀), *Z. mays* (4♀♀), *A. cepa* (1♀), *A. porrum* (2♀♀, 1♂).

Open field red form:

Material examined: Akkuş, 15.09.2014, *P. vulgaris* (1♂); Çamaş, 05.08.2014, *P. vulgaris* (5♀♀, 1♂), *C. sativus* (2♀♀, 2♂♂), *Z. mays* (11♀♀, 1♂); Çatalpınar, 21.08.2014, *P. vulgaris* (4♂♂); Çaybaşı, 10.10.2014, *P. vulgaris* (6♀♀, 2♂♂); Fatsa, 28.08.2014, *C. sativus* (3♀♀, 2♂♂); Gürgentepe, 02.10.2014, *P. vulgaris* (2♂♂), *S. melongena* (1♂); Kabadüz, 26.09.2014, *P. vulgaris* (5♀♀, 5♂♂); Ünye, 07.08.2014, *P. vulgaris* (10♀♀, 4♂♂).

Greenhouse green form:

Material examined: Çaybaşı, 10.10.2014, *C. sativus* (3♀♀), *C. annuum* (1♀); Fatsa, 13.09.2013, *P. vulgaris* (8♀♀, 2♂♂), *S. lycopersicum* (1♀), *S. melongena* (1♀1♂), *C. annuum* (1♀), 28.08.2014, *C. sativus* (2♀♀, 2♂♂); Gülyalı, 27.06.2013, *P. vulgaris* (23♀♀), *C. sativus* (15♀♀), *S. lycopersicum* (1♀); Gürgentepe, 02.10.2014, *P. vulgaris* (13♀♀, 8♂♂), *C. annuum* (7♀♀, 2♂♂), *C. sativus* (3♀♀, 1♂); Altınordu, 23.08.2013, *C. sativus* (5♀♀, 1♂); Perşembe, 23.07.2013, *P. vulgaris* (21♀♀, 2♂♂), *C. sativus* (17♀♀, 1♂), *S. lycopersicum* (3♀♀); Ulubey, 11.09.2014, *C. sativus* (2♀♀); Ünye, 26.07.2013, *C. sativus* (30♀♀, 2♂♂), *S. melongena* (33♀♀, 1♂), *S. lycopersicum* (13♀♀), 07.08.2014, *C. sativus* (10♀♀), *S. lycopersicum* (3♀♀).

Greenhouse red form:

Material examined: Gürgentepe, 02.10.2014, *P. vulgaris* (1♀); Ünye, 07.08.2014, *S. lycopersicum* (2♀♀, 1♂), *S. melongena* (7♀♀, 1♂).

Distribution: *T. urticae* is a cosmopolitan polyphagous mite species (Migeon et al., 2011). In previous studies, it was commonly found on crops in Turkey (Öngören et al., 1975; Soysal & Yayla, 1988; Güven & Madanlar, 2000; Tokkamış & Yanar, 2011; Çobanoğlu & Kumral, 2014, 2016; Akyazı et al., 2017; İnak & Çobanoğlu, 2018).

Tenuipalpidae

***Brevipalpus obovatus* Donnadieu, 1875**

Material examined (open field): Çamaş, 05.08.2014, *P. vulgaris* (1♀); Gülyalı, 19.08.2015, *S. lycopersicum* (1pn), *C. sativus* (1♀); Kabadüz, 26.08.2014, *S. melongena* (1♀); Altınordu, 29.08.2013, *S. melongena* (1♀, 3pn); Perşembe, 27.09.2013, *S. melongena* (2♀♀), *Z. mays* (1♀); Ulubey, 06.09.2013, *C. sativus* (1pn).

Distribution: *B. obovatus* has been found on many types of plants worldwide (Beard et al., 2012). In Turkey, this species was determined for the first time on lemon trees (Düzgüneş, 1952), and subsequently in various localities in Turkey (Çobanoğlu et al., 2016).

***Brevipalpus lewisi* McGregor, 1949**

Material examined (open field): Fatsa, 28.08.2014, *S. melongena* (1♀); Perşembe, 27.09.2013, *P. vulgaris* (1♀, 1pn), *S. melongena* (1♀), *C. annuum* (5♀♀).

Distribution: *B. lewisi* occurs on many plant groups around the world and Turkey (Jeppson et al., 1975; Soylu & Ürel, 1977; Ghai & Shenhmar, 1984; Göven et al., 2009; Khanjani et al., 2013; Yanar & Erdoğan, 2013; Çobanoğlu et al., 2016; Ueckermann & Ripka, 2016; Ueckermann et al., 2018).

Tarsonemidae

***Polyphagotarsonemus latus* (Banks)**

Material examined (open field): Çamaş, 05.08.2014, *P. vulgaris* (4♀♀), *C. sativus* (2♀♀, 1♂); Çatalpınar, 21.08.2014, *P. vulgaris* (4♀♀, 3♂♂), *Cucurbita* sp. (1♂), *C. annuum* (2♀♀); Fatsa, 13.09.2013, *P. vulgaris* (9♀♀), *C. annuum* (2♀♀); Gürgentepe, 02.10.2014, *P. vulgaris* (1♀); Kabadüz, 26.09.2014, *P. vulgaris* (1♀), *Cucurbita* sp. (8♀♀, 1♂), *S. melongena* (14♀♀), *C. annuum* (1♀); Altınordu, 20.07.2013, *P. vulgaris* (11♀♀, 3♂♂), *S. lycopersicum* (1♀), 23.08.2013, *P. vulgaris* (19♀♀, 4♂♂), *C. annuum* (11♀♀, 1♂), *S. melongena* (1♀); Perşembe, 23.07.2013, *P. vulgaris* (7♀♀, 1♂), *C. annuum* (3♀♀, 2♂♂); Ulubey, 06.09.2013, *P. vulgaris* (43♀♀, 2♂♂), *Cucurbita* sp. (3♀♀), *C. annuum* (13♀♀, 1♂), *Z. mays* (1♀); Ünye, 07.08.2014, *P. vulgaris* (5♀♀, 3♂♂), *C. annuum* (1♀).

Material examined (greenhouse): Fatsa, 13.09.2013, *P. vulgaris* (4♀♀).

Distribution: *P. latus* is a very common species around the world (Binisha & Bhaskar 2013; CABI, 2014). In Turkey, it has been recorded in association with several vegetable crops (Çobanoğlu, 1995; Tunç & Göçmen, 1995; Yabaş & Ulubilir, 1995; Bulut, 1999; Can & Çobanoğlu, 2010).

***Daidalotarsonemus* sp. De Leon, 1956**

Material examined (open field): Çatalpınar, 21.08.2014, *Cucurbita* sp. (1♀).

***Tarsonemus waitei* Banks, 1912**

Material examined (open field): Fatsa, 13.09.2013, *Cucurbita* sp. (7♀♀), *C. sativus* (2♀♀); Ünye, 26.07.2013, *A. cepa* (1♀).

Material examined (greenhouse): Fatsa, 13.09.2013, *S. lycopersicum* (1♀); Ulubey, 06.09.2013, *S. lycopersicum* (2♀♀).

Distribution: *T. waitei* previously recorded in Brazil, Canada, China, Congo, Costa Rica, Hungary, New Zealand, Poland, Portugal and Ukraine (Lin & Zhang, 2002; Ripka et al., 2005). It has also been found in Turkey (Çobanoğlu, 1995; Tokkamış, 2011).

***Tarsonemus confusus* Ewing, 1939**

Material examined (open field): Gürgentepe, 20.10.2014, *S. lycopersicum* (15♀♀, 1♂).

Material examined (greenhouse): Fatsa, 28.08.2014, *S. melongena* (1♀); Ulubey, 06.09.2013, *S. lycopersicum* (1♀).

Distribution: *T. confusus* was reported in the USA, Canada, Italy, Ireland, Germany, Poland, Ukraine, Russia, Japan, Korea, China, Egypt by Lin & Zhang (2002) and Hungary by Ripka et al. (2005). In Turkey, it was first identified on *Pyracantha coccinea* Roem (Rosaceae) in Edirne by Çobanoğlu (1995). *C. annuum*, *C. sativus* (Tokkamış, 2011) and *S. lycopersicum* (Çobanoğlu & Kumral, 2014) are among the hosts of *T. confusus*.

***Xenotarsonemus* sp.**

Material examined (open field): Gülyalı, 10.08.2015, *C. annuum* (2♀♀).

***Tarsonemus* sp. 1**

Material examined (open field): Gürgentepe, 20.10.2014, *S. lycopersicum* (1♀); Kabadüz, 26.09.2014, *S. lycopersicum* (1♀).

***Tarsonemus* sp. 2**

Material examined (open field): Gülyalı, 10.07.2013, *S. melongena* (1♀).

***Tarsonemus* sp. 3**

Material examined (open field): Fatsa, 28.08.2014, *Cucurbita* sp. (1♀).

Eriophyidae

***Aculops lycopersici* (Tryon, 1917)**

Material examined (open field): Çatalpınar, 05.08.2014, *S. lycopersicum* (10♀♀); Fatsa, 13.09.2013, *S. lycopersicum* (9♀♀); Kabadüz, 26.09.2014, *S. lycopersicum* (4♀♀); Altınordu, 20.07.2013, *S. lycopersicum* (26♀♀), 23.08.2013, *S. lycopersicum* (3♀♀); Perşembe, 27.09.2013, *S. lycopersicum* (13♀♀); Ulubey, 06.09.2013, *S. lycopersicum* (7♀♀); Ünye, 07.08.2014, *S. lycopersicum* (8♀♀).

Material examined (greenhouse): Fatsa, 13.09.2013, *S. lycopersicum* (9♀♀).

Distribution: *A. lycopersici* is widespread worldwide (Denizhan et al., 2015). It has also been recorded in association with tomato plants in many localities in Turkey (Şekeroğlu & Özgür, 1984; Madanlar & Öncüer, 1994; İnal, 2005; Yanar et al., 2008; Can & Çobanoğlu, 2010; Çobanoğlu & Kumral, 2014; Denizhan et al., 2015).

Tydeidae

***Brachytydeus mali* (Oudemans, 1929)**

Material examined (open field): Çatalpınar, 21.08.2014, *P. vulgaris* (1♀); Gülyalı, 19.08.2015, *C. annuum* (1♀).

Distribution: *B. mali* was collected in Scotland (Baker & Wharton, 1952), Serbia (Stojnic et al., 2002), Iran (Jalilirad et al., 2012), Spain and Greece (Anonymous, 2015). It was reported in Turkey (Istanbul) by Yeşilayer (2009).

***Tydeus californicus* (Banks, 1904)**

Material examined (open field): Akkuş, 15.09.2014, *C. annuum* (1♀); Perşembe, 13.09.2013, *C. annuum* (1♀).

Distribution: *T. californicus* is very common around the world (Tempfli et al., 2015). The first record of *T. californicus* in Turkey was on citrus leaves in Adana (Düzgüneş, 1963).

***Tydeus goetzi* Schruft, 1972**

Material examined (open field): Akkuş, 15.09.2014, *C. annuum* (1♀); Gülyalı, 27.06.2013, *C. sativus* (1♀), 10.07.2013, *P. vulgaris* (2♀♀), *C. annuum* (2♀♀); Perşembe, 27.09.2013, *P. vulgaris* (1♀), *C. annuum* (2♀♀), 09.07.2014, *Cucurbita* sp. (1♀); Altınordu, 20.07.2013, *P. vulgaris* (2♀♀), *C. sativus* (2♀♀), *S. melongena* (1♀).

Distribution: *T. goetzi* is a rare species. This mite has been determined only in Germany (Schruft, 1972), France (Andre, 2011) and Turkey (Akyazı et al., 2017).

Triophtydeidae

***Triophtydeus triophthalmus* (Oudemans, 1929)**

Material examined (open field): Ulubey, 11.09.2014, *P. vulgaris* (1♀).

Distribution: *T. triophthalmus* has been reported from Italy (Sabbatini Peverieri et al., 2009), Ukraine (Ripka et al., 2005), Turkey (Özman-Sullivan et al., 2005), Germany and Sweden (Tempfli et al., 2015). However, the feeding habits of *T. triophthalmus* is contradictory (Tempfli et al., 2015).

***Triophtydeus immanis* Kuznetzov, 1973**

Material examined (open field): Altınordu, 21.07.2013, *S. lycopersicum* (1♀), 23.08.2013, *S. lycopersicum* (1♀).

Distribution: *T. immanis* has been reported from South Africa (Ueckermann & Grout, 2007), Turkey (Özman-Sullivan et al., 2005) and Hungary (Ripka et al., 2002, 2005).

Iolinidae

***Homeopronematus staerki* (Schruft, 1972)**

Material examined (open field): Akkuş, 15.09.2014, *P. vulgaris* (1♀), *C. annuum* (7♀♀); Çamaş, 05.08.2014, *P. vulgaris* (2♀♀), *Cucurbita* sp. (1♀); Çatalpınar, 21.08.2014, *S. lycopersicum* (2♀♀); Fatsa, 13.09.2013, *P. vulgaris* (2♀♀), *Cucurbita* sp. (3♀♀), *C. annuum* (1♀); Gülyalı, 10.07.2013, *P. vulgaris* (2♀♀); Gürgentepe, 02.10.2014, *P. vulgaris* (6♀♀); Kabadüz, 26.09.2014, *P. vulgaris* (3♀♀, 1tn), *S. lycopersicum* (7♀♀), *S. melongena* (2♀♀); Altınordu, 20.07.2013, *P. vulgaris* (3♀♀), *S. lycopersicum* (5♀♀), *C. sativus* (1♀), 23.08.2013, *P. vulgaris* (5♀♀), *S. lycopersicum* (1♀); Perşembe, 23.07.2013, *S. lycopersicum* (2♀♀), 27.09.2013, *S. lycopersicum* (4♀♀), *C. annuum* (2♀♀), 09.07.2014, *P. vulgaris* (1♀); Ulubey, 14.07.2013, *P. vulgaris* (2♀♀), 06.09.2014, *P. vulgaris* (5♀♀), *C. sativus* (1♀), *S. melongena* (1♀); Ünye, 26.07.2013, *P. vulgaris* (3♀♀), 07.08.2014, *P. vulgaris* (2♀♀).

Material examined (greenhouse): Perşembe, 23.07.2013, *P. vulgaris* (1♀), *S. lycopersicum* (3♀♀); Ünye, 26.07.2013, *C. annuum* (2♀♀); Fatsa, 13.09.2013, *S. melongena* (1♀).

Distribution: *H. staerki* has been reported in Serbia (Stojnic et al., 2002), Germany (Schruft, 2006), Hungary (Ripka et al., 2005, 2013; Tempfli et al., 2015). Özman-Sullivan et al. (2005) recorded *H. staerki* on hazelnut in Turkey.

***Pronematus sextoni* Baker, 1968**

Material examined (open field): Fatsa, 13.09.2013, *P. vulgaris* (3♀♀); Altınordu, 23.08.2013, *S. melongena* (1♀), *C. annuum* (1♀); Ulubey, 06.09.2013, *C. sativus* (1tn), *C. annuum* (1♀).

Distribution: *P. sextoni* has been found in India (Gupta, 1985), Africa (Gupta et al., 2015) and Turkey (Çobanoğlu & Kazmierski, 1999).

Cheyletidae

***Cheletomimus berlesei* (Oudemans)**

Material examined (open field): Altınordu, 23.08.2013, *S. melongena* (1♀).

Distribution: *C. berlesei* has been reported from America, France, Italy, Israel and New Zealand (Summers & Price, 1970). It Turkey, it was first identified in Istanbul by Yeşilayer & Çobanoğlu (2012) and it was found in association with *Cenopalpus lineola* Canestrini & Fanzago (an ornamental plant). During this study, it was collected with *T. urticae, P. latus* and *P. sextoni* on *S. melongena*.

***Cheletogenes ornatus* (Canesterini & Fanzago, 1876)**

Material examined (open field): Perşembe, 09.07.2014, *S. melongena* (1♀).

Distribution: This mite is found in Southern Europe, South Africa, Italy, Israel, China, Australia and America (Volgin, 1989). *C. ornatus* was reported by Düzgüneş (1963) on lemon trees in Antalya, Turkey. In this study, it was found together with *T. urticae* on *S. melongena*.

Stigmaeidae

***Zetzellia mali* (Ewing)**

Material examined (open field): Akkuş, 15.09.2014, *P. vulgaris* (1♀); Gürgentepe, 02.10.2014, *S. melongena* (1♀); Altınordu, 20.07.2013, *S. lycopersicum* (2♀♀), 21.07.2015, *Cucurbita* sp. (1♀); Perşembe, 27.09.2013, *S. melongena* (2♀♀); Ünye, 07.08.2014, *P. vulgaris* (1♀).

Distribution: *Z. mali* has an extensive worldwid range (Gerson et al., 2003). It has been widely found in many provinces in Turkey by many researchers (Düzgüneş, 1963; Akyazı & Ecevit, 2003; Çobanoğlu et al., 2003; İnal, 2005; Kumral, 2005; Kasap & Çobanoğlu, 2007; Denizhan & Çobanoğlu, 2009; Yeşilayer, 2009; Karagöz, 2010; Sağlam & Çobanoğlu, 2010; Dönel & Doğan, 2013; Çobanoğlu & Kumral, 2014; Kumral & Çobanoğlu, 2015b; Akyazı et al., 2016a, 2017; İnak & Çobanoğlu, 2018). During this study, it was collected together with *T. urticae, H. staerki* and *A. lycopersici* on *P. vulgaris*.

Eupodidae

***Eupodes* sp.**

Material examined (open field): Ulubey, 11.09.2014, *Cucurbita* sp. (3♀♀); Gürgentepe, 02.10.2014, *Cucurbita* sp. (2♀♀); Gülyalı, 27.06.2013, *Cucurbita* sp. (1♀).

Bdellidae

***Cyta* sp.**

Material examined (greenhouse): Fatsa, 28.08.2014, *S. melongena* (1♀).

Trombidiidae

***Allothrombium pulvinum* Ewing, 1917**

Material examined (open field): Kabadüz, 26.07.2013, *S. melongena,* (1dn), 26.09.2014, *P. vulgaris* (1♂), *C. annuum* (1♂).

Distribution: *A. pulvinum* was first discovered in North America by Ewing (1917). Later, it was determined in North America, Europe and Asia (Zhang & Norbakhsh, 1995). It has also been reported in Turkey (Çobanoğlu et al., 2003; Yeşilayer, 2009).

Erythraeidae

***Abrolophus* sp.**

Material examined (open field): Ünye, 07.08.2014, *C. sativus* (1♀), *C. lanatus* (1♀).

Material examined (greenhouse): Fatsa, 13.09.2013, *C. sativus* (1♀).

Distribution: In Turkey, *Abrolophus* sp. was identified in Bursa (Kumral, 2005), İzmir (Kılıç et al., 2012) and Ankara (Kumral & Çobanoğlu, 2015a).

Acaridae

***Tyrophagus putrescentiae* (Schrank, 1781)**

Material examined (open field): Çatalpınar, 21.08.2014, *P. vulgaris* (1♀); Altınordu, 20.07.2013, *S. melongena* (1tn); Perşembe, 27.09.2013, *P. vulgaris* (1♂).

Distribution: The cosmopolitan *T. putrescentiae* has been found in New Zealand, Australia, China, Ecuador, Germany, Japan, the Netherlands and the USA (Fan & Zhang, 2007). In Turkey, this species was collected from vegetables including *C. annuum*, *C. sativus* (Tokkamış & Yanar, 2011), *A. cepa* (Kılıç et al., 2012), *S. lycopersicum* (Çobanoğlu & Kumral, 2014).

***Tyrophagus palmarum* (Oudemans, 1924)**

Material examined (open field): Çatalpınar, 21.08.2014, *Z. mays* (1♀), Ulubey, 06.09.2013, *C. sativus* (1♀).

Distribution: *T. palmarum* has been reported from Czechoslovakia (Zdarkova, 1967), Germany (Franz et al., 1997), Australia, Netherland, Tuvalu and New Zealand (Fan & Zhang, 2007). It was detected for the first time in Turkey in dust samples taken from the homes of allergic asthmatic patients in Samsun Province of Turkey by Çelik (2009).

Phytoseiidae

Key to species of the genus *Amblyseius* Berlese in Turkey based on adult females (based on Faraji et al., 2011; Akyazı et al., 2016b)

1. Ventrianal shield vase-shaped. 2

—Ventrianal shield not vase-shaped. 3

2. Calyx of spermatheca tubular. . . . . *A. largoensis* (Muma, 1955)

—Calyx of spermatheca fundibular. . . . . *A. herbicolus* (Chant, 1959)

3. Seta Z5 longer than width of dorsal shield; spermatheca with calyx annulated, flared distally. . . . . .*A. obtusus* (Koch, 1839)

—Seta Z5 shorter than width of dorsal shield; spermatheca with calyx not annulated. 4

4. Ventrianal shield with large elliptical (crescent shaped) preanal solenostomes. 5

—Ventrianal shield with small round preanal solenostomes. 8

5. Dorsal shield reticulated. 6

—Dorsal shield smooth. 7

6. Fixed digit with 10 teeth; GeII with 8 setae. . . . . *A. bryophilus* Karg, 1970

—Fixed digit with 7-8 teeth; GeII with 7 setae. . . . . .*A. rademacheri* Dosse, 1958

7. Seta Z5 102-116 long; atrium of spermatheca relatively long; StiIV at most reaching the insertion of StIV. . . . . .*A. swirskii* Athias-Henriot, 1962

—Seta Z5 longer than 150; atrium of spermatheca short and c-shaped; StiIV passing well behind the insertion of StIV. . . . . *A. andersoni* (Chant, 1957)

8. Seta Z4 almost reaching insertion of seta S4. . . . . *A. armeniacus* Arutunjan and Ohandjanian, 1972

—Seta Z4 short, less than 1/3 of distance between setae Z4 and S4. . . . .*A. kadzhajai* Gomelauri, 1968

***Amblyseius andersoni* (Chant, 1957)**

Material examined (open field): Perşembe, 23.07.2013, *C. sativus* (2♀♀), 27.09.2013, *C. annuum* (3♀♀, 1♂).

Material examined (greenhouse): Fatsa, 13.09.2013, *S. melongena* (1♀).

Distribution: *A. andersoni* is a very common predatory mite with a worldwide distribution (Demite et al., 2017). It has been reported together with various mite species by many researchers in Turkey (Faraji et al., 2011). In this study, it was collected with *B. lewisi, T. urticae* and *H. staerki* from *C. annuum* and *C. sativus* leaves in the open fields and with *T. urticae* and *H. staerki* on *S. melongena* in the greenhouses.

***Amblyseius rademacheri* Dosse, 1958**

Senior synonym: *Amblyseius khnzoriani* Wainstein & Arutunjan (Wainstein, 1975)

Material examined (open field): Ulubey, 11.09.2014, *S. melongena* (2♀♀).

Description (n = 2)

Dorsum (Figure 2A) - Dorsal shield elongate, strongly reticulated; length (j1-J5) 343-345; width (s4-s4) 165-167; seven pairs of solenostomes (gd1, gd2, gd4, gd5, gd6, gd8, gd9); 17 pairs of setae. Dorsal setae, short and minute regular, except for j1, j3, s4, Z4 and Z5. Z4 and Z5 strongly serrated. Peritremes extending beyond bases of setae j1 (Figure 2A).

Measurements of dorsal setae - j1 22-24, j3 33-35, j4 5-6, j5 4, j6 8-9, J2 9, J5 8, z2 12-13, z4 14, z5 6, Z1 9, Z4 86-89, Z5 108-109, s4 65-68, S2 11, S4 9, S5 8-9, r3 12-14, R1 8-9.

Venter (Figure 2B) - Sternal shield reticulated; length (ST1-ST3) 65-67; width (ST2-ST2) 66-68; two pairs of solenostomes (pst1-pst2); threepairs of setae (ST1, ST2 and ST3). Metasternal shield is located separately and bearing ST4. Genital shield length 76 (ST5-ST5). Ventrianal shield slightly reticulated; length 108-109, width (ZV2-ZV2) 98-105; three pairs of preanal setae (JV1, JV2 and ZV2); one pairs of crescentic solenostomes (gv3) located between setae JV2. Additionally, setae ZV1, ZV3, -JV4 and JV5 surrounding ventrianal shield. Setae JV5 smooth, 50 in length.

Spermatheca (Figure 3A) - Calyx cup shaped; atrium C-like.

Chelicerae (Figure 3B) - Fixed digit of chelicerae with seven teeth and movable digit with two teeth, both 30-31 long.

Legs (Figure 3C) - Length of legs as follows: legI 341-358, legII 275-296, legIII 299-303 and leg IV 405-411. Three macrosetae on Leg IV (SgeIV 58-59, StiIV 39-40, StIV 82). Leg I, II and III with one macrosetae on genu (SgeI 26-34, SgeII 30-32, SgeIII 34).

Male - Not found in this study.

Remarks: In previous studies, seven species belonging to the genus *Amblyseius* (Faraji et al., 2011; Döker et al., 2014a; Akyazı et al., 2016b) were recorded for the Turkish fauna. Here, *Amblyseius rademacheri* is reported for the first time in Turkey.

Based on the female specimens, *A. rademacheri* is similar *Amblyseius bryophilus* Karg. However, it differs from the latter in having seven setae on genu II. *A. bryophilus* has 8 setae on genu II.

Amblyseius rademacheri is also similar to *A. swirskii*. However, it differs from the latter by having dorsal shield strongly reticulated, setae Z4 and Z5 long and serrate and two teeth on movable digit of chelicerae. Dorsal shield of *A. swirskii* is smooth, setae Z4 and Z5 slender and lightly serrate and three teeth on movable digit of chelicerae. Additionally, setae S2 of *A. rademacheri* are 11 µm long while in that of *A. swirskii* approximately twice as long.

Amblyseius rademacheri was found together with *T. urticae* on *S. melongena* in this study. In previous studies, it was found on various fruits, weeds, forest trees in association with tetranychid and eriophid mites (Hajizadeh, 2007). Tixier et al. (2013) also reported *A. rademacheri* on *Vitis vinifera* L. (Vitaceae). Komi et al. (2008), found this species on pepper and eggplant in Japan.

Distribution: *A. rademacheri* has been recorded in Armenia, Austria, Azerbaijan, China-Jiangxi, Czech Republic, Denmark, Georgia, Germany, Hungary, Iran, Italy, Japan, Latvia, Moldova, Netherlands, Poland, Russia-Moscow Province, Primorsky Territory; Yaroslavl Province; Slovakia, Slovenia, South Korea, Spain, Switzerland, Ukraine (Demite et al., 2017) and Turkey (this study).

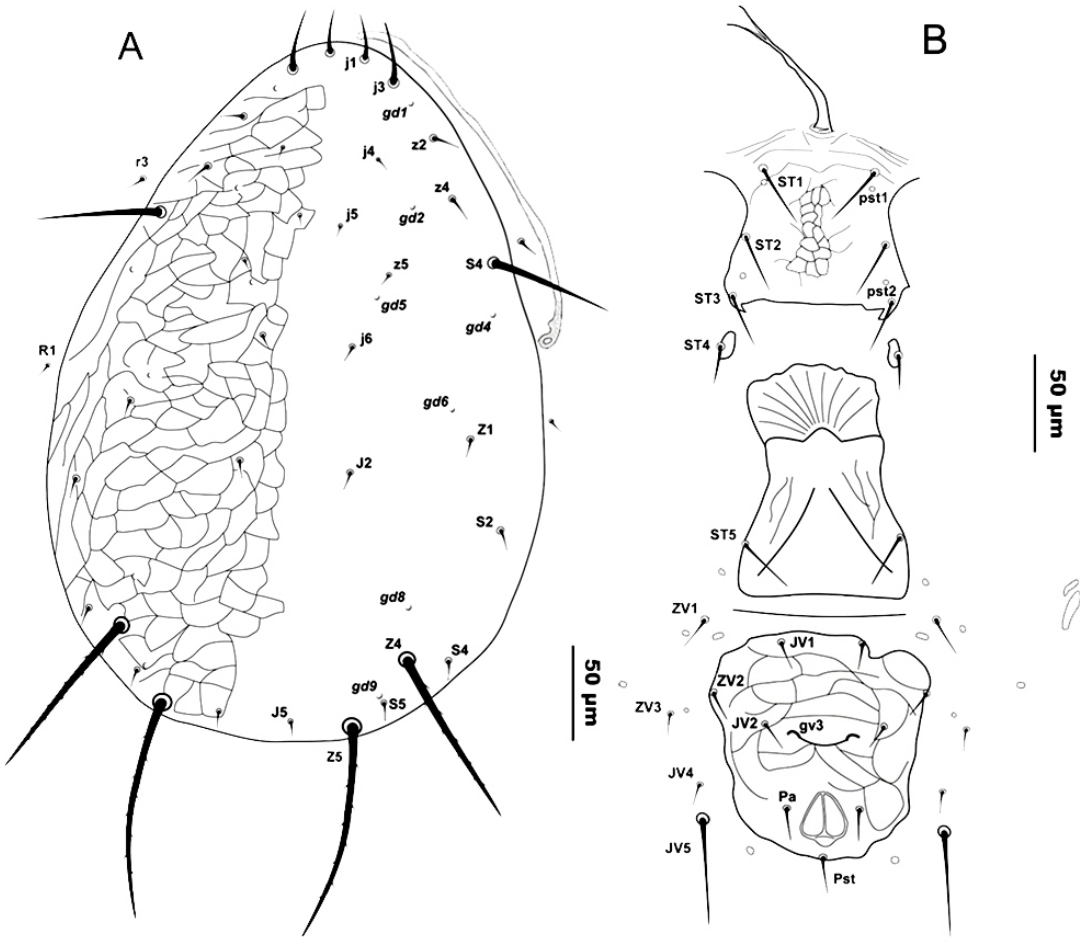

Figure 2. Dorsum (A) and venter (B) of *Amblyseius rademacheri* (♀).

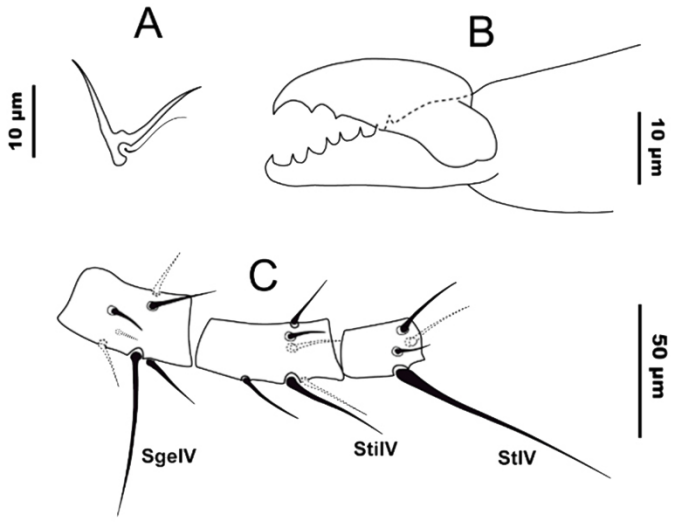

Figure 3. Spermatheca (A), chelicerae (B), and leg IV (C) of *Amblyseius rademacheri* (♀).

***Amblyseius swirskii* Athias-Henriot, 1962**

Material examined (open field): Çaybaşı, 10.10.2014, *P. vulgaris* (2♀♀), *C. sativus* (13♀♀), *S. melongena* (2♀♀, 1♂), *C. annuum* (2♀♀, 2♂♂); Fatsa, 28.08.2014, *C. annuum* (1♀); Gülyalı 17.09.2015, *C. sativus* (3♀♀), *S. melongena* (1♀); Altınordu, 21.07.2015, *P. vulgaris* (3♀♀), *C. sativus* (9♀♀); Perşembe, 27.09.2013, *C. annuum* (1dn), 09.07.2014, *C. annuum* (1♀); Ulubey, 06.09.2013, *P. vulgaris* (3♀♀), *Cucurbita* sp. (9♀♀, 2♂♂, 1dn), *C. sativus* (3♀♀), *C. annuum* (2♀♀, 2♂♂, 1dn), 11.09.2014, *Cucurbita* sp. (5♀♀, 1♂), *C. sativus* (1♀), *C. annuum* (2♀♀); Ünye, 07.08.2014, *Cucurbita* sp. (1♀).

Distribution: *A. swirskii* is an important biological control agent of mites and small insects (Demite et al., 2017). This predator was reported for the first time on *P. vulgaris, S. melongena* and *C. sativus* in Adana (Kibritçi et al., 2007). In present study, it was collected in association with colonies of *B. lewisi* on *C. annuum* and *Cucurbita* sp., *B. obovatus* and *T. palmarum* on *C. sativus* and with *T. urticae* on all examined vegetable species.

***Aristadromips masseei* (Nesbitt, 1951)**

Material examined (open field): Altınordu, 20.07.2013, *Cucurbita* sp. (5♀♀, 2♂♂), 21.07.2015, *C. sativus* (1♀); Gülyalı, 10.07.2013, *C. sativus* (1♀).

Material examined (greenhouse): Gürgentepe, 02.10.2014, *C. sativus* (2♀♀, 4♂♂).

Distribution: *A. masseei* was determined in northwestern countries of the Palearctic region (Demite et al., 2017). This predatory was found for the first time in Giresun Province, Turkey (Çobanoğlu, 1991-1992). In this study, it was collected together with *T. urticae* from *C. sativus* and *Cucurbita* sp. leaves.

***Euseius finlandicus* (Oudemans, 1915)**

Material examined (open field): Çaybaşı, 10.10.2014, *C. annuum* (2♀♀); Çatalpınar, 21.08.2014, *P. vulgaris* (1♀); Kabadüz, 26.09.2014, *C. annuum* (3♀♀); Perşembe, 27.09.2013, *C. sativus* (1♀).

Distribution: *E. finlandicus* is common around the world (Demite et al., 2017). In Turkey, this predatory mite was also determined by many researchers (Faraji et al., 2011). During this research, *E. finlandicus* was collected with *T. urticae* on *P. vulgaris* and *C. sativus*.

***Euseius gallicus* Kreiter and Tixier, 2010**

Material examined (open field): Gülyalı, 19.08.2015, *P. vulgaris* (7♀♀), *C. sativus* (10♀♀, 2♂♂), *C. annuum* (1♀).

Distribution: *E. gallicus* has been reported in Tunisia (Kreiter et al., 2010), Belgium, France, Germany, the Netherlands and Turkey (Tixier et al., 2009; Döker et al., 2014b). In this research, it was found together with *Xenotarsonemus* sp., *T. goetzi* and *B. mali* on *C. annuum.*

***Kampimodromus aberrans* (Oudemans, 1930)**

Material examined (open field): Kabadüz, 26.09.2014, *C. sativus* (1♀); Perşembe, 09.07.2014, *Cucurbita* sp. (1♂).

Distribution: Cosmopolitan *K. aberrans* has been recorded worldwide (Demite et al., 2017). It is one of the most common species in Turkey (Faraji et al., 2011). During this study, it was collected together with *T. goetzi* on *Cucurbita* sp. and *T. urticae* on *C. sativus*.

***Neoseiulus barkeri* Hughes, 1948**

Material examined (open field): Fatsa, 28.08.2014, *Cucurbita* sp. (2♀♀); Perşembe, 27.09.2013, *P. vulgaris* (1♀), *S. lycopersicum* (1♀), *S. melongena* (1♀); Ulubey, 11.09.2014, *S. melongena* (2♀♀).

Material examined (greenhouse): Perşembe, 23.07.2013, *C. sativus* (1♀).

Distribution: *N. barkeri* has been found in many countries around the world (Demite et al., 2017). In Turkey, it was determined on *S. melongena* in Antalya (Çobanoğlu, 1989a) and *A. cepa* in İzmir (Kılıç et al., 2012). It was found in association with *T. urticae, B. obovatus* on *S. melongena* and *T. urticae* on *Cucurbita* sp. in this study.

***Neoseiulus bicaudus* (Wainstein, 1962)**

Material examined (open field): Ünye, 07.08.2014, *A. cepa* (1♀).

Distribution: *N. bicaudus* has been reported in Palearctic region (Asali Fayaz & Khanjani, 2012). In Turkey, it was found on *C. sativus* (İnal, 2005) and *S. melongena*, *Cucurbita* sp. (Can & Çobanoğlu, 2010) with *T. cinnabarinus*. In this study, *N. bicaudus* was collected together with population of *T. urticae* on *A. cepa*.

***Neoseiulus californicus* (McGregor, 1954)**

Material examined (open field): Fatsa, 13.09.2013, *S. melongena* (1♀); Perşembe, 09.07.2014, *Z. mays* (1♀).

Material examined (greenhouse): Perşembe, 23.07.2013, *C. annuum* (1♀); Ünye, 07.08.2014, *C. sativus* (13♀♀, 1♂).

Distribution: *N. californicus* is widely used as an effective biological control agent around the world. It has been found in many countries of the world (Demite et al., 2017). In Turkey, it was reported on *C. annuum* and *P. vulgaris* in association with *T. urticae* in Aydın (Çakmak & Çobanoğlu, 2006). Çobanoğlu & Kumral (2014) found *N. californicus* in association with populations of tetranychids in tomato fields in Ankara and Bursa. Döker et al. (2016) also determined this predatory mite with *T. urticae* on *S. melongena.*

***Phytoseius finitimus* Ribaga, 1904**

Material examined (open field): Çatalpınar, 21.08.2014, *S. melongena* (1♀); Gülyalı, 19.08.2015, *P. vulgaris* (1♀), *S. lycopersicum* (2♀♀); Kabadüz, 26.09.2014, *P. vulgaris* (2♀♀); Altınordu, 23.09.2013, *P. vulgaris* (3♀♀, 1♂), 20.07.2014, *Cucurbita* sp. (1♂), *S. lycopersicum* (1♂), *C. sativus* (2♀♀), *S. melongena* (3♀♀); Perşembe, 23.07.2013, *P. vulgaris* (1♀), 09.07.2014, *P. vulgaris* (1♀, 1♂), *Cucurbita* sp. (1pn), *C. sativus* (1♀); Ulubey, 06.09.2013, *P. vulgaris* (1♀), *Cucurbita* sp. (1♀), 11.09.2014, *C. annuum* (1♀); Ünye, 26.07.2013, *C. sativus* (1♀), 07.08.2014, *C. annuum* (1♀).

Material examined (greenhouse): Fatsa, 28.08.2014, *C. sativus* (1♀); Ünye, 26.07.2013, *C. sativus* (1♂).

Distribution: *P. finitimus* is a common predatory mite species (Demite et al., 2017). It has been collected together with several mite species in many provinces of Turkey by many researchers (Faraji et al., 2011). In the current study, it was collected with *T. urticae, A. pulvinum, P. latus, T. goetzi, T. putrescentiae, B. obovatus, T. immanis* and *H. staerki* on *C. annuum, P. vulgaris*, *S. melongena, S. lycopersicum* and *C. sativus.*

***Phytoseiulus persimilis* Athias-Henriot, 1957**

Material examined (open field): Çaybaşı, 10.10.2014, *P. vulgaris* (5♀♀, 1pn), *C. sativus* (1♀); Çatalpınar, 21.08.2014, *P. vulgaris* (10♀♀, 1♂), *S. melongena* (1♀); Fatsa, 13.09.2013, *S. lycopersicum* (1♂), 28.08.2014, *P. vulgaris* (1♀, 4♂♂, 1pn); Gülyalı, 19.08.2015, *P. vulgaris* (1♀), 17.09.2015, *P. vulgaris* (7♀♀, 1♂); Altınordu, 21.07.2015, *C. sativus* (1♀); Perşembe, 29.07.2014, *Cucurbita* sp. (2♀♀); Ünye, 07.08.2014, *P. vulgaris* (1♀, 2pn, 4tn), *C. lanatus* (1♀).

Material examined (greenhouse): Ünye, 26.07.2013, *C. sativus* (15♀♀, 1♂), *C. annuum* (1♀).

Distribution: *P. persimilis* is a common predatory species around the world (Demite et al., 2017) and has been successfully used for years for the biological control of *T. urticae* in many countries. Natural populations of *P. persimilis* have been recorded in Turkey (Şekeroğlu & Kazak 1993; İnal, 2005; Akyazı & Ecevit, 2008; Kasap et al., 2013; Çobanoğlu & Kumral, 2014).

***Proprioseiopsis okanagensis* (Chant, 1957)**

Material examined (open field): Kabadüz, 26.09.2014, *Cucurbita* sp. (1♀).

Distribution: *P. okanagensis* was reported in Europe, North America (Demite et al., 2017) and Turkey (Çobanoğlu, 1989c; Çobaoğlu & Bayram, 1999).

***Transeius wainsteini* (Gomelauri, 1968)**

Material examined (open field): Çaybaşı, 10.10.2014, *C. annuum* (1♀); Gürgentepe, 02.10.2014, *Cucurbita* sp. (1♀); Perşembe, 23.07.2013, *C. sativus* (1♀), 27.09.2013, *C. annuum* (1♀); Ünye, 07.08.2014, *Cucurbita* sp. (1♀).

Distribution: Demite et al. (2017) listed *T. wainsteini* in Armenia, Azerbaijan, Denmark, Georgia, Germany, Russia, Iran and Poland and Turkey. In this study, it was collected together with *B. lewisi, H. staerki* and *T. urticae* on *C. annuum, Cucurbita* sp. and *C. sativus.*

***Typhlodromus athiasae* Porath and Swirski, 1965**

Material examined (greenhouse): Ünye, 26.07.2013, *C. sativus* (28♀♀, 7♂♂), *C. annuum* (2♀♀, 1♂).

Distribution: *T. athiasae* is widespread around the world (Azerbaijan, Cyprus, Egypt, France, Greece, Iran, Israel, Jordan and Syria) (Demite et al., 2017). In Turkey, this predatory mite has been found by many researchers (Faraji et al., 2011).

Conclusion

A total of 43 mite species were identified during this study with 27% these found in both open fields and greenhouses. The others were found only in the open fields. Our results showed that the vegetables growing areas of Ordu Province, especially in open field conditions, are extremely rich with regard to beneficial mite fauna. This may be due to the limited usage of pesticides in the area. Therefore, the results obtained from the study may help to evaluate potential of the predators for biological control of phytophagous mites, including *T. urticae* and *P. latus* in Ordu. *Amblyseius rademacheri,* a promising predator was also found for the first time in Turkey. Therefore, in further studies it should be determined if *A. rademacheri* can be used in biological control programs in Turkey.

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Original article (Orijinal araştırma)

An evaluation on host discrimination and superparasitism in *Trissolcus semistriatus* (Nees, 1834) (Hymenoptera: Scelionidae), egg parasitoid of *Eurygaster integriceps* Put., 1881 (Hemiptera: Scutelleridae)[1]

Eurygaster integriceps Put., 1881 (Hemiptera: Scutelleridae)'in yumurta parazitoiti *Trissolcus semistriatus* (Nees, 1834) (Hymenoptera: Scelionidae)'da konukçuyu ayırt etme ve süperparazitizm üzerine bir değerlendirme

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Abstract

Trissolcus semistriatus (Nees, 1834) (Hymenoptera: Scelionidae) is the most common and important egg parasitoid of *Eurygaster integriceps* Put., 1881 (Hemiptera: Scutelleridae). This study was conducted to determine discrimination *E. integriceps* eggs parasitized by either self or a conspecific female of *T. semistriatus* in choice and no-choice tests, and to establish effect of adult parasitoid density and host density on parasitism in the laboratory in 2016. Female parasitoids did not superparasitize self-parasitized host eggs. However, superparasitism of 18% (significantly lower than the 81% parasitism rate) of the eggs parasitized by another female occurred within 1 h of first parasitism. Superparasitism was significantly lower at 1% in no-choice tests than the 23% recorded in choice tests within 24 h of first parasitism. Parasitism significantly decreased with increasing host egg number, but parasitism did not change with increasing parasitoid density. Therefore, it is concluded that *T. semistriatus* can discriminate between parasitized and unparasitized host eggs, with superparasitism infrequent when females encounter preparasitized host eggs within 48 h of first parasitism.

Keywords: *Eurygaster integriceps*, host discrimination, intraspecific competition, parasitism, *Trissolcus semistriatus*

Öz

Trissolcus semistriatus (Nees, 1834) (Hymenoptera: Scelionidae) *Eurygaster integriceps* Put., 1881 (Hemiptera: Scutelleridae)'in en yaygın ve önemli yumurta parazitoitidir. Bu çalışma, 2016 yılında laboratuvarda tercihli ve tercihsiz denemelerle *T. semistriatus*'un kendisi ya da diğer bir dişisi tarafından parazitlenmiş *E. integriceps* yumurtasını ayırt etme durumunu tespit etmek ve ergin parazitoit ve konukçu yoğunluğunun parazitlenme üzerindeki etkisini saptamak için yürütülmüştür. Dişi parazitoit kendisi tarafından parazitlenmiş konukçu yumurtasını süperparazitlememiştir. Ancak ilk parazitlenmeden sonraki 1 saat içinde diğer bir dişi tarafından parazitlenmiş yumurtaların %18'inin (%81'lik parazitizm oranından önemli olarak daha düşük oranda) süperparazitlendiği görülmüştür. İlk parazitlenmeden 24 saat sonrasındaki tercihli denemelerde kaydedilen %23'lük orana göre tercihsiz denemelerde önemli olarak daha düşük %1'lik bir oranda süperparazitizm meydana gelmiştir. Parazitizm artan konukçu yumurtası sayısı ile önemli oranda düşmüş, ancak artan parazitoit yoğunluğu ile değişmemiştir. Bu nedenle, *T. semistriatus*'un parazitlenmiş konukçu yumurtasını ayırt edebildiği, dişilerin ilk parazitlenmeden 48 saat sonrasına kadar önceden parazitlenmiş konukçu yumurtalarıyla karşılaştığında nadiren bir süperparazitizm meydana geldiği kanısına varılmıştır.

Anahtar sözcükler: *Eurygaster integriceps*, konukçuyu ayırt etme, tür içi rekabet, parazitizm, *Trissolcus semistriatus*

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Introduction

The Sunn pest, *Eurygaster integriceps* Put., 1881 (Hemiptera: Scutelleridae) has a wide distribution in the Palearctic Region, that is Eastern Europe, Near and Middle East, and Western Asia. It is the most important species in cereal fields and it causes severe qualitative and quantitative damage on wheat and barley. The scelionid egg parasitoids have demonstrated a great potential to suppress the Sunn pest below the economic threshold, but this can vary among regions and from year to year (Brown, 1962; Safavi, 1968; Lodos, 1982; Öncüer & Kıvan, 1995). *Trissolcus semistriatus* (Nees, 1834) (Hymenoptera: Scelionidae) is the most common egg parasitoid of the Sunn pest.

The scelionid egg parasitoids of certain species of Lepidoptera and Heteroptera have been used in both augmentative and classical biological control applications (Orr, 1988; Hoffmann et al., 1991; Corrêa-Ferreira & Moscardi, 1996; Weber et al., 1996; Ehler, 2002). They are solitary parasitoids and only one parasitoid can develop within one host egg (van Lenteren, 1981). When a female lays an egg in a host egg, she marks it with pheromone after oviposition so females can discriminate between parasitized and unparasitized eggs (Okuda & Yeargan, 1988; Roitberg & Mangel, 1988). These pheromone marks are generally species specific and reduce superparasitism, but sometimes they can affect related species (Agboka et al., 2002). However, it has been observed that parasitoid females frequently oviposited an egg into a host preparasitized by herself (self-superparasitism), by a conspecific female (superparasitism) or by another species (multiparasitism) (van Alphen & Visser, 1990; Visser et al., 1990, 1992; Godfray, 1994). Superparasitism is more likely to occur when the parasitoid to host ratio is high (van Alphen & Vet, 1986). When host eggs are fewer in number, more than one female *Trissolcus grandis* (Thomson, 1861) (Hymenoptera: Scelionidae) oviposits into the same host egg (Kozlov, 1968). In such cases, solitary parasitoids waste both time and eggs.

An earlier study of female behaviour in *Trissolcus viktorovi* Kozlov, 1968, *Trissolcus djadetshko* (Ryach., 1959) and *T. grandis* during attack on eggs of *Eurydema ornatum* (L., 1758) (Hemiptera: Pentatomidae) already parasitized by the same species showed that intraspecific reinfestation occurs only rarely, whereas interspecific multiple parasitism is frequent, but varies between the different species (Buleza, 1971a). Buleza (1971b) also researched the interspecific relationships between *T. viktorovi*, *T. djadetshko* and *T. grandis*. According to another study, *T. grandis, Trissolcus simoni* (Mayr, 1879) and *T. viktorovi* could distinguish between parasitized and non-parasitized eggs of *E. integriceps, Graphosoma lineatum* (L., 1758) and *Eurydema ventralis* (Kolenati, 1846) (Hemiptera: Pentatomidae); in the absence of non-parasitized eggs, once-parasitized eggs were preferred to twice-parasitized eggs (Kartsev, 1985). Todoroki & Numata (2017) recently studied about effects of mating experience on superparasitism by female *T. semistriatus*. It was observed that virgin females discriminated between parasitized *Eurydema rugosum* Motshulsky, 1861 (Hemiptera: Pentatomidae) eggs from unparasitized eggs and avoided complete superparasitism. In contrast, mated females superparasitized more parasitized eggs than virgin females did.

The primary aim of this study was to determine intraspecific host discrimination of *T. semistriatus* and if its females superparasitized host eggs. Similar studies (mentioned above) have been done on *T. grandis*. Recently, *T. semistriatus* was synonymized with *T. grandis* (Talamas et al., 2017). In this study, host discrimination of *T. semistriatus* will be detailed and compared with former findings from different *Trissolcus* spp. and different host species. The secondary aim was to determine the effect of parasitoid and host density on parasitism efficiency. Thus, intraspecific competition could be considered their adaptive value.

Material and Methods

Insect rearing

Eurygaster integriceps adults were collected from wheat fields in Tekirdağ at the end of April 2016 when they have migrated to fields after overwintering area. They were cultured on potted wheat in a growth room at 26±1ºC, 60±10% RH and 16:8 h L:D photoperiod (Kıvan & Kılıç, 2002). Eggs of *E. integriceps* were collected daily for use in the experiments.

Laboratory progeny of *T. semistriatus* were used in the experiments. They were reared on *E. integriceps* eggs in glass tubes in an incubator (26±1ºC, 60±10% RH and 16:8 h L:D photoperiod) and fed with a sugar solution (30%) absorbed into filter paper (1 x 5 cm) (Kıvan, 1998).

In choice superparasitism experiments

Egg masses of *E. integriceps* (1 d old) was placed individually in a Petri dishes (6 cm diameter), and a sketch of each egg mass was drawn under a stereomicroscope to record the number and location of its eggs (Mahmoud & Lim, 2008). For evaluation of self-discrimination, individual females (24 h old) previously mated and inexperienced were released onto an egg mass in a Petri dish, allowed to parasitize half of the eggs in the mass, and then removed. One h later, the same female was introduced again to the same preparasitized egg mass. For evaluation of conspecific discrimination, 1 h later another female of *T. semistriatus* was released onto the preparasitized egg mass. All observations were made under a stereomicroscope and ended when the female parasitoid had parasitized all the eggs in the mass or had stopped ovipositing for more than 30 min. The number of parasitized and self superparasitized eggs were counted then incubated at 26±1ºC for the development of parasitoids. The eggs marked after oviposition was considered to be parasitized (Rabb & Bradley, 1970; Weber et al., 1996; Mahmoud & Lim, 2008). The experiment was repeated 12 times.

Effect of time after first oviposition on superparasitism in choice tests

To examine the possible effects of the length of time after first oviposition, the female of *T. semistriatus* was allowed to parasitize half of the eggs in an egg mass. Another female of the same species was then introduced onto the same egg mass after 1, 24 and 48 h from first parasitism. Observations were under a stereomicroscope. The number of parasitized or conspecific superparasitized eggs were recorded, and eggs were incubated at 26±1ºC for the development of the host or the parasitoids. These procedures were repeated 10 times with different individual parasitoids.

No-choice superparasitism experiments

One female was allowed to parasitize all the eggs in an egg mass and, after 24 h from the first oviposition, another female of the same species was released on the same egg mass. Observations were under a stereomicroscope. The number of parasitized or conspecific superparasitized eggs were recorded, and eggs were incubated at 26±1ºC for the development of the host or the parasitoid. The experiment was repeated with 10 parasitoid females.

Adult competition experiments

Two treatments were used to examine adult competition and the effect of parasitoid and host density on parasitism by *T. semistriatus*. Firstly, the host density was kept constant and female parasitoid density was varied. Two, four and eight females were released on two egg masses. After 24 h of parasitism, the females were removed and parasitized eggs were kept for incubation. Secondly, female parasitoid density was kept constant and host density was varied. This time, two females were released on two, four and eight egg masses. Each treatment was replicated five times.

Statistical analysis

Parasitism rate was calculated by dividing the number of once-parasitized eggs by the total number of host eggs offered. Superparasitism rate was the proportion of the number of twice-parasitized eggs to total number of host eggs offered. Adult emergence rate was the proportion of the number of emerged adult parasitoids to number of parasitized eggs. Parasitism, superparasitism and adult emergence rate were analyzed as pooled data by the X^2 test of contingency table ($p < 0.05$), and to compare choice with no-choice test results, t-test was used ($p = 0.05$). To evaluate the effect of time for data collected after 1, 24 and 48 h from first parasitism and data on adult competition effects ANOVA was applied and Tukey's test for multiple comparing (SPSS, 2006).

Results

Intraspecific host discrimination and larval competition

There was no superparasitism when *T. semistriatus* female encountered host eggs preparasitized by herself after 1 h (Table 1). However, another female deposited a few eggs in the host eggs preparasitized by a conspecific female. The number of superparasitized eggs was significantly different from once-parasitized (X^2 = 66.5, p < 0.05). In choice tests, the superparasitism rate (18%) was significantly lower than the parasitism rate (81%) (Table 1). Although the rate of adult emergence from conspecific superparasitized eggs was seemed lower in compared to emergence from unparasitized eggs, it was not significant according to the t-test. When unparasitized and preparasitized host eggs in the same egg mass were provided to a female *T. semistriatus*, she did not usually oviposit eggs in parasitized host eggs but occasionally eggs were oviposited in host eggs previously parasitized by other females.

Table 1. Host discrimination of *Trissolcus semistriatus* on *Eurygaster integriceps* eggs previously parasitized by themselves or conspecific females (mean±SD)

Host egg status	Number of eggs (masses) tested	Self-discrimination		Conspecific discrimination	
		Parasitism rate	Emergence rate	Parasitism rate	Emergence rate
Unparasitized	167 (12)	0.97±0.06	0.97±0.06	0.81±0.14	0.99±0.02
Preparasitized	137 (10)	0	-	0.18±0.15	0.76±0.41

The number of superparasitized host eggs were significantly lower after 1 (X^2 = 66.5, p < 0.05), 24 (X^2 = 56.5, p < 0.05) and 48 h (X^2 = 65.5, p < 0.05) from first parasitism. The parasitism rates after 1, 24 and 48 h were similar at 8, 70% and 81%, respectively (Table 2). The parasitism and superparasitism rates, and also percentage adult emergence of once-parasitized eggs did not change with time elapsed after first parasitism. Although the emergence rate from superparasitized eggs (67%) decreased after 48 h from first oviposition, it was not significant. The survival of superparasitized eggs was relatively low.

Table 2. Effect of time after first oviposition on host discrimination of *Trissolcus semistriatus* on preparasitized *Eurygaster integriceps* eggs by conspecific females (mean±SD)

Time (h) after pre-parasitization	Number of eggs (masses) tested	Parasitism rate	Superparasitism rate	Emergence rate	
				Once parasitized	Superparasitized
1	137 (10)	0.81±0.14	0.18±0.15	0.99±0.02	0.76±0.41 ab
24	123 (9)	0.70±0.18	0.23±0.14	0.96±0.11	0.92±0.17 a
48	137 (10)	0.81±0.24	0.10±0.11	0.99±0.03	0.47±0.50 b

*Same letters in the column indicate that means are not significantly different (p ≤ 0.05, Tukey's test).

In the no-choice test, the number of superparasitized host eggs was significantly low (X^2 = 65.3, p < 0.05) from first parasitism. The superparasitism rate in no-choice test was significantly low (0.01±0.04) than that of choice test (0.23±0.14) (t = 4.35, p = 0.05) (Figure 1). The females could discriminate between parasitized and unparasitized host eggs, and only one female deposited two eggs into two preparasitized eggs. As a result of hatching all two superparasitized eggs, the emergence rate was 1.0±0.00 and was not significantly different from that of choice test (0.92±0.17) (t = -0.232, p = 0.05).

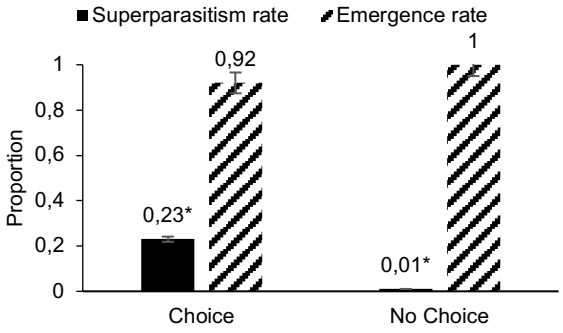

Figure 1. Comparison of the superparasitism and emergence rate of *Trissolcus semistriatus* in choice and no-choice tests. The asterisks indicate significant differences between proportions (p = 0.05, t-test).

Adult competition

The parasitism and adult emergence significantly decreased increasing density when two females encountered with a high density of host eggs (Table 3).

Table 3. Influence of host density on parasitism and emergence rate of *Trissolcus semistriatus* (mean±SD)

Number of host egg masses (Mean number of eggs tested)	Parasitism rate	Emergence rate
2 (26.75)	0.91±0.17 a	0.97±0.05 a
4 (53.00)	0.51±0.28 b	0.91±0.05 a
8 (98.80)	0.31±0.22 b	0.66±0.25 b

*Same letters in the same column indicate that means are not significantly different (p ≤ 0.5, Tukey's test).

When two egg masses were exposed to a different number of the female parasitoid, the parasitism rate was low (Table 4). Parasitism rate was 91% when two host egg masses were exposed to two females of *T. semistriatus* and 80% with eight females, but, this decrease was not significant. However, emergence rate was significantly affected at different parasitoid density (Table 4).

Table 4. Influence of parasitoid female density on host parasitism and emergence rate of *Trissolcus semistriatus* (mean±SD)

Number of female parasitoids	Mean number of eggs tested	Parasitism rate	Emergence rate
2	26.8	0.91±0.17	0.97±0.05 ab
4	27.6	0.91±0.10	0.89±0.07 b
8	25.3	0.80±0.21	1.00±0.00 a

*Same letters in the same column indicate that means are not significantly different (p ≤ 0.5, Tukey's test).

Discussion

Trissolcus semistriatus females could discriminate between host eggs that where unparasitized or preparasitized by self or conspecific parasitoids. No females deposited eggs in host eggs preparasitized by herself and self superparasitism was not observed. However, one female deposited a few eggs in host eggs preparasitized by conspecific another female. Thus, conspecific superparasitism was much lower in both choice and no-choice experiments. Intraspecific reinfestation of *E. ornatum* by *T. grandis* occurred very rarely (Buleza, 1971a). Mated females of *T. semistriatus* superparasitized more parasitized eggs of *E. rugosum* than virgin females (Todoroki & Numata, 2017). Agboka et al. (2002) also reported that self

superparasitism was only 4% for *Telenomus busseolae* Gahan, 1922 and about 6% for *Telenomus isis* Polaszek, 1993 (Hymenoptera: Scelionidae). Self superparasitism was considerably lower than intraspecific superparasitism, suggesting that the females are able to discriminate between the marking of their own and conspecific females (Agboka et al., 2002). Mahmoud & Lim (2008) suggested that host discrimination has evolved to increase the fitness of female that marks the host eggs, and showed that *Trissolcus nigripedius* Nakagawa, 1900 and *Telenomus gifuensis* Ashmead, 1904 (Hymenoptera: Scelionidae) discriminated between host egg preparasitized by either self or conspecific. They reported that superparasitism rates were 19 and 31% for *T. nigripedius* and *T. gifuensis*, respectively. Female parasitoids avoid superparasitism and disperse to search unparasitized host eggs (Okuda & Yeargan, 1988).

Low emergence rates for conspecific superparasitized eggs was observed in this study, but it was not significant unlike some reports in the literature (Agboka et al., 2002, Mahmoud & Lim, 2008). The low rates for both *T. nigripedius* and *T. gifuensis* have been attributed to the intraspecific competition between similar aged larvae (Mahmoud & Lim, 2008).

Trissolcus semistriatus tended to avoid host eggs that had been parasitized by conspecifics 1, 24, and 48 h previously, so the superparasitism rate was low and did not change with increasing time after first oviposition. However, Agboka et al. (2002) determined that superparasitism was significantly higher when eggs were offered directly and after the 0 and 48 h than after 24 h for both *T. busseolae* and *T. isis*. They suggested that the parasitoids recognized eggs parasitized at least 24 h earlier via an internal marker; when they insert their ovipositor in parasitized eggs, they abruptly removed it as if startled.

Adult emergence from superparasitized eggs was not significantly different at 1, 24 or 48 h after first oviposition. The eggs of *Telenomus solitus*, Johnson, 1983 hatched less 20 h after deposition (Navasero & Oatman, 1989), for example *Trissolcus basalis* (Wollaston, 1858) (Hymenoptera: Scelionidae) requires, on average, 17 h for egg development (Corrêa-Ferreira, 1993). When a female deposits an egg in an already parasitized host, larval competition begins in the host (Bakker et al., 1985). The emergence was higher at 24 h after the first oviposition since the older larvae might outcompete the younger one (Okuda & Yeargan, 1988). However, Volkoff & Colazza (1992) reported that second instar platygastrid larvae, although larger, are less mobile and have no jaws, which makes them more susceptible to attack by first-instar larvae. So, second larvae have a better chance of surviving if the time between oviposition events is enough for the first larvae to molt to the second instar (Cingolani et al., 2013). The first instar of the larvae has well-developed mandibles and is very mobile, so it is possible for older instars to be killed by younger, first instar larvae. It was not possible to determine whether first larvae or second larvae were dominant in the study because they are from the same species. So adult emergence from host eggs superparasitized at the different interval time was a similar and high ratio.

Superparasitism occurred at low percentage when female exposed to parasitized eggs in no-choice test, it was even lower than that observed in the choice test. A single female superparasitized just two eggs in a whole parasitized egg mass in no-choice test and the emergence was 100% in these eggs. If females had no chance to choose unparasitized host egg, they did not prefer to oviposit in the host. Female showed different behavior in choice and no-choice tests when a female encountered preparasitized egg masses. She spent a shorter time on the mass in no-choice tests, although she stayed for a longer time on the eggs and searched for suitable host eggs in the choice tests. Adult emergence rates have been reported to be 93 and 75% for *T. nigripedius* and *T. gifuensis*, respectively, in no-choice tests (Mahmoud & Lim, 2008), which is similar to the current study.

In adult competition tests, parasitism rate decreased with increasing host egg numbers because females encountered many host eggs which were preparasitized by herself. It is known that a female can parasitize 12 to 24 host eggs per day (Kıvan & Kılıç, 2006a, b). Emergence rate also decreased with increasing host egg numbers. It is suggested that host eggs may be multiparasitized two, three or more

times when the number of females is increased, however, emergence rate can decrease. However, parasitism rate was not changed when parasitoid density was increased. This may be due to the female *T. semistriatus* discriminating between host eggs parasitized by other conspecific females and avoids parasitizing these eggs resulting in higher adult emergence from parasitized eggs. In this experiment, all emergence rates were close to one however there was some significant difference. It appears that a smaller number of host eggs parasitized by eight females but all of these parasitized eggs hatched while similar rate of emergence.

In summary, the present study indicates that *T. semistriatus* had intraspecific host discrimination abilities, so superparasitism was very low. Future studies should aim to determine host discrimination and interspecific competition of different *Trissolcus* species under both laboratory and field conditions.

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Original article (Orijinal araştırma)

Coexistence of four orb-web spiders in an oil palm plantation in Peninsular Malaysia

Batı Malezya'da yağlık palmiye plantasyonunda küresel ağlı dört örümcek türünün bir arada bulunması

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Abstract

Thirty adult female individuals of each of the four orb-web spider species namely *Araneus* sp. (Araneidae), *Gasteracantha kuhli* Koch, 1837 (Araneidae), *Gasteracantha hasselti* Koch, 1837 (Araneidae) and *Opadometa grata* (Simon, 1877) (Tetragnathidae) were randomly sampled between 1000 and 1400 h from May to July 2017 in an oil palm plantation in Perak, Malaysia. Morphological and web characters of these orb-web spiders were obtained and analyzed using principal component analysis (PCA) and bootstrapping methods. For the morphological characters, the PCA results captured a total of 99% of the variance and indicate that the Araneid species have distinct clustering. For the web characters, the PCA captured 76% of the total variance and did not show any distinct clustering with significant overlapping between them. Moreover, the mean and 95% confidence intervals using bootstrapping identified significant differences in the morphological and web characters for most spider species with little overlap. This study indicates that the four orb-web spider species could coexist in terms of their spatial territory and food resources in the oil palm plantation, suggesting that these resources were not a limiting factor.

Keywords: Agriculture, co-occurrence, homogeneous habitat, niche overlap

Öz

Araneus sp. (Araneidae), *Gasteracantha kuhli* Koch, 1837 (Araneidae), *Gasteracantha hasselti* Koch, 1837 (Araneidae) ve *Opadometa grata* (Simon, 1877) (Tetragnathidae), isimli küresel ağlı dört örümcek türünden her birinden otuz yetişkin dişi birey, Perak, Malezya'daki bir yağlık palmiye plantasyonunda Mayıs-Temmuz 2017 tarihleri arasında 1000 ila 1400 saat arasında gündüz rastgele olarak örneklenmiştir. Bu küresel ağlı örümcek türlerinin, morfolojik ve ağ karakterleri, temel bileşen analizi ve önyükleme yöntemleri kullanılarak elde edilmiş ve analiz edilmiştir. Morfolojik karakterler için, temel bileşen analiz sonuçları, varyansın toplam %99'unu yakalamış ve Araneid türlerinin ayrı bir grup oluşturduğunu göstermiştir. Ağ karakterleri için, temel bileşen analizi toplam varyansın %76'sını yakalamış ve aralarında belirgin bir örtüşmeyle ayrı bir grup özelliği göstermemiştir. Öte yandan, önyüklemeyi kullanan ve ortalama %95 güven aralığında, örümcek türlerinin çoğunda küçük çakışmalar için morfolojik ve ağ karakterlerinde önemli farklılıklar tespit etmiştir. Bu çalışma, küresel ağlı dört örümcek türünün, yağlık palmiye plantasyonundaki alansal topraklar ve besin kaynakları açısından bir arada bulunabileceğini ve bu kaynakların sınırlayıcı bir faktör olmadığını göstermektedir.

Anahtar sözcükler: Tarım, birlikte bulunma, homojen habitat, niş örtüşmesi

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Introduction

In agricultural pest management, beneficial organisms were used for biological control of insect pest populations (Norma-Rashid et al., 2014). Spiders, a highly successful group of natural predators have been identified as efficient natural predators in agriculture ecosystems (Maloney et al., 2003). Their polyphagous diet (Marc & Canard, 1997), high resistance to insecticides (Hoque et al., 2002) and coexistence capability in different niches (Norma-Rashid et al., 2014) make them potential agents for effective biological control in agriculture ecosystems. Previous studies have demonstrated the importance and benefits of different spider species in pest control (Riechert & Lawrence, 1997; Motobayashi et al., 2007; Tahir et al., 2009). These studies revealed that different spider species have specific roles and thus, higher spider species diversity can provide more effective control of pest populations (Marc et al., 1999).

Orb-web spiders of similar guild living in the same habitat often compete for the same resources so may differentiate their niches (Richardson & Hanks, 2009; Dzulhelmi, 2016). They can be distinguished morphologically, and portray distinctive web characteristics and/or web placement within a particular vegetation type, which results in a confined spatial use and non-interfering target and amount of prey (Richardson & Hanks, 2009; Tahir et al., 2009, 2012; Dzulhelmi et al., 2017). Spiders divide their habitat niches at fine scales, facilitating different hunting strategies, attuned naturally to capture specific types of prey (Wise, 1993; Schmitz & Suttle, 2001; Malumbres-Olarte et al., 2013). As sit-and-wait type of predators, orb-web spiders opt to regularly alter their web characteristics and/or relocating the placement of the web to a more profitable locations to increase prey-capture efficiency (Scharf et al., 2011). Factors that influence the process include the maturity, competition, predation risks and prey types of spiders within a particular habitat (Dzulhelmi, 2016). However, there is no defined optimum capture efficiency in the mechanism for changing the placement of the web, except through lifelong trial and error (Scharf et al., 2011). This strategy will create niche partitioning that reduces the amount of niche overlaps between the competitor species and allows the spiders to coexist within the same area (Tahir et al., 2012). Moreover, when the habitat is less complex, there is likely to be fewer niches available (Jimenez-Valverde & Lobo, 2007). In this study we focus on four common diurnal open-hub orb-web spider species, *Araneus* sp. (Araneidae), *Gasteracantha kuhli* Koch, 1837 (Araneidae), *Gasteracantha hasselti* Koch, 1837 (Araneidae) and *Opadometa grata* (Simon, 1877) (Tetragnathidae). Our objective was to determine if there are any niche overlaps between these species in an oil palm plantation using morphology and web characteristics differences as proxy measures of the species niche spaces.

Material and Methods

Study area

The fieldwork was conducted at an oil palm plantation (4°02’51” N, 101°01’08” E) in Perak State, Peninsular Malaysia. The sampled plot of 10 ha consists of palms aged 10-years old with tree height of about 10 m growing in peat soil.

Data collection

Thirty adult female individuals for each of the four orb-web spider species were randomly sampled between 1000 and 1400 h from May to July 2017. The vertical distance of the orb-web from the ground (height) was measured from the soil surface to the center of the orb-web *in-situ*. The webs were then dusted with white powder to increase visibility and to enhance photography resolution. Then, a measuring tape was held next to the webs to ensure proper scaling of the web during photography (Dzulhelmi et al., 2017). Other web characteristics, i.e., web-area, free-zone area, hub-area, mesh-size, number of spirals and number of radii, were measured directly from the photographs with the scale calibrated using KLONK Image measurement software. The occupant of the individual on each web were collected, stored in vials containing 75% ethanol and brought to the laboratory. The individuals collected were photographed, and body length

and carapace width measured using a Portable Capture Pro v2.1 Dinolite (China). Wet weights of each individual were measured with weighing scale. Species determinations were made according to Koh & Ming (2013) and Dzulhelmi & Suriyanti (2015).

Statistical analysis

The mean and standard deviation of each morphological and web characteristic variable of each spider species were plotted to examine the spread of the data. Principal component analysis (PCA) was used on the multivariate data sets to be easily visualize by reducing the number of dimensions within the dataset (PCA has been widely used to describe the niche space of spider species). In this study, the morphological and web characteristics were analyzed separately. PCA analysis was used to determine if there was distinct clustering in terms of morphological and web characteristics. The variables were standardized by taking the log and default settings were used for the PCA. Also, the bootstrap is a statistical tool to quantify uncertainty associated with a given estimator. From relatively small datasets, it can produce accurate estimates and standard errors of the parameters in the original distribution by resampling the dataset. Unlike null hypothesis statistical tests such as ANOVA, bootstraps can be applied to data in which there are violations of parametric assumptions and yield standard error estimates which do not depend on the parametric assumptions of traditional tests. The 95% confidence intervals (CI) derived from each parameter can be used as a hypothesis test, in which significant differences between estimates can be detected when there is no overlap between 95% CI. The bootstrap estimates of the mean and 95% CI were plotted on a radar chart to visualize the fundamental niche space of each of these spider species. The vegan package was used for PCA (Oksanen et al., 2017), boot package for bootstrapping (Canty & Ripley, 2017) and FMSB was used to plot the radar graphs (Nakazawa, 2017). All the analysis was performed using R 3.2.2 (R Core Team, 2015).

Results

A total of 120 adult females representing four orb-web spider species with 30 individuals for each species were collected in the study area. The morphological characteristics of these four-spider species were distinctive especially in terms of total length and carapace width, when these two characters are examined together the spider species can be quite easily differentiated (Figure 1). The four-spider species differed significantly in wet weight ($df = 3$, $F = 169$, $P < 0.0001$), total length ($df = 3$, $F = 152$, $P < 0.0001$) and carapace width ($df = 3$, $F = 427$, $P < 0.0001$).

For PCA on morphological characteristics, the PC1 captured 97% of the variance while the PC2 captured 2% of the remaining variance, resulting in a cumulative total of 99% of the variance explained (Figure 2, Table 1). The PCA biplot of morphological characteristics indicates that the Araneid spider species form distinct clustering. The *G. kuhli* is smaller than *G. hasselti* while *Araneus* sp. is distinctly larger than the two former spider species. However, *O. grata* did not form a distinctive cluster, overlapping with *G. kuhli* in terms of body size. There was no significant difference between *G. hasselti* and *O. grata* in body length. *Gasteracantha hasselti* has longer body protruding from the side equipped with long spines while *O. grata* has a pyriform abdomen and strongly overhang the carapace. Absence of significant differences in wet weight and carapace width between *G. kuhli* and *O. grata* may have contributed to obvious overlap between the two spider species in the PCA biplot.

Whereas, most web characteristics of these four-spider species had overlaps in standard deviation (Figure 1). For PCA on web characteristics, the percentage of variance captured in PC1 was 56% while PC2 captured 20% of the remaining variance for a cumulative total of 76% of the variance (Figure 3, Table 2). The PCA biplot of web characteristics show that the four orb-web spider species did not show any distinct clustering in their web characteristics, with large amount of overlap between them.

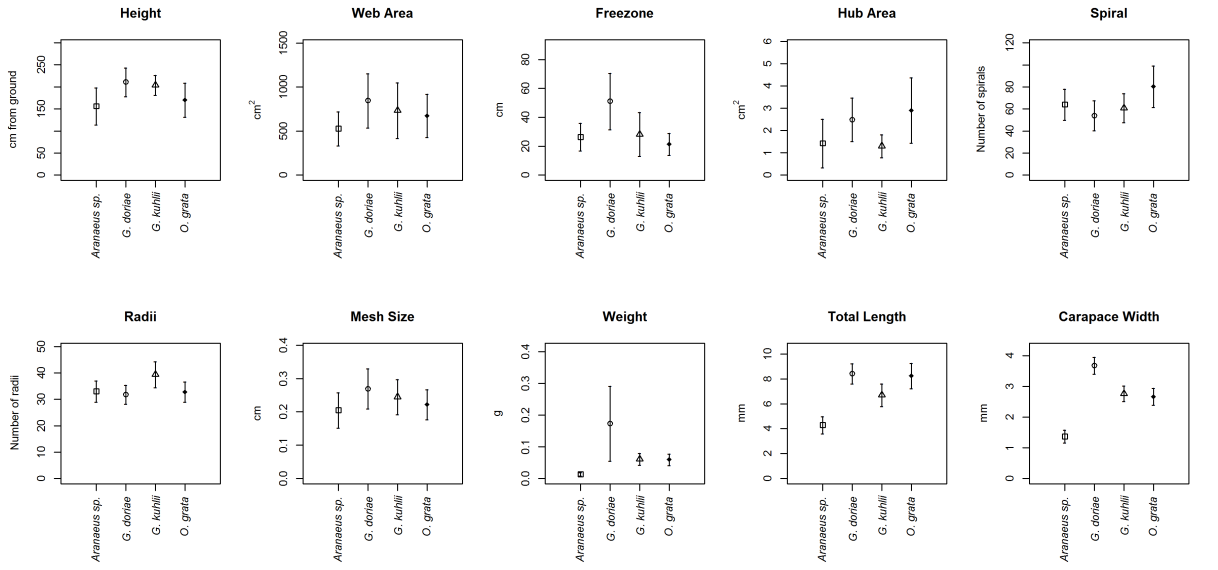

Figure 1. The means and the standard deviations of each species.

Table 1. The loadings for the principle component analysis for morphological characteristics

Morphological characteristics		
Variables	PC1	PC2
Weight	3.0666	0.1670
Total length	0.8729	-0.3438
Carapace width	1.1494	-0.1845

Table 2. The loadings for the principle component analysis for web characteristics

Web characteristics		
Variables	PC1	PC2
Height	0.1902	0.1550
Web-area	1.0375	0.0857
Freezone-area	1.5520	0.8676
Hub-area	1.6011	-0.9806
Number of spirals	-0.0364	-0.6061
Number of radii	-0.0160	0.0000
Mesh-size	0.3180	0.2612

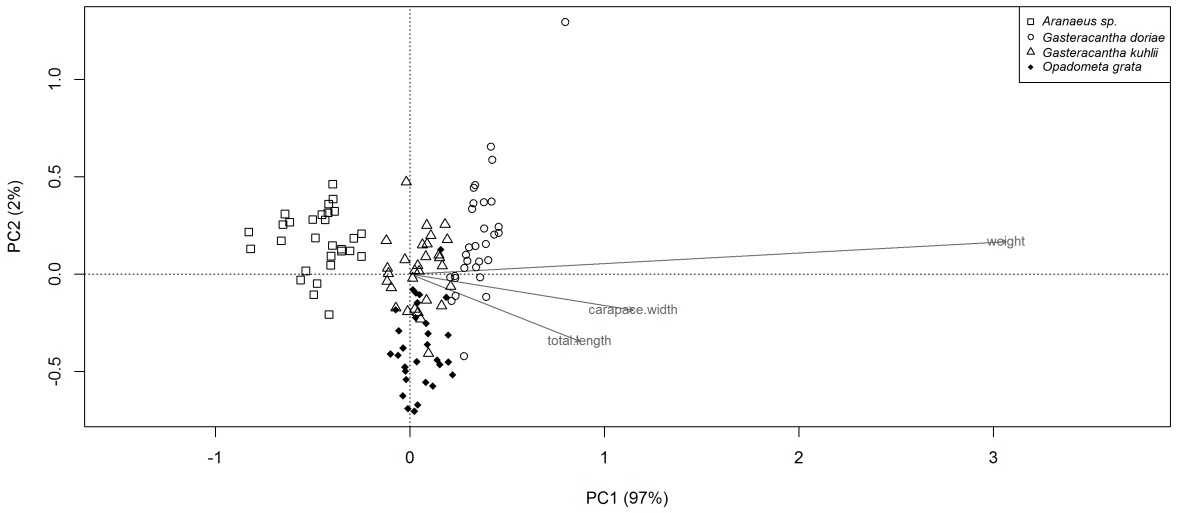

Figure 2. The principle component analysis biplot of morphological characters.

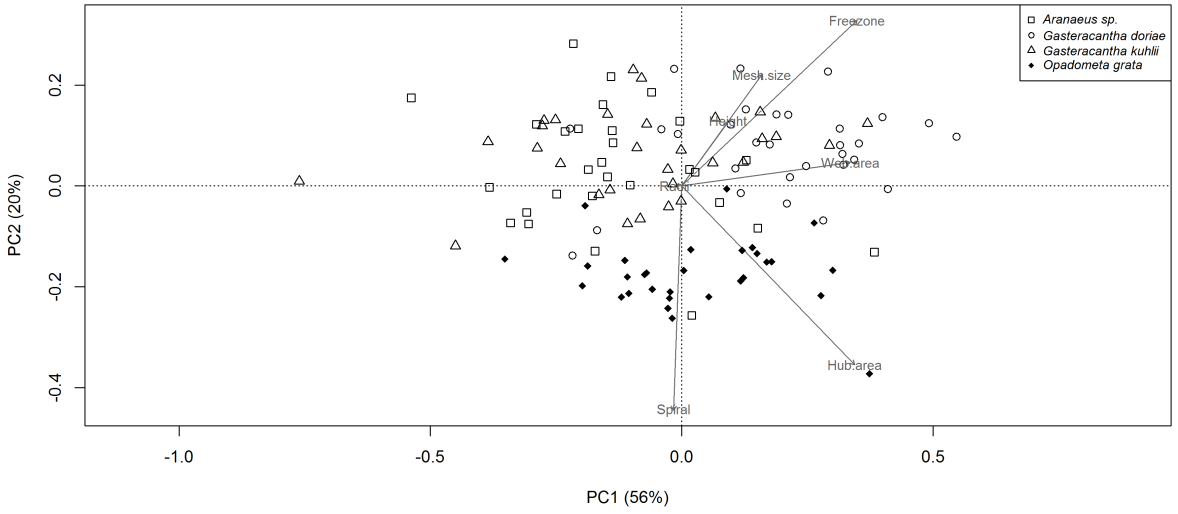

Figure 3. The principle component analysis biplot of web characters.

The means and 95% CI using bootstrapping for the morphological and web characteristics were estimated (Figure 4). Unlike the means and standard deviations, the nonparametric 95% CI were significantly different for most species with little overlap. This indicates that the means that generate the distribution of web characteristics are distinctly different between these orb-web spider species.

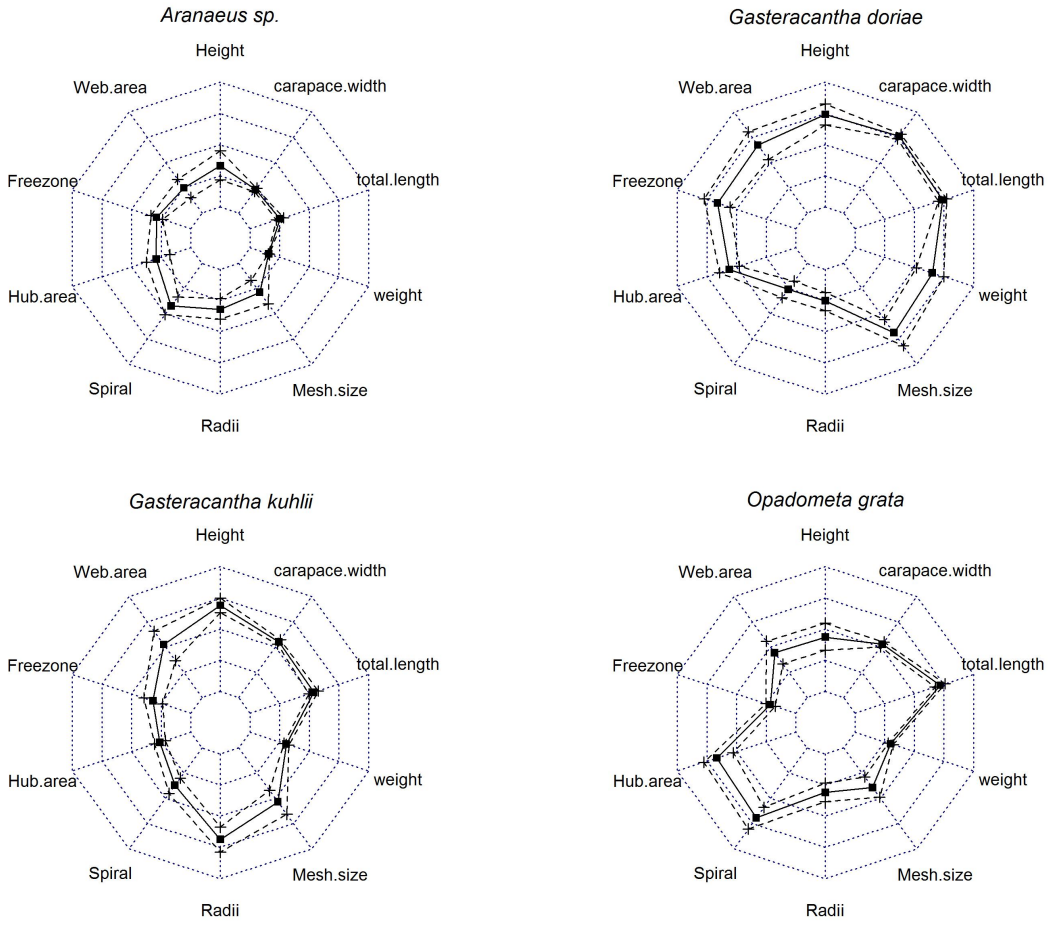

Figure 4. Radar plots of the means and 95% confidence intervals for the web and morphological characters of each species. The square represents the mean while the dotted lines are the 95% confidence intervals for each estimate.

Discussion

Web-building spider species differ in where webs are positioned within the vegetation, which is driven by differences in web type and web structure (e.g., spacing of mesh, web size, height of web placement, and the sizes of prey captured) (Gómez et al., 2016). Orb-web spider species found at higher distance from the ground are normally larger and heavier than the lower ones (Henaut et al., 2006; Tahir et al., 2010). They may target larger prey types that can be captured at higher sites. The prey types captured were associated with the body size of the orb-web spiders, where larger spider tends to capture larger prey types (Richardson & Hanks, 2009). In this study, the PCA biplot on morphological characteristics indicate that the three Araneid spider species had distinct clustering, while *O. grata* overlaps with *G. kuhli* in their body sizes. There was no significant difference between *G. hasselti* and *O. grata* in body length. *G. hasselti* has longer body protruding from the side equipped with long spines while *O. grata* has a pyriform abdomen and strongly overhang the carapace. Absence of significant differences in wet weight and carapace width between *G. kuhli* and *O. grata* may have contributed to obvious overlap between the two spider species in the PCA biplot.

In order to coexist, orb-web spider species need to segregate in other niche dimensions such as food, time and microhabitat resources in a shared environment (Butt & Tahir, 2010). They need to avoid competition by specializing in specific niche spaces that do not overlap with those of other orb-web spider

species (Richardson & Hanks, 2009; Dzulhelmi et al., 2017). However, in this study, this does not appear to have been the case as indicated by both the PCA biplots, and the mean and standard deviations. In some of the web characteristics, there were many overlaps between the four orb-web spider species. For instance, *G. hasselti* with *G. kuhli* and *Araneus* sp. with *O. grata* constructed their webs at similar locations. *Araneus* sp., *G. hasselti* and *O. grata* were close in number of radii, while *Araneus* sp. and *G. kuhli* had about the same freezone-area and hub-area. The extent of the overlap in web characteristics among these orb-web spider species implies that prey availability is not a limiting factor in this particular habitat. Hence, two communities can share the same resource without competing with one another, and niche overlap may be higher (Uetz et al., 1978; Butt & Tahir, 2010). They would maintain their webs at that particular locations especially when there is enough available space to construct their webs with excessive food resources within reach and less competition (Dzulhelmi et al., 2018). However, when prey availability becomes a limiting factor, some orb-web spider species might exploit different food and occupy different space resources to reduce competition (Enders, 1974; Uetz et al., 1978; Butt & Tahir, 2010).

In contrast, the nonparametric 95% confidence intervals for web characteristics showed that there was a significant difference in the means for most orb-web spider species with little overlap. This indicates that while the spiders were building webs that overlapped in terms of size, shape and placement, many of the parameters that were generating the variation of web design in each species were significantly different from one another. It could be that those mean values represent an optimum web design for the natural habitat of the spiders, while the observed webs in the PCA were webs that were free of the resource constraints of the original habitat. These mean values suggest that webs of each species were designed for their natural niche constraints. The *Araneus* sp. constructed small webs at lower height, while *O. grata* constructed larger web but at almost similar height to *Araneus* sp. Lower height provided vegetation with suitable attachment points for smaller webs (McCravy & Hessler, 2012). Larger orb-web spider species tend to construct larger web-area, but do not necessarily construct webs at greater height (Tahir et al., 2012). Then again, *G. hasselti* and *G. kuhli* constructed their webs with comparable web-area and at greater height. This little overlap in either web-area or distance from the ground has been identified as contributor to resource partitioning and may reflect difference in use of space resources for *Micrathena gracilis* and *M. mitrata* (McCravy & Hessler, 2012). This demonstrates that the spiders may have evolved to avoid niche overlap in certain types of habitat. Anthropogenic disturbance caused by oil palm plantation may have caused the spiders to refine their web structures to suit the new environment, though a stable equilibrium point has yet been reached.

The shape, structure and sites in which webs are placed are in response to prey availability (Moore, 1977; Blackleadge et al., 2003), where orb-web spiders may relocate their webs in response to these prey types (Moore, 1977; McReynolds, 2000; Henaut et al., 2006). It is possible in an oil palm plantation there is an altered availability of prey compared to the spiders natural habitats, and deviation from evolutionarily optimized web designs are necessary to take advantage of these new conditions. However, web design may still be influenced by evolutionary trends. This study was not able to quantify the prey captured by these orb-web spider species, but understanding what prey the spiders consume in oil palm plantations may give better insights into the niche plasticity of these species as well as possible commercial benefits of using spiders for integrated pest management.

Conclusion

The results of this study showed obvious overlap of web characteristics despite the morphological differences in the four orb-web spider species. This study indicates that the four orb-web spider species were tolerant of their spatial territory and food resources in the oil palm plantation, suggesting that these were not a limiting factor. The naturally occurring spider in the oil palm plantation should also be further investigated and manipulated for insect pest management.

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Original article (Orijinal araştırma)

New application method for entomopathogenic nematode *Heterorhabditis bacteriophora* (Poinar, 1976) (Rhabditida: Heterorhabditidae) HBH strain against *Locusta migratoria* (Linnaeus, 1758) (Orthoptera: Acrididae)

Entomopatojen nematod *Heterorhabditis bacteriophora* (Poinar, 1976) (Rhabditida: Heterorhabditidae) HBH hibrit ırkının *Locusta migratoria* (Linnaeus, 1758) (Orthoptera: Acrididae)'ya karşı yeni bir uygulama yöntemi

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Abstract

Entomopathogenic nematodes (EPNs) of the families Heterorhabditidae and Steinernematidae are being used as biocontrol agents against many soil borne insect pests in agriculture. Above-ground applications against the insects are usually unsuccessful due to the lack of humidity. Therefore, EPNs rapidly lose their effectiveness. In this study, conducted in 2018 under laboratory conditions in Bursa-Turkey, a new application method was developed for the use of *Heterorhabditis bacteriophora* (Poinar, 1976) (Rhabditida: Heterorhabditidae) HBH hybrid strain against the migratory locust, *Locusta migratoria* (Linnaeus, 1758) (Orthoptera: Acrididae). A new trap system is coated with hydrophilic cotton fabric to provide the necessary humidity to allow the use of EPNs above-ground. Three different application rates of *H. bacteriophora* (5000, 25000 and 50000 IJs) were applied to the trap system. The fabric was inoculated with the nematodes and combined with a reservoir containing 200 ml of ringer solution. The dead and live nematodes were recorded periodically to determine their persistence on the fabric. The mortality of *L. migratoria* were also recorded to determine the infectivity of *H. bacteriophora*. The infectivity and persistence of the nematodes was sustained for more than 4 weeks by this method.

Keywords: Above-ground application, entomopathogenic nematodes, hydrophilic fabric, *Locusta migratoria*

Öz

Entomopatojen nematodlar (EPN) Heterorhabditidae ve Steinernematidae familyalarına bağlı olan, toprak altında yaşayan zararlı böceklere karşı kullanılan biyolojik mücadele etmenleridir. Toprak üzerine yapılan uygulamalar nemli ortam sağlanamaması nedeniyle başarısız olmaktadır. Bu nedenle toprak üzerinde EPN etkinliği çok kısa sürede yok olmaktadır. Bursa'da laboratuvar koşullarında 2018 yılında yapılan bu çalışmada, *Heterorhabditis bacteriophora* (Poinar, 1976) (Rhabditida: Heterorhabditidae) HBH hibrit ırkının uygulaması üzerine yeni bir teknik geliştirilmiş ve *Locusta migratoria* (Linnaeus, 1758) (Orthoptera: Acrididae) üzerindeki etkinliği incelenmiştir. Yeni geliştirilen tuzak sistemi hidrofil bir kumaş ile kaplanmış ve bu kumaş sayesinde tuzak yüzeyinin sürekli olarak nemli kalması sağlanmıştır. Tuzak sisteminde *H. bacteriophora*'nın üç farklı uygulama dozu (5000, 25000 ve 50000 IJs) kullanılmıştır. Tuzak yüzeyindeki hidrofil kumaş üzerinde belirli dozlardaki nematod solüsyonları uygulanmış ve tuzak sistemi 200 ml Ringer solüsyonu içeren rezervuar ile birleştirilerek kullanıma hazır hale getirilmiştir. Belirli aralıklarla ölü ve canlı EPN bireyleri sayılarak kumaş üzerindeki kalıcılık hesaplanmıştır. Buna ek olarak çekirgelerin ölüm oranları da periyodik olarak hesaplanmış ve tuzağın etkinliği belirlenmiştir. Çalışma sonucunda bu tuzak sistemi sayesinde bu nematodun kalıcılığının artırıldığı ve toprak üzerine yapılan uygulama ile etkinlikte başarı sağlandığı tespit edilmiştir.

Anahtar sözcükler: Toprak üstü uygulama, entomopatojen nematodlar, hidrofil kumaş, *Locusta migratoria*

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Introduction

Locusta migratoria (Linnaeus, 1758) (Orthoptera: Acrididae) is one of the most destructive agricultural insect pests worldwide (Zhang et al., 2009; Wang et al., 2014). It is a polyphagous pest (Showler, 1995) that can migrate over thousands of kilometers (Zhang et al., 2009). *Locusta migratoria* is distributed over a larger area than any another locust or grasshopper and can spread over the entire eastern hemisphere (Australia, Africa, Europe and Asia) (Zhang et al., 2009). Locust invasions destroyed nearly 8 Mha of agricultural plants in 12 of the 30 provinces of China between 1995 and 2001 (Ma et al., 2005). Given the difficulty of predicting locust outbreaks, the affected countries use pesticides excessively, which are toxic to the environment and non-target organisms (Vos et al., 2000; Rai & Chavhan, 2015). Development of environmentally-safe alternatives to locust control using biological agents including nematodes, protozoa, bacteria, fungi and viruses have been reported (Rai et al., 2013; Rai & Chavhan, 2015).

Entomopathogenic nematodes (EPNs) from the families Steinernematidae and Heterorhabditidae are used for biological control of insects instead of using insecticides (Cross et al., 1999; Lacey & Shapiro-Ilan, 2008) to reduce the negative impact on the environment (Lewis & Clarke, 2012; Shapiro-Ilan et al., 2014). They are mostly used against soil borne insect pests (Ehlers, 1996; Gaugler, 2002; Wright et al., 2005). Various formulations have been developed to use EPNs against above-ground pests, (Schroer & Ehlers, 2005; Georgis et al., 2006; Beck et al., 2013). Some important factors that make the above-ground application unsuccessful are temperature, ultraviolet radiation and humidity. Of these, humidity is the most restrictive factor for EPNs because current formulations do not provide a moist environment for sufficient time (Georgis et al., 2006; Lacey & Georgis, 2012). It has been shown that some additives can improve EPN persistence and effectiveness on foliage, but this enhancement have not been adequate (Baur et al., 1997; Schroer & Ehlers, 2005). Many laboratory studies have been conducted to test the persistence of EPNs on the foliage. The persistence of EPNs were usually hours rather than days (Schroer & Ehlers, 2005). Although remarkable improvements have been made for the above-ground application up to date, they are still not sufficiently efficacious to be recommended for commercial use (Grewal, 2002).

This study aimed to develop a method for above-ground application with the trap system using hydrophilic cotton fabric against the *L. migratoria*. For this purpose, the HBH hybrid strain of *Heterorhabditis bacteriophora* (Poinar, 1976) (Heterorhabditidae: Rhabditida) patented by Susurluk (TPMK Patent No: TR 2013 06141 B) was used against *L. migratoria* in the new trap system.

Material and Methods

Insect, entomopathogenic nematode and trap

The HBH hybrid strain of *H. bacteriophora* was selected for this study. The hybrid strain was obtained after hybridization of Turkish native *H. bacteriophora* isolates from different climatic regions of Turkey (including hot, cold, rainy and semi-arid). The HBH strain was patented due to its superior biological characters (high effectiveness, long persistence and high reproduction capacity). Under laboratory conditions, EPN populations were reproduced as infective juveniles (IJs) using *in vivo* methods according to Kaya & Stock (1997). The final instar of great wax moth, *Galleria mellonella* (Linnaeus, 1758) (Lepidoptera: Pyralidae), larva was used for EPN reproduction at 25ºC. The juveniles were extracted using the White trap method (White, 1927). Freshly-harvested 2-3 d-old IJs were used in the experiment. Commercially produced adult locusts, *Locusta migratoria* L. (Orthoptera: Acrididae), was purchased from a local store (Mira Limited Şti., Aksu, Antalya, Turkey) and divided into experimental groups before trap preparation. The trap consisted a hemispherical pot with a diameter of 10 cm and a surface area of 150 cm². The surface was covered with cotton fabric and no attractants were used for the locusts.

Experimental design

Ringer solution (200 ml) was placed into the hemispherical pots to provide a liquid reservoir for the nematodes. The fabric used in this study was a 100% cotton gauze bandage with about 40 threads per cm and was applied with folds to cover the entire water reservoir. After nematode inoculation of the fabric, it was combined with the reservoir and the pot was closed with a plastic lid (Figure 1). As evaporation occurred from the trap surface, the hydrophilic fabric absorbed liquid continuously from the reservoir.

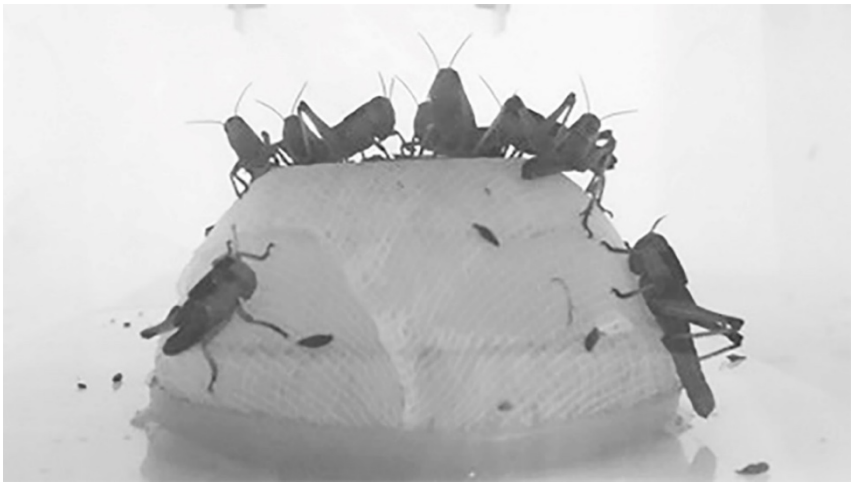

Figure 1. The trap system that contains the reservoir and fabric inoculated with the EPNs.

Three different doses of nematodes (5000, 25000 and 50000 IJs) were inoculated to three different traps and 10 adult *L. migratoria* transferred to each trap. The dead and alive locust adults were recorded 2 d after each locust placement. All locusts were removed from the trap after mortality was recorded. On days 15 and 30, a further 10 locusts were placed on the same traps. Thus, each trap had three cohorts of locusts that were transferred 1, 15 and 30 d after nematode inoculation. The timeline is shown in Figure 2. As a control, 10 locusts were transferred to the trap systems with no nematodes applied.

In addition to the effectiveness of *H. bacteriophora* HBH against the migratory locust, locust-free traps were used to detect persistence of the nematodes on the fabric. The mortality of the nematodes (persistence) in the traps was recorded 1, 15 and 30 d after inoculation. All experiments were repeated three times under laboratory conditions at 25ºC in the Nematology Laboratory in Department of Plant Protection, Faculty of Agriculture, Bursa Uludağ University, Turkey in 2018.

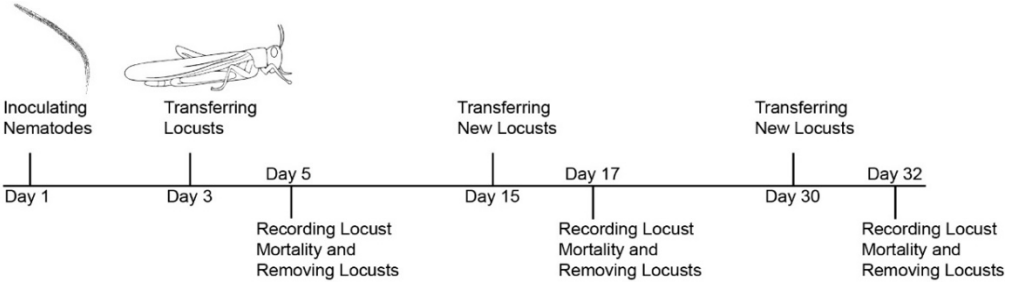

Figure 2. Trap experiment timeline.

Statistical analysis

Statistical differences of the mortality of EPNs were analyzed using one-way ANOVA with JMP®7.0 software. LSD test ($P < 0.05$) was used to determine the difference between means.

Results and Discussion

Infectivity of *Heterorhabditis bacteriophora* HBH against *Locusta migratoria*

The highest mortality of *L. migratoria* was recorded on day 3 of the experiment with 50000 IJs per trap. The effect of 25000 and 50000 IJs on day 17 was not statistically significant. On day 32, the highest mortality of *L. migratoria* was detected with 50000 IJs. On day 1, the effect of 5000 IJs was not statistically significant compared to 25000 IJs, but it was significantly lower than other doses on days 17 and 32 ($F = 35.3$, $df = 11; 24$, $P > 0.0001$).

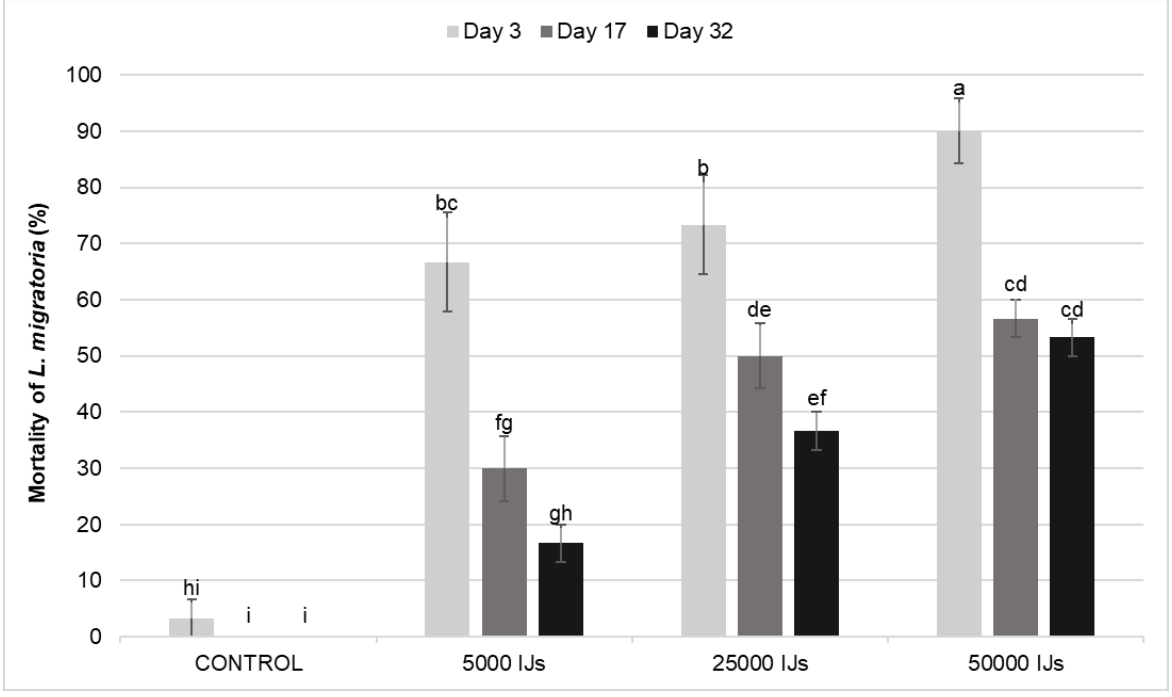

Figure 3. Mortality of *Locusta migratoria* in the doses of 5000, 25000 and 50000 IJs of *Heterorhabditis bacteriophora* HBH strain on days 3, 17 and 32 ($F = 35.3$; $df = 11, 24$; $P > 0.0001$).

Persistence of *Heterorhabditis bacteriophora* HBH on the traps

On day 1, the mortality (persistence) of IJs of *H. bacteriophora* HBH was not significantly different between inoculation rates. On day 15, the mortality of *H. bacteriophora* HBH with 25000 IJs dose was statistically higher than with 50000 IJs. On day 30, it was the opposite of this situation. The mortality of *H. bacteriophora* HBH with 5000 IJs was not significantly different from the other doses at any assessment time. The results of the persistence experiment are given in Figure 4 ($F = 66.7$; $df = 8, 18$; $P > 0.0001$). As expected, dead individuals increased over time. However, peak of the mortality was mostly below 40% of the population after 30 days, which meant that persistence of the nematode at the end of the experiment was nearly 60%. This result indicates that the new trap was effective persistence of the nematode over an extended period.

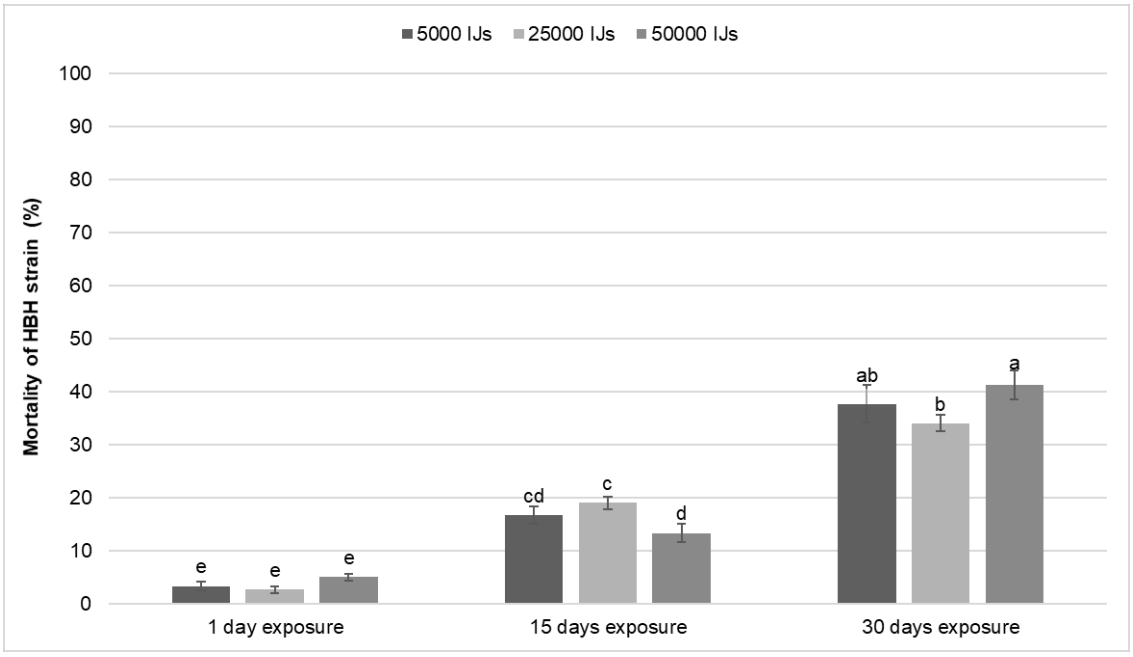

Figure 4. Mortality of *Heterorhabditis bacteriophora* HBH strain in the doses of 5000, 25000 and 50000 IJs per trap (F = 66.7; df = 8, 18; P > 0.0001).

Although there is a demand for foliar applications, EPNs are rarely used against above-ground insects because of the inability to achieve the desired success above-ground (Arthurs et al., 2004). For above-ground application, various formulations have been developed to protect EPNs from ultraviolet light and to prevent desiccation (Georgis et al., 2006; Beck et al., 2013). However, the effect of these formulations lasts only a few hours or days and the EPNs rapidly lose their effectiveness (Glazer, 2002; Arthurs et al., 2004; Schroer & Ehlers, 2005). The most critical feature of the trap system used in this experiment is the maintenance of humidity which allows it to provide a sustained effect of EPNs in above-ground application over an extended period.

In this study, the moist environment, which is necessary for EPNs, was maintained using hydrophilic cotton fabric. In several other studies, it was found that some additives usually enhanced the persistence and effectiveness of EPNs on foliage, but this improvement was insufficient (Baur et al., 1997; Schroer & Ehlers, 2005). Many laboratory studies have been conducted to test the persistence (mortality) of EPNs above ground. These demonstrated that persistence of EPNs lasted only for hours rather than days (Schroer & Ehlers, 2005). However, in the present study, the persistence of *H. bacteriophora* HBH strain extended for over 4 weeks. By day 30, the mortality of *H. bacteriophora* had reached about 40%, however, might be possible to improve persistence using better fabrics or having a larger fluid reservoir.

The method of storing and transporting EPNs in a polyether-polyurethane sponge-based formulation inspired for this work. EPNs require a thin layer of water for movement and cannot move effectively under waterlogged conditions (Grewal, 2002). The cotton fabric selected for this work provided a thin layer of water to facilitate nematode movement and also maintained a moist environment. In a study to improve the efficacy of entomopathogenic nematodes for above-ground application, Dito et al. (2016) used a protective gel covers that lasted for 8 h. In some other studies, *Steinernema feltiae* (Filipjev, 1934) was used weekly against damaging above-ground pests, *Thrips tabaci* Lindeman, 1889 and *Trialeurodes vaporariorum* Westwood 1856 (Trdan et al., 2007; Laznik et al., 2011; Beck et al., 2015).

Steinernema carpocapsae (Weiser, 1955) is considered to be more suitable for foliar application than *H. bacteriophora* because the former is more resistant to desiccation, inclined to stay in the upper soil layers

and has an ambusher host-finding strategy (Salame & Glazer, 2015). Although *H. bacteriophora* has a less active host-finding strategy and is prone to go deeper into the soil (Grewal et al., 1994), it is evident from the current study that *H. bacteriophora* can also be used for above-ground application using hydrophilic cotton fabric. Also, the chemical-physical nature of the soil may not be suitable for EPNs when they are applied inundatively (Kaya, 1990; Barbercheck, 1992; Koppenhöfer & Fuzy, 2006), so deployment of EPNs using this trap system would avoid this problem.

With the method developed in this study, *H. bacteriophora* HBH strain remained active for over 4 weeks on an above-ground surface and infected *L. migratoria*. For more realistic results, the trap system needs to be tested under field conditions, which is a more challenging environment then laboratory. Our trap system serves as a basic prototype for initial assessment, but could be modified using different fabrics, trap shapes, and different substances to protect EPNs or attract hosts. Through evaluating such modifications, application of EPNs with a trap system has a reasonable likelihood of becoming an effective option for field applications.

Conclusions

The trap system developed in this study enabled *H. bacteriophora* HBH strain to be successfully used against the *L. migratoria* above ground under laboratory conditions, with the infectivity and persistence were maintained for more than 4 weeks. In order to be successful in the field, a fabric must be selected that protects the EPNs from ultraviolet radiation and slows evaporation. In addition, enhancements such as pheromone or color could be combined with the fabric to attract target pests to the traps. Through this approach, more economical control might be achieved without the need to apply the EPNs over a large area. The findings presented here indicate that this method could be a solution to rapid desiccation of EPNs applied above ground, if confirmed by field studies.

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Original article (Orijinal araştırma)

Response of tomato plants carrying *Mi-1* gene to *Meloidogyne incognita* (Kofoid & White, 1919) Chitwood, 1949 under high soil temperatures[1]

Yüksek toprak sıcaklıklarında *Mi-1* genini taşıyan domates bitkilerinin *Meloidogyne incognita* (Kofoid & White, 1919) Chitwood, 1949'a tepkisi

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Abstract

The *Mi-1* gene conferring resistance to root-knot nematodes in tomato breaks down at soil temperatures above 28ºC. To understand this phenomenon, the reactions of susceptible and resistant tomatoes to *Meloidogyne incognita* (Kofoid & White, 1919) Chitwood, 1949 were separately investigated under four soil temperatures, 25, 28, 30 and 32ºC, and at six time periods, 6, 12, 24, 48, 120 and 168 h. The study was conducted between 2015 and 2016 in growth chambers. In the first experiment, the plants were separately exposed to soil temperatures for the same six periods before nematode inoculation and then transferred to a growth chamber with 25ºC. Reproduction factor (Rf) for nematode on resistant plants was <1, while the Rf for susceptible plants was >1. Results indicated that the resistance provided by *Mi-1* persisted under all soil temperatures. In the second experiment, the seedlings were simultaneously inoculated with *M. incognita* when soil temperatures reached 25, 28, 30 and 32ºC, and held in soil temperatures for the same six periods, then transferred to a growth chamber with 25ºC soil temperature. Rf in heterozygous resistant plants exposed to 32ºC soil temperature for ≥48 h was >1. This study indicated that the resistance in plants held at 32ºC soil temperature for ≥48 h lost its effect.

Keywords: Duration, *Mi-1* gene, resistance, root-knot nematodes, soil temperature, tomato

Öz

Domateste kök ur nematodlarına karşı dayanıklılık sağlayan *Mi-1* geni 28ºC'nin üzerindeki toprak sıcaklılarında kırılmaktadır. Bu durumu anlamak için *Meloidogyne inco*gnita (Kofoid & White, 1919) Chitwood, 1949' ya hassas ve dayanıklı bitkiler, dört toprak sıcaklığında (25, 28, 30 ve 32ºC) ve 6 sürede (6, 12, 24, 48, 120 ve 168 saat) ayrı ayrı incelenmiştir. Bu çalışma 2015 ve 2016 yılları arasında iklim odalarında yürütülmüştür. İlk denemede bitkiler nematod inokulasyonundan önce aynı altı zaman periyodu için 25, 28, 30 ve 32ºC toprak sıcaklıklarına maruz bırakılmış, daha sonra 25ºC'deki iklim odasına aktarılmıştır. Nematodların üreme faktörü (Rf) dayanıklı bitkilerde 1'den büyük hassas bitkilerde ise 1'den küçük bulunmuştur. İlk denemenin sonucu, *Mi-1* tarafından sağlanan dayanıklılığın belirtilen toprak sıcaklıklarında kırılmadığını göstermiştir. İkinci denemede toprak sıcaklıkları 25ºC, 28ºC, 30ºC ve 32ºC'ye ulaştığında eş zamanlı olarak *M. incognita* inokulasyonu yapılmış ve adı geçen sürelerde toprak sıcaklığına maruz bırakılmıştır. Daha sonra bitkiler 25ºC toprak sıcaklığına sahip iklim odasına aktarılmıştır. 32ºC toprak sıcaklığına, 48 saat ve üzerinde maruz bırakılan heterozigot dayanıklı bitkilerde Rf değeri >1 olarak bulunmuştur. Bu sonuçlar 32ºC toprak sıcaklığına, 48 saat ve üzeri maruz bırakılan bitkilerdeki dayanıklılığın etkisini yitirdiğini göstermiştir.

Anahtar sözcükler: Süre, *Mi-1* geni, dayanıklılık, kök-ur nematodları, toprak sıcaklığı, domates

[1] This study represents first author's master thesis.

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Introduction

Tomato is a widely grown vegetable with an annual worldwide yield of about 173 million ton/year (FAO, 2014). It is also a major dietary source of lycopene, which reduces the risk of developing heart disease and cancer (Clinton et al., 1996; Arab & Steck, 2000). As with other crop plants, many pests and pathogens attack cultivated tomatoes, damaging both quality and quantity of production. Root-knot nematodes (RKN), *Meloidogyne* spp., are considered a major pest in tomato-growing areas. They feed and develop on plant roots, resulting in the formation of galls or knots, which cause reduced water and nutrients uptake (Duncan & Noling, 1998). As a result, plants may exhibit unspecific symptoms, which can be confused with water and nutrient deficiency (Duncan & Noling, 1998). In addition, plants can become more susceptible to fungal and bacterial diseases (Taylor & Sasser, 1978; Siddiqui et al., 2014; Al-Hazmi & Al-Nadary, 2015; Lobna et al., 2016).

Management of RKN is difficult due to their polyphagous nature (Siddiqi, 2000), reproduction capacity (Moens et al., 2009), being soilborne pathogens (Starr et al., 1989; Manzanilla-López & Starr, 2009) having a wide range of host (Hussey, 1985). Pesticides, resistant cultivars and rootstocks are commonly used to manage RKN (Devran et al., 2010). Nematicides have been widely used to RKN control. However, the use of some nematicides has been restricted owing to human health and environmental concerns (Devran et al., 2008; Moens et al., 2009; Devran et al., 2013). Therefore, growing resistant cultivars is considered an alternative and environmentally-friendly strategy to manage RKN (Devran et al., 2013). In tomatoes, *Mi-1* controls resistance to RKN. This gene was introgressed into cultivated tomato from wild tomato *Solanum peruvianum* L. (Solanaceae) in the 1940s (Smith, 1944). It confers resistance against three species of RKN: *Meloidogyne incognita* (Kofoid & White, 1919) Chitwood, 1949, *Meloidogyne javanica* (Treub, 1885) and *Meloidogyne arenaria* (Neal, 1889) Chitwood, 1949 (Roberts & Thomason, 1986). *Mi-1* gene-mediated resistance is characterized by a localized hypersensitive response to the attempt of a nematode to initiate a feeding site in root cells (Dropkin, 1969a). This gene has been successfully incorporated into many commercially available tomato cultivars and is currently the only source of RKN resistance in commercial tomatoes (Devran et al., 2010; Seid et al., 2015). This gene also confers resistance to the aphid *Macrosiphum euphorbiae* (Thomas, 1878) (Homoptera: Aphididae) and *Bemisia tabaci* (Gennadius, 1889) (Hemiptera: Aleyrodidae) biotypes Q and B (Rossi et al., 1998; Nombela et al., 2003). However, the resistance conferred by *Mi-1* gene has limited. The gene loses its effectiveness at soil temperatures of above 28ºC (Dropkin, 1969b). However, there are discrepancies in information about the recovery and the duration of resistance breakdown provided by *Mi-1* gene at high soil temperatures. Dropkin (1969b) reported that the phenotypic expression of resistance provided by the gene changed at temperatures above 28ºC. Araujo et al. (1982a) observed numerous egg masses on roots of plants incubated for 3 d at 25ºC, then moved to 32.5ºC for 27 d in their reciprocal experiment. In contrast, Haroon et al. (1993) reported that there were no galls on in vitro root explant tissues of resistant tomatoes maintained at 28ºC or 30ºC for 10 d; however, galls were present on roots at 33ºC and increased at 37 and 40ºC. Similarly, when in vitro root explants without *Mi-1* gene and resistant genotypes carrying *Mi-1* gene were inoculated with *M. incognita* and *M. arenaria* at 28, 31, 34 and 37ºC, heterozygous and homozygous genotypes were equally resistant to both RKN species and genotypes lacking *Mi-1* gene were susceptible to all temperatures. In addition, the resistance level was maintained fully at 31ºC, maintained partially at 34ºC and lost at 37ºC (Abdul-Baki et al., 1996). In several studies, tomato plants with the *Mi-1* gene were inoculated with *M. incognita* after heat treatment at five temperatures between 27 and 38ºC. Consequently, the seedlings showed increased susceptibility to *M. incognita* above 30ºC with the maximum reached at 34ºC (Zacheo et al., 1995). Carvalho et al. (2015) reported that for plants exposed to ambient temperatures of 35ºC for 3 h daily (midday), resistance level could be recovered by maintaining them in a low temperature for 6 d. Unlike under controlled conditions, when the soil temperature reached above 28ºC under greenhouse, *Mi-1*-mediated resistance reduced greatly (Cortada et al., 2008).

Although the effect of temperatures on RKN resistance under different temperatures has been extensively studied, the results remain inconclusive. Unlike many previous studies, in this study, the soil temperature was constantly monitored with a probe, and the inoculation time and heat exposure periods recorded. This study examined the reactions of (a) tomatoes bearing *Mi-1* gene to *M. incognita* at high soil temperatures, and (b) tomatoes cultivars with or lacking the *Mi-1* gene to *M. incognita* exposed to soil temperatures of 25, 28, 30 and 32ºC for 6, 12, 24, 48, 120 and 168 h. The purpose was to determine the time of resistance break down in heterozygous and homozygous tomato cultivars at the *Mi* locus exposed to high soil temperatures and then simultaneously inoculated with *M. incognita*.

Material and Methods

The study was conducted at Plant Protection Department, Faculty of Agriculture Akdeniz University, Antalya, Turkey between 2015 and 2016.

Plant material

The homozygous tomato cv. Tueza F1 without the *Mi-1* gene (*mimi*), the heterozygous cv. Seval F1 at the *Mi-1* locus (*Mimi*) and the homozygous cv. Browny F1 at the *Mi-1* locus (*MiMi*) were used in the bioassay. Tomato seedlings were provided by Multi Tohum Tar. San Tic. A.Ş. (Antalya, Turkey). Individual tomato seedlings were transferred to 250-mL pots including sterilized sandy soil.

Nematode isolate

The S6 isolate of *M. incognita* race 2 was used in the experiments. This isolate was previously described and characterized (Devran & Söğüt, 2009, 2011). The isolate was maintained in the susceptible tomato cv. Tueza F1. Tomato plants were inoculated with 1000 J2 (Devran et al., 2010; Devran & Söğüt, 2014; Mıstanoğlu et al., 2016) and maintained in a growth chamber at 25±0.5ºC with 16:8 h L:D photoperiod and 65% RH. Eight weeks after nematode inoculation the tomato plants were uprooted and the roots were washed free of soil. Then, the egg masses were handpicked and incubated in a petri dish at room temperate. The juveniles (J2) were collected at first 24 h, counted and inoculated in new tomato plants in the same day or stored in refrigerator at 4ºC for 2 d, until inoculation.

DNA isolation

Plant genomic DNA and nematode DNA were isolated according to previous studies (Devran & Söğüt, 2009; Devran et al., 2013).

PCR amplification

The S6 isolate of *M. incognita* race 2 was verified the use of Inc14F/Inc14R primers (Randig, 2002). DNA of *M. arenaria* (K18) and *M. javanica* (AKS2) isolates was included as controls. The K18 and AKS2 isolates were identified and characterized in previous studies (Devran & Söğüt, 2009, 2011). The presence of *Mi-1* gene in tomato plants was verified using the *Mi23* marker (Seah et al., 2007). All PCR reactions were performed according to previous studies (Devran & Söğüt, 2009, 2014; Devran et al., 2013).

Effect of high temperatures on tomato resistance against *M. incognita*

Two different experimental designs were used, a) plants heat treated before nematode inoculation, and b) plants heat treated and inoculated simultaneously. Soil temperature in pots was continuously monitored with a probe and registered.

Experiment 1: Tomato seedlings (five replicates/treatment) with four true leaves individually exposed to soil temperatures of 25, 28, 30 and 32ºC for 6, 12, 24, 48, 120 and 168 h (Figure 1a). Then, the plants were transferred to a growth chamber at 25ºC with 16:8 h L:D photoperiod and 65% RH. When the soil temperature reached 25ºC, tomato seedlings were inoculated with 1000 freshly hatched *M. incognita* J2s (Figure 1b). The experiment was set up, performed and then repeated.

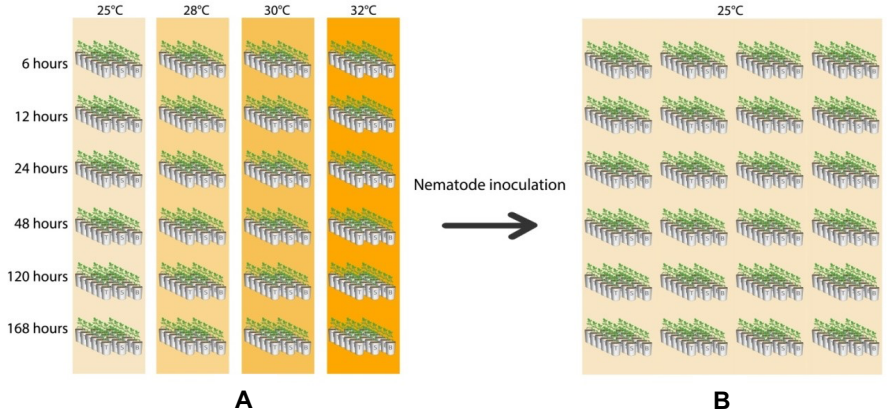

Figure 1. Design of the experiments to study the effect of high temperatures on tomato resistance. A) Tomato seedlings were individually exposed to four soil temperatures for different time periods, before inoculation with *Meloidogyne incognita* second-stage juveniles (J2); B) Tomato seedlings were exposed to different soil temperatures, inoculated with J2 and maintained for different time periods at 25 ºC.

Experiment 2: The tomato plants (five replicates/treatment) were exposed to soil temperatures of 25, 28, 30 and 32ºC for 6, 12, 24, 48, 120 and 168 h. When soil reached the designated temperature, plants were inoculated with 1000 freshly hatched (<24 h) *M. incognita* J2s (Figure 2a). The plants were held in soil temperatures for the same six periods and then transferred to a growth chamber at 25ºC, as referred before, until the end of the experiment (Figure 2b). The experiment was designed, conducted and then repeated.

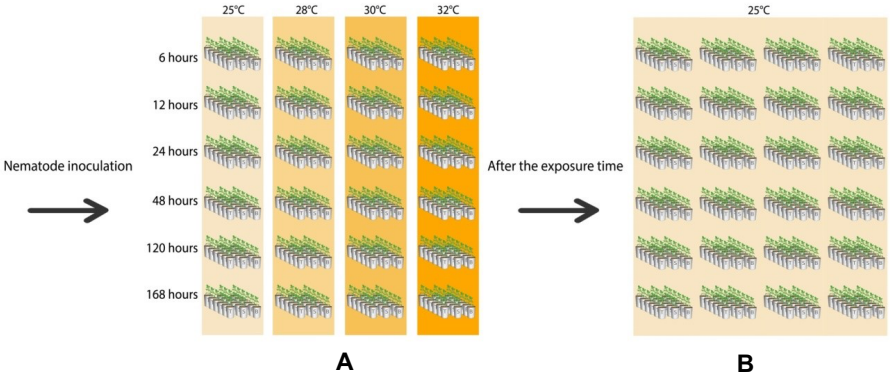

Figure 2. Design of the experiments to study the effect of high temperatures on tomato resistance A) The tomato seedlings separately exposed to four soil temperatures for different time periods and inoculated with *Meloidogyne incognita* second-stage juveniles; B) Plants transferred to a growth chamber with 25ºC.

Sixty day after inoculation the plants were evaluated. The number of galls and egg masses per root system was recorded in all experiments. Galls and egg masses were counted under a stereomicroscope. J2 were recovered from the soil of each pot (100 g soil/pot) using a modified Baermann funnel technique within 2 d (Hooper, 1986). Reproduction factor (RF; i.e., final J2 population density/initial nematode population, 1000 J2s) was calculated (Ferris, 1985). The data (number of egg masses, galls and J2s) data were log transformed [log10(x+1)] and analyzed by ANOVA. The significant differences within treatments were tested using LSD. The statistical analysis was performed according to SAS program (v. 9.0 for Windows; SAS Institute Inc., Cary, NC, USA).

Results and Discussion

Confirmation of nematode isolate identification and detection of *Mi-1* gene

The *M. incognita* isolate S6 identification was confirmed by PCR using species-specific primers (Inc14F/Inc14R), which produced an amplicon of about 400 bp, as expected (Figure 3a).

The *Mi-1* gene was verified using the *Mi23* marker. This marker allows the amplification of 430-bp and 380-bp fragments for tomato cultivars without the *Mi-1* gene (*mimi*) and homozygous tomato cultivars at the *Mi* locus (*MiMi*), respectively. Heterozygous tomato cultivars (*Mimi*) presented the two fragments, as expected (Figure 3b).

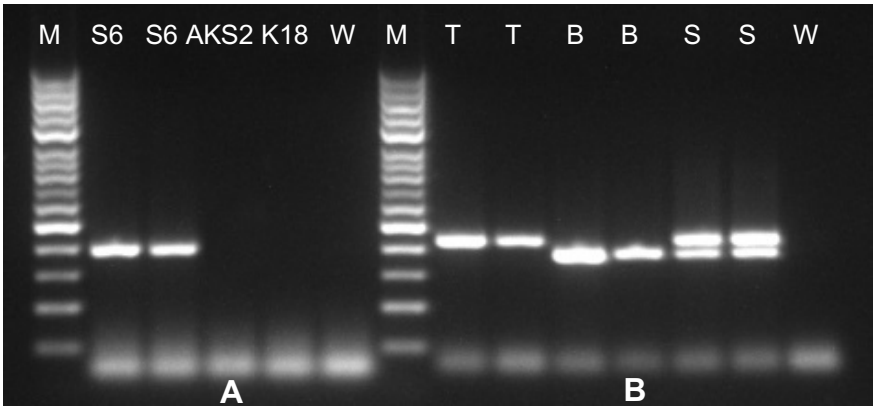

Figure 3. A) Amplified DNA of *Meloidogyne incognita* using primers Inc14F/Inc14R and B) PCR products of tomato cultivars using primers Mi23F/R. M: Marker (100 bp DNA Ladder, GeneAll, Seoul, Korea), S6: *M. incognita*, AKS2: *M. javanica*, K18: *M. arenaria*, T: Tueza F1 (*mimi*), B: Browny F1 (*MiMi*), S: Seval F1 (*Mimi*), W: Negative control.

Effect of high temperatures on tomato resistance

Experiment 1: At the end of the experiment, the number of J2s, egg masses and galls was evaluated for all plants (Table 1). The data indicated the resistance conferred by *Mi-1* persisted at the soil temperatures tested. Rf values of *M. incognita* on tomato plants (*mimi*) was >1 while on tomato cultivars (*MiMi* or *Mimi*) were <1, as expected (Table 1). Rf value of plants exposed to heat treatment at 32ºC is shown in Table 1.

Experiment 2: The tomato seedlings exposed to soil temperatures for the six time periods at the time of inoculation and parameters were evaluated (Table 2). The data showed marginal increases in the number of galls and egg masses on the roots of tomato plants (*MiMi*) held at 30ºC soil temperature for >120 h (Table 2). However, resistance did not break down and Rf values of *M. incognita* on homozygous (*MiMi*) and heterozygous (*Mimi*) tomato plants were <1 (Table 2). The number of egg masses on the roots of cv. Browny F1 (*MiMi*) held at 32ºC did not change at 6 and 12 h but increased to statistical significance at 24 h and markedly increased >48 h. The Rf of *M. incognita* on tomato seedlings (*Mimi*) at 32ºC for ≥48 h was >1. However, the results also demonstrated that the Rf of nematodes on tomato plants (*MiMi*) exposed to 32ºC soil temperature for 120 and 168 h were >1 (Table 2). So, the present study demonstrates that *Mi-1* gene-mediated resistance in tomato breaks down in plants held at 32ºC for ≥48 h.

Table 1. Number of galls, egg masses and Rf of *Meloidogyne incognita* on tomato cultivars exposed to different temperatures before inoculation with 1000 second-stage juveniles

Soil temperature	Parameters	Tomato cultivars*	Hours of exposure					
			6	12	24	48	120	168
25ºC	Gall	Tueza F1	148.4 a**	127.8 a	158.0 a	136.6 a	168.2 a	95.0 a
		Seval F1	4.8 b	0.4 e	0.8 de	2.6 bcd	2.0 bcde	3.4 bc
		Browny F1	0.4 e	3.6 b	2.0 cde	1.6 bcde	1.6 cde	2.0 cde
	Egg Mass	Tueza F1	83.0 a	44.4 b	65.2 a	70.4 a	77.8 a	61.8 a
		Seval F1	0.6 c	0.0 d	0.2 cd	0.4 cd	0.0 d	0.2 cd
		Browny F1	0.0 d	0.2 cd	0.2 cd	0.0 d	0.0 d	0.0 d
28ºC	Gall	Tueza F1	122.4 a	127.0 a	87.4 b	111.2 a	81.0 ab	89.6 ab
		Seval F1	1.2 de	4.8 c	3.4 cd	0.0 e	1.4 de	2.2 de
		Browny F1	0.2 e	0.4 e	0.0 e	0.6 e	0.0 e	0.0 e
	Egg Mass	Tueza F1	76.0 a	65.8 ab	50.0 c	58.6 ab	40.2 bc	40.8 abc
		Seval F1	0.2 de	0.6 de	0.4 de	1.6 d	0.0 e	0.2 de
		Browny F1	0.2 de	0.0 e	0.0 e	0.0 e	0.0 e	0.0 e
30ºC	Gall	Tueza F1	149.2 a	89.2 ab	106.4 ab	86.0 ab	66.2 b	124 a
		Seval F1	2.0 de	1.8 de	5.6 c	4.0 cd	1.8 de	1.4 de
		Browny F1	0.2 e	1.2 de	1.4 e	0.6 e	1.2 e	1.0 e
	Egg Mass	Tueza F1	46.6 a	58 a	52.4 a	48.8 a	14.6 b	74.4 a
		Seval F1	0.2 c	0.4 c	0.2 c	0.4 c	0.4 c	0.8 c
		Browny F1	0.2 c	0.6 c	0.4 c	0.0 c	0.0 c	0.4 c
32ºC	Gall	Tueza F1	81.8 a	124.8 a	98.6 a	126.2 a	125.4 a	79.5 a
		Seval F1	0.6 de	4.6 b	4.4 b	3.2 bc	0.0 e	1.2 cde
		Browny F1	1.2 cd	0.4 de	0.6 de	1.2 cd	0.4 de	0.0 e
	Egg Mass	Tueza F1	42.8 a	48.2 a	50.4 a	54.2 a	57.4 a	54.5 a
		Seval F1	0.0 b	0.0 b	0.0 b	0.2 b	0.4 b	0.0 b
		Browny F1	0.4 b	0.0 b	0.0 b	1.0 b	0.2 b	0.0 b
	Rf	Tueza F1	2.63 c	3.90 b	4.12 b	1.12 d	5.08 b	7.91 a
		Seval F1	0.004 e	0.012 e	0.0 e	0.0 e	0.04 e	0.004 e
		Browny F1	0.0 e	0.004 e	0.008 e	0.008 e	0.0 e	0.0 e

* Tueza F_1: without *Mi* gene (*mimi*), Seval F_1: heterozygous (*Mimi*), Browny F_1: homozygous carrying the Mi gene (*MiMi*).

** Means with in the temperature sharing the same letter are not significantly different from each other at $P = 0.05$ according to the LSD. Untransformed data shown, statistical analysis was performed on log(x+1) transformed data.

Table 2. Number of galls, egg masses and Rf of *Meloidogyne incognita* on tomato cultivars exposed to different temperatures after inoculation with 1000 second-stage juveniles

Soil temperature	Parameters	Tomato cultivars*	Hours of exposure					
			6	12	24	48	120	168
25ºC**	Gall	Tueza F1	-	-	-	-	-	168.2 a
		Seval F1	-	-	-	-	-	2.0 b
		Browny F1	-	-	-	-	-	0.0 c
	Egg Mass	Tueza F1	-	-	-	-	-	84.4 a
		Seval F1	-	-	-	-	-	0.0 b
		Browny F1	-	-	-	-	-	0.0 b
28ºC	Gall	Tueza F1	81.0 a***	66.2 a	71.0 a	75.5 a	69.5 a	63.2 a
		Seval F1	3.2 bcd	1.0 ef	3.0 bcd	4.5 bc	2.5 cde	5.0 b
		Browny F1	0.0 f	1.5 cde	0.7 ef	1.0 ef	2.0 cde	1.2 de
	Egg Mass	Tueza F1	54.2 a	47.5 a	62.7 a	57.5 a	51.5 a	45.5 a
		Seval F1	0.7 b	0.5 bc	0.0 c	1.0 bc	0.0 c	0.5 bc
		Browny F1	0.0 c	0.2 bc	0.0 c	0.2 bc	0.0 c	0.5 bc
30ºC	Gall	Tueza F1	158.6 a	138.6 a	153.2 a	157.0 a	138.2 a	126.0 a
		Seval F1	3.0 bcde	3.6 bcd	1.4 defg	1.2 efgh	4.6 bc	4.0 bc
		Browny F1	1.0 fgh	0.4 gh	0.6 gh	0.2 h	5.2 b	2.0 cdef
	Egg Mass	Tueza F1	89.2 a	77.4 a	74.4 a	78.4 a	78.6 a	58.2 a
		Seval F1	0.6 de	0.2 e	0.2 e	0.2 e	1.6 cd	2.8 c
		Browny F1	0.0 e	0.0 e	0.0 e	0.0 e	4.8 b	0.6 de
32ºC	Gall	Tueza F1	108.2 a	129.8 a	131.2 a	132.8 a	94 ab	101.2 a
		Seval F1	0.8 hı	1.0 hı	4.0 g	17.4 f	56.2 bc	37.6 cd
		Browny F1	1.4 hı	0.8 ı	2.0 h	18.0 ef	39.4 dc	26.6 de
	Egg Mass	Tueza F1	71.2 a	109.4 a	73.6 ab	88.4 a	35.8 d	63.4 abc
		Seval F1	0.4 h	0.4 h	2.4 g	16.2 f	33.2 de	41.4 bcd
		Browny F1	0.2 h	0.2 h	1.2 h	19.8 ef	39.6 cd	22.2 def
	Rf	Tueza F1	12.75 abc	27.49 a	15.51 ab	9.27 bcd	5.64 cde	7.59 bcd
		Seval F1	0.07 ij	0.39 hij	0.33 hij	2.78 efg	3.86 def	4.72 def
		Browny F1	0.004 j	0.01 ij	0.07 ij	0.86 ghij	1.52 fgh	1.05 ghi

* Tueza F_1: without *Mi* gene (*mimi*), Seval F_1: heterozygous (*Mimi),* Browny F_1: homozygous carrying the Mi gene (*MiMi).*
** The experiment was continuously conducted at 25 ºC. Only one data was given in a column (168 hours).
*** Means with in the temperature sharing the same letter are not significantly different from each other at $P = 0.05$ according to the LSD. Untransformed data shown, statistical analysis was performed on log(x+1) transformed data.

It has been shown that some accessions of *S. peruvianum* are resistant to some RKNs at high soil temperature (Ammati et al., 1986). However, these genes are not commercially available for tomato cultivation. Therefore, *Mi-1* gene is the only commercially available resistance gene against some RKN and is widely used to RKN management in tomato-growing areas (Devran et al., 2010; Seid et al., 2015). However, this gene breaks down at high soil temperatures. Therefore, the effective utilization of the gene is restricted in tomato-growing areas.

Validation of *M. incognita* isolate and presence/absence of *Mi-1* gene on tomato cultivars is required before experiments. In the present study, this validation was performed using specific primers and our findings were in accordance with earlier studies (Randig et al., 2002; Devran & Söğüt, 2009, 2014; Devran et al., 2013).

Several studies have been conducted on the breakdown of *Mi-1* gene at high soil temperatures; however, results indicate a discrepancy in information about the duration of resistance breakdown at high soil temperatures. In this study, two experiments were conducted to clarify this phenomenon. In the first experiment, when plants were exposed to soil temperatures for different time periods and inoculated with *M. incognita* J2 at 25ºC, the resistance provided by *Mi-1* gene did not break down. Therefore, the resistance did not break down in plants exposed to high soil temperatures before nematode inoculation. Conversely, in other previous study, seedlings were exposed to high soil temperatures before nematode inoculation and then infected with *M. incognita*. Results indicated that the seedlings became increasingly susceptible to *M. incognita* above 30ºC and were completely susceptible at 34ºC (Zacheo et al., 1995). The difference may be due to *M. incognita* inoculum level. Araujo et al. (1982b) reported that temperature and inoculum level produced quantitative differences in resistance for both species of *Meloidogyne* with 28 d of incubation. In another study, Carvalho et al. (2015) reported that for plants exposed to 35ºC for 3 h daily (midday), resistance level could be recovered by maintaining them in a low temperature for 6 d. However, other studies reported that resistance could not be recovered (Araujo et al., 1982a; Abdul-Baki et al., 1996). This study revealed that resistance did not break down in plants exposed to high soil temperatures before nematode inoculation. This study showed that the resistance broke down in plants held at 32ºC soil temperature for ≥48 h, which is consistent with Dropkin (1969b). Although susceptible and resistant seedlings were inoculated with 20, 100 and 200 *M. incognita* J2 at 32.5ºC soil temperature, there were fewer egg masses on resistant plant roots than on susceptible plant roots. However, when plants were inoculated with 1000 and 2000 *M. incognita* J2, both plants showed comparable number of egg masses (Araujo et al., 1982b). Our results showed that egg masses in resistant tomato seedlings (*MiMi*) inoculated with 1000 *M. incognita* J2 were fewer than in susceptible plants (*mimi*). In another study, Abdul-Baki et al. (1996) reported that the resistance level in in vitro root explants was fully maintained at 31ºC, partially maintained at 34ºC and lost at 37ºC.

Some studies have also been conducted on the effect of temperatures on plants carrying *Mi-1* gene under greenhouse conditions. Cortada et al. (2008) reported that resistance conferred by *Mi-1* gene reduced greatly in soil temperature above 28ºC under greenhouse conditions in summer. The results showed differences on performance of plants carrying *Mi-1* gene under greenhouse conditions.

In conclusion, the resistance provided by *Mi-1* gene in tomato breaks down at soil temperatures above 28ºC in tomato-growing areas in many parts of the world. Therefore, planting time is important for tomato plants carrying *Mi-1* gene. These results showed that resistance did not break down in plants exposed to high soil temperatures before nematode inoculation. Therefore, the *Mi-1* gene-mediated resistance in tomato does not break down in seedling facilities, which are not usually infested with RKN, if the soil temperature in nursery is above 28ºC. In addition, results showed resistance break down in nematode-inoculated plants held at 32ºC for ≥48 h. The findings could help in the effective use of *Mi-1* gene and indicate the appropriate planting times of tomatoes bearing *Mi-1* gene for cultivation in fields prone to high soil temperatures (≥32ºC).

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