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Orijinal araştırma (Original article)

Effects of some plant extracts on root-knot nematodes *in vitro* and *in vivo* conditions[1]

Bazı bitki ekstraktlarının kök-ur nematodlarına karşı etkinliğinin *in vitro* ve *in vivo* koşullarda araştırılması

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Summary

One of the major pests of the vegetables, root knot nematodes (*Meloidogyne* spp.) (Tylenchina: Meloidogynidae) (RKNs) can cause economic losses by forming knots on the host plant roots. RKNs are more prevalent in the greenhouse vegetable growing areas of the coastal regions. In this study, the effects of plant extracts from five different plants; *Capsicum frutescens*, *Hyoscyamus niger* (Solanaceae), *Melia azedarach* (Meliaceae), *Xanthium strumarium* and *Achillea wilhelmsii* (Asteraceae) were evaluated against RKNs. In the first studies; the effects of plant extracts (0.5, 1.0, 1.5, 3.0, 6.0 and 12.0% concentrations) on eggs, egg masses and second stage juveniles (J2s) of the *Meloidogyne incognita* were evaluated in laboratory conditions (*in vitro* tests). Concentrations of 3.0, 6.0 and 12.0% for *H. niger*, *X. strumarium* and *M. azedarach* caused 100% inhibition of egg hatching and J2s mortality. Effects of the same plant extracts against *M. javanica* on egg mass of nematode (I), the plant height (II), the plant age (III) plant dry weight (IV), root fresh weight (V) and root dry weight (VI) were also evaluated in greenhouse-pot studies. Result of pot trials, 12.0% of *H. niger* and *X. strumarium* has shown high effect on hatching studies. In the toxicity studies, 6.0% and 12.0% of *X. strumarium* and *M. azedarach* were found effective.Three plant extracts (*X. strumarium, H. niger* and *M. azedarach*), against mixed populations of *M. incognita* and *M. javanica* on tomatoes under natural greenhouse conditions (*in vivo*) were evaluated. Treatmets were repeated 4 times by watering for each pot (extract were applied 1 ml plant^{-1}). In greenhouse trials, root gal indicies (root knot) and crop yield (tomatoes) (kg plant^{-1}) values and effect of root gal indicies and yield (%) were evaluated. *In vivo* studies, it could be concluded that, only the *M. azedarach* has effected the galling indicies and consequentelly crop yield significantly.

Keywords: Root knot nematode, *Meloidogyne incognita*, *M. javanica,* plant extracts, tomato

Özet

Sebzelerin önemli zararlılarından biri olan ve bitki köklerinde urlar meydana getirerek ekonomik bağlamda ürün kayıplarına neden olan kök-ur nematodları (*Meloidogyne* spp.) (Tylenchina: Meloidogynidae) (KUN) kıyı bölgelerimiz başta olmak üzere örtü altı sebze yetiştiriciliği yapılan tüm alanlarda yaygın olarak görülmektedir. Bu çalışmada, *Capsicum frutescens*, *Hyoscyamus niger* (Solanaceae), *Melia azedarach* (Meliaceae), *Xanthium strumarium* ve *Achillea wilhelmsii* (Asteraceae) bitkilerinden elde edilen bitkisel ekstraktların KUN'lere karşı etkileri araştırılmıştır. İlk çalışmalar laboratuvar-petri (*in vitro*) denemleri olarak yürütülmüştür. Denemlerde *Meloidogyne incognita* kültüründen elde edilen yumurta, yumurta paketleri ve 2. dönem larvalar (L2)'ına karşı ekstraktlar 6 farklı konsantrasyonda (0.5, 1.0, 1.5, 3.0, 6.0 ve %12.0) kullanılmıştır. *H. niger*, *X. strumarium* ve *M. azedarach'nın* 3.0, 6.0 ve %12.0 konsantrasyonları yumurta açılımına ve L2 (larvaya toksisite)'ye %100 etkili bulunmuştur. Diğer çalışmalar olan sera-saksı denemelerinde *M. javanica* kullanılmış; her bir bitki köküdeki yumurta paketi sayısı (I), bitkinin boyu (II), bitkinin yaş (III) ve kuru ağırlığı (IV), kök yaş (V) ve kuru ağırlığı (VI) parametreleri değerlendirilmiştir. Sera-saksı denemelerinden elde edilen sonuçlarına göre; kök-ur nematodu yumurta açılımına etkisi çalışmalarında, *H. niger* ve *X. strumarium*'un %12'lik konsantrasyonu yüksek etki göstermiştir. Nematod larvalarına karşı olan toksisite çalışmalarında ise *X. strumarium* ve *M. azedarach*'ın %6.0 ve 12.0'lik konsantrasyonları etkili bulunmuştur. Son çalışmalar doğa-sera çalışmaları (*in vivo*) olarak yürütülmüştür. Üç bitki ekstraktı (*X. strumarium, H. niger* ve *M. azedarach*) *M. incognita* ve *M. javanica* ile karışık popülasyonlar olarak bulaşık olduğu tespit edilen seralarda uygulanmıştır. Parsellere 1 ml bitki^{-1} olacak şekilde süzgeçli kovayla uygulanmıştır. Çalışmalar; kök indeks ve verim (kg bitki^{-1}) değerleri ile kök ur indeksi etki ve verim artışı (%) açısından değerlendirilmiştir. Doğa-sera çalışmaları sonucu, bitkisel ekstraktlardan sadece *M. azedarach*'nın köklerde meydana gelen urlanmalar ve verim açısından etkili olduğu söylenebilir.

Anahtar sözcükler: Kök ur nematodu, *Meloidogyne incognita*, *M. javanica,* bitkisel ekstraktlar, domates

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Introduction

Plant-parasitic nematodes are major pests in many countries, particularly in the tropics and subtropics, where they are recognized as the cause of serious yield losses on a wide range of crops (Luc et al., 2005; Sasser & Freckman, 1987). Among all plant-parasitic nematodes, root-knot nematodes (*Meloidogyne* spp.) (Tylenchina: Meloidogynidae) (RKNs), are economically the most important and agriculture productivity and quality limiting pathogens (Javed et al., 2006). The most destructive species is *Meloidogyne incognita* (Kofoid & White) Chitwood (Tylenchina: Meloidogynidae), which causes serious problems to tremendous number of economically important crops (Tsay et al., 2004). Nematode control is largely based on synthetic nematicides, which is expensive and potential risk to environment, consequentely non-target organisms. For more acceptable alternatives to chemicals, the possibilities are being investigated of exploiting nematode-antagonistic plants for the management of plant parasitic nematodes (Chitwood, 2002; Akhtar, 2004). Current management of nematodes are focused on plant resistance, crop rotation, cultural practices or chemical nematicides (Chitwood, 2002). Because of these disadvanteges, scientists found natural product with nematicidal activity such as plant extract, root exudates, plant volatiles etc. Linford et al., (1938) were the first to study the nematicidal effect of chopped pine-apple [*Annanas comosus* (L.) Merr. (Poales:Bromeliaceae)] leaves used as organic amendment against *Meloidogyne* spp., while a review of phytochemical strategies for the control of nematodes was given by Chitwood (2002). Numerous plant species, representing 57 families including Lamiaceae, Asteraceae, Myrtaceae, Rutaceae, Lauraceae, can contain nematicidal compounds (Sukul, 1992; Andres et al., 2012).

In Turkey, *M. incognita*, *M. arenaria* Chitwood, *M. javanica* (Treub) Chitwood and *M. hapla* Chitwood are the most commonly found RKN species, with *M. incognita* being the most pathogenic and widespread (Kepenekci, 2012). The use of plant extracts as an alternative to synthetic pesticides for control of RKNs is becoming important. In recent years, research on this topic has increased rapidly in the Mediterranean coast (Ntallie et al., 2011; Andres et al., 2012).

The objective of current study was to determine the efficacy of plant extracts derived from *Capsicum frutescens* L., *Hyoscyamus niger* L. (Solanaceae), *Melia azedarach* L. (Meliaceae), *Xanthium strumarium* L. and *Achillea wilhelmsii* C.Koch (Asteraceae) as alternative to chemical nematicides. The effects of five different plants extracts on mortality of RKNs were investigated *in vitro* and *vivo* conditions.

Material and Methods

Plant material

Five indigenous plants namely; pepper (*Capsicum frutescens*), henbane (*Hyoscyamus niger*) (Solanaceae), bead-tree (*Melia azedarah*) (Meliaceae), common cocklebur (*Xanthium strumarium*) and yarrow (*Achillea wilhelmsii*) (Asteraceae) were collected from various ecological zones of Anatolia, Turkey.

Extraction

Plant leaves were plucked from their branches and spread on polythene sheets on benches in the laboratory for ten days to air dry. Then plants were dried at 80°C for 3-4 days. The dried materials were ground to fine particles using a blender. Ethanol was added to the ground plant material and shakenon arotary in a shaker at 120 rpm for 48 hours. The solution was filtered and the material was vacuumed in a rotary evaporator at 50-60°C to obtain organic crude extracts (ethanol is eliminated) (Brauer & Davkota, 1990). Each plant extract was prepared in 200 g/200 ml and were used immediately in all tests. Concentrations of 0.5, 1.0, 1.5, 3.0, 6.0 and 12.0% was prepared with distilled water (Orisajo et al., 2007).

Culture root-knot nematodes

SC-2121 varieties of tomato [*Solanum lycopersicum* L. (Sloanaceae)] are known to be susceptible to RKNs (*Meloidogyne incognita* for labarotory-petri dish studies and *M. javanica* for greenhouse-pot studies), were sown into pots containing steriled soil and sand in greenhouse conditions at 25±1ºC temperature. Roots were well washed and were cut 1 centimeter in length and were shaken for 3.0-3.5 minutes in 1 liter of 0.525% NaOCl (Sodium hypochlorite) solution to extract nematodes eggs. Optained solution was sieved through 75 and 26 µm (200 and 500 mesh) sieves and nematode eggs were retained on 500 mesh opening sieve were collected in 100 ml size glass beakers (Hussey & Barker, 1973). The egg suspension was poured on to a cotton-wool filter and incubated at 26±2ºC. Emerged second stage juveniles (J2s) were collected daily for up to 4 days and stored fridge (4ºC) until used for experiment. To collect egg masses for labarotory-petri studies, tomato plants infected with a RKN (*M. incognita*) were carefully washed by tap water and egg-masses were hand-picked into Petri dishes containing distilled water.

Laboratory-petri dish studies (*in vitro*)

Nematicidal effect of plant extracts was evaluated on *M. incognita* under laboratory conditions. Plant suspensions of concentrations of 0.5, 1.0, 1.5, 3.0, 6.0 and 12.0% were prepared with distilled water. A suspension of eggs, medium size 5 egg masses and J2s in distilled water was prepared.

Effects of plant extract on egg hatching**,** one ml of RKN eggs suspension containing 101–123 (110±5.5) eggs ml^{-1} added to 1 ml of selected plant extract and 3 ml of distilled water were transferred to sterilized Petri dishes. Distilled water used as a control. All treatments were kept at 28±2°C. After seven days of exposure, the numbers of hatched eggs were counted. The toxicity of plant extract was assessed as the mean percentage of the dead nematodes. Medium sizes of 5 egg masses [1712–1794 (1768.3±25.3) J2s 5 egg masses ml^{-1}] added to 1 ml of the selected plant extract and 3 ml of distilled water were transferred to sterilized Petri dishes. Egg masses kept in distilled water as control. Each treatment was replicated 5 times. After 7 days exposure, the number of juveniles hatched was counted with the aid of inverted microscope at magnification 40×.

Effects of plant extracts (0.5, 1.0, 1.5, 3.0, 6.0 and 12.0% concentrations) on eggs and J2s of the *M. incognita* were evaluated in laboratory conditions. Suspensions of J2s [97-115 (103.7±4.1) J2s ml^{-1}] and eggs [101-123 (110±5.5) eggs ml^{-1}] in distilled water were prepared. One ml of J2s or eggs suspension, 1 ml of extract and 3 ml of distilled water was transferred in sterilized Petri dishes in five replicates while, distilled water used as a control and kept at 28±2°C. After 7 days of exposure, the numbers of dead RKN was counted. The toxicity of plant extract was assessed as the mean percentage of the dead nematodes. Nematodes were considered dead if they did not move when probed with a fine needle (Abbasi et al., 2008).

Greenhouse-pot studies

Tomato seeds (SC-2121 variety) were planted in 80 ml (5 cm diam. 4 cm height) pots filled with autoclaved peat. Two-four leaves, tomatoes seedlings were transferred individually to the approximately 340 ml plastic pots (7 cm diam. 7 cm height) containing 320 g sterilized loamy sand soil (80% sand, 15% silt and 5% clay). After 2 weeks, tomato seedlings (approximately 10 cm height) were inoculated with 3000 *M. javanica* eggs or 1000 J2s in 1 ml suspension (egg hatching and mortality test) (Adekunle & Akinlua, 2007; Liman et al., 2010). Seedlings which showed either development disorders or larger than average size were not used in the experiments. *Meloidogyne javanica* eggs or J2s were applied with a pipet to 2 cm deep holes around the seedling roots. Plant extracts and nematodes were applied the same time of holes explained above with the concentration of 3.0, 6.0 and 12.0% 1 ml extract per plant or pot. The control pots received only water (- control) and only nematodes (+ control) containing [negative (-)

control, water only (no nematodes were applied); positive (+) control, (nematodes only, 3000 eggs or 1000 J2s)]. The experiments contained five replicates (pots) for each treatment. The parameters; total number of egg masses (I), plant height (II), fresh (III) and dry weight of the green parts of plants (IV) and fresh (V) and roots dry weight (VI).

The experiment contained five replicates (pots) for each treatment, arranged in a randomized block design (blocked by row on the greenhouse bench in controlled conditions). Temperature [13.32-33.59ºC (22.02ºC ±4.14)] and humidity [23.40-77.10% (36.34%±9.25)] was monitored throughout the experimental periods in the trial. Bioassays were conducted in a greenhouse between October 2012 and May 2013.

Nine weeks after treatments, the plants were harvested, plants were cut one cm above the soil level to divide the roots from the above-ground portion of the plants and each section was weighed, then dried at 70°C for 48 hours and weighed again (Mohammad et al., 2007). To count egg masses, plant roots were stained red with phloxine B (Fenner, 1962; Dickson & Struble, 1965; Holbrook et al., 1983). Roots with nematod galls were placed in an aqueous solution of phloxine B (0.15 g L tap water^{-1}) for 15-20 min (Daykin & Hussey, 1985). Root systems were rinsed in tap water to remove residual stain from the roots, and egg masses were counted under a dissecting microscope or a magnifying glass with light (8×).

Nature-greenhouse studies (*in vivo*)

The results obtained from the greenhouse-pot studies caaried out in two different areas of Mediterranean (Kepez-Antalya) and Aegean (Fethiye-Muğla) Regions of Turkey. These regions where known to have planty of greenhouse crop production. All the studies conducted in the greenhouses were based on the “Standart Pesticide Trial Methods” (Anonymous, 2009).

Due to the non-homogenius distribution of the nematodes in nature, the trials were conducted as four replicates for each treatment and arranged in a randomized complete block designs in a naturally mixed populations of *M. incognita* and *M. javanica* occurring greenhouse. The characteristics of the trials were formed by plant extracts which had given promising results after greenhouse-pot studies, one of which has (+) control [nematicide, *Quillaja saponaria* (QL Agri ®), had been applied to this extract as comparison pesticide] and (-) control (only periodically upkept and watered).

All applications were performed with the filtered bucket. The trial plots were 8.0 m in length1.5 m in width and containing total of two rows and thirty-two plants. Two trial plots were seperated by a 2.5 m border line. Plant extracts (12% concentration *of M. azedarach, X. strumarium* and *H. niger*’s) were applied 4 times (early planting, with planting, 15th day after planting, 30th day after planting) with watering pot (extracts were applied 1 ml plant^{-1}). After application, drip irrigation was opened approximately 1 hour for diffusing the biopreparats in the soil.

In the greenhouses, Care F1 (in Kepez) and Ilgın F1 (in Fethiye) tomato cultivars had been used. In the nature-greenhouse applications; during the growing period of the tomato plant, the mature tomatoes had been weighed and the effects of the applications to the efficiency had been evaluated. At the end, twenty tomato plants were removed for each parcel and, the root galling of plants were evaluated according to the 0-10 Zeck scale (Zeck, 1971).

Regarding the effect of the applications to the efficiency the weighings had been started on the 22nd of September, 2014 in Kepez and on the 11th of January, 2015 the last weighing were recorded. The same day the tomato plants had been removed and evaluated for the root knots. In Fethiye the tomatoes that had become mature between the dates 12th of September, 2014 and 08th of January, 2015 had been weighed and 2 days after the last weighing the plants had been removed and had been evaluated regarding the root knots. For both greenhouses, not only the weighing data were recorded 8 to 10 times during the growing season but also in the intermittent periods the markatable tomatoes were recorded.

The galling index (I), the percentage effect (%) (II), efficiency (yields) (kg plant^{-1}) (III) and efficiency increase percentage (%) (IV) points of view were statistically evaluated.

During the trials, growing temperature and the humidity conditions in the greenhouse were recorded using HOBO (the temperatue and the humidity recorder) [Kepez, 14.46-42.22ºC (27.28ºC ±7.71) and 62.26-84.12% (76.57%±5.13); Fethiye, 15.57-43.12ºC (24.85ºC ±6.88) and 67.33-96.97% (86.24%±8.70)].

Nematodes diagnosis studies

Morphological and molecular diagnosis of nematodes taken from infected roots of tomatoes from natural greenhouses –from Kepez (Antalya), Mediterranean Region and Fethiye (Muğla), Aegean Region– were performed. Our result indicated that both greenhouse locations were contaminated with mixed population of *M. incognita* and *M. javanica*. Greenhouses in Fethiye were sustained mostly *M. incognita* and in Kepez however, sustained mostly *M. javanica*.

Statistical analysis

Analyses of varians were applied to the obtained data by using SPSS (1999) software. The found effects were compared to the controls and means of data groups were separated by Duncan multiple range test.

Results and Discussion

In this study, the nematicidal activities of pepper (*Capsicum frutescens*), henbane (*Hyoscyamus niger*), bead-tree (*Melia azedarach*), common cocklebur (*Xanthium strumarium*) and yarrow (*Achillea wilhelmsii*) were evaluated by root-knot nematodes (*Meloidogyne* spp.) (RKNs) hatching and mortality test.

Laboratory-petri dish studies (*in vitro*): In trials performed at concentrations of 0.5%, 1.0% and 1.5%, the effect of treatments against the J2s (larva toxicity to second stage juveniles) was determined to be too low. The effect was stronger when the concentrations were raised to 3%, 6% and 12%. Compared with the other plant extracts, the *A. wilhelmsii* and *C. frutescens* were found to be less effective in terms of killing capacity. The 6% and 12% concentrations of *H. niger*, *M. azedarach* and *X. strumarium* had the highest killing capacity by killing all applied J2s. Higher concentrations were associated with higher death rates (Table 1). Similar results were observed with the egg hatching trial rate, and the 6% and 12% concentrations of *H. niger*, *M. azedarach* and *X. strumarium* as well as the 3% concentrations were found to be 100% effective on egg hatching (no J2s hatching) (Table 1). According to these results of laboratory-petri dish studies, we decided to use 3%, 6% and 12% concentrations for all the plant extracts in the greenhouse-pot trials (Table 1).

Greenhouse-pot studies: The effect to the egg hatching and J2 mortality of nematodes in the greenhouse-pot trials were evaluated separately. When we evaluate the number of egg masses on the roots of the tomato; regarding the egg hatching; with the maximum concentration (12%) of *X. strumarium* has the maximum effect (10.4 egg masses per plant) and followed by *H. niger* (19.8 egg masses per plant). Although the effect to the larval toxicity of the *M. azedarach* was great, it has low effect on the egg hatching. There counted 102.2 egg masses per plant in the 12% concentration of *M. azedarach* application. In the control group there determined 196.2 egg masses per plant (Figure 1A). Regarding the larval toxicity the 12% concentration of *M. azedarach* has the maximum effect with 3.6 egg masses. The 12% concentration of *X. strumarium* and 6% *M. azedarach* followed by 33.8 and 38.0 egg masses per plant. *H.n*, which is effective to the egg hatching has low effect for the larval toxicity. In the control group 166.2 average number of egg masses had been determined ($F= 11.92$; df: 16.68; $P<0.05$) (Figure 1 A).

Table 1. Effect of various concentrations of some plant extracts *Melia azedarach, Xanthium strumarium, Hyoscyamus niger, Achillea wilhelmsii* and *Capsicum frutescens* on *Meloidogyne incognita* survival under laboratory conditions. Data are expressed as mean±SD

Treatment		Hatch inhibition and mortality of J2s after 7 days					
		Tested concentration					
		0.5%	1%	1.5%	3%	6%	12%
C. frutescens	E*	108±4.3 b	97±5.2 b	22.6±8.9 b	8±5.2 a	3.6±1.1 a	2.8±1.6 a
	EM**	1737±62.1 *c*	1546.4±71.8 *c*	66.2±27 *c*	8.6±5.4 *a*	4.4±3.7 *a*	3.8±2.4 *a*
	J2s***	104.4±4.8 B	98.4±5.3B	36.6±16.3 B	12.4±3.2 ab	9.2±2.6 a	5.2±1.9 a
H. niger	E	42±9.13 a	50.8±12.1 ab	16±11.8 ab	0 a	0 a	0 a
	EM	1212.2±5.8 *b*	853±94.1 *b*	8.6±5.4 *a*	0 *a*	0 *a*	0 *a*
	J2s	49.2±11.7 A	38.6±11.1 AB	13.4±7.8 AB	0 A	0 A	0 A
M. azedarach	E	99±12.2 b	80.4±1.36 ab	14.4±6.4 ab	0 a	0 a	0 a
	EM	1695.4±61.5 *c*	1007.8±103.3 *b*	24±13.7 *ab*	0 *a*	0 *a*	0 *a*
	J2s	86.8±16 AB	36.4±4.9 AB	7.2±3 A	0 A	0 A	0 A
X. strumarium	E	46±19.3 a	18.4±7.8 a	4.6±4.5 a	0 a	0 a	0 a
	EM	1153.8±51.3 *a*	608.2±171.2 *a*	9.8±5.9 *a*	0 *a*	0 *a*	0 *a*
	J2s	53.4±13.7 A	18.8±8.3 A	8.6±5.8 A	0 A	0 A	0 A
A. wilhelmsii	E	107.8±5.4 b	85±6.6 ab	27±10.1 b	7±5.3 a	3.8±1.3 a	3.4±1.5 a
	EM	1730±32 *c*	1518.4±122 *c*	40.4±13.1 *bc*	12.2±4.8 *a*	7.6±4.5 *a*	4.4±2.5 *a*
	J2s	103.2±3.8 B	90.8±7 B	38.6±4.5 B	15.6±3.3 AB	10.6±2.9 AB	5.4±2.1 A
Control (water only)	E	114.2±2.58 b	109.4±5.2 b	111.4±6.9 c	108.4±6 b	107.4±4.9 b	109.2±6.3 a
	EM	1758.8±28.9 *c*	1766.6±28.3 *d*	1777.8±17.4 *d*	1763.8±29.8 *b*	1775.8±21.7 *b*	1767±31.7 *b*
	J2s	106.8±7 B	103.2±4.2 B	104.4±4.9 B	102.4±1.6 B	103.2±3 B	102.2±1.7 B

* Eggs ml^{-1} (lower case letters indicate significant, $P<0.05$), eggs suspension [101-123 (110±5.5) eggs ml^{-1}] (E)
** 5 egg masses which J2s out of ml^{-1} (italicized letters indicate significant differences, $P<0.05$), medium size 5 egg masses [1712-17.94 (1768.3±25.3] juvenile 5 egg masses ml^{-1}) (EM)
*** Second stage juveniles (upper case letters indicate significant differences, $P<0.05$), juvenile suspension [97-115 (103.7±4.1) juvenile ml^{-1}] (J2s).

Regarding the plant length, from the egg hatching point of view the best effect comes from the 12% concentration of *X. strumarium* which is same with the negative control (44.8 cm). The same concentration of *H. niger* followed this with 42.3 cm but had taken place in a different group with the control group statistically. In the same trials the applications which had been under positive control (33.6 cm) which are *C. frutescens* at 3%, 6%; *M. azedarach* at 3%; *X. strumarium* at 3%, 6%; and *A. wilhelmsii* at 6% concentrations (28.6, 29.1, 28, 32.2, 26.2, 24.6 and 28.8 cm respectively) (F=7.98; df:16.68; P<0.05) (Figure 1B). In the larval toxicity trials; although the maximum effect had come from 12% concentration of *M. azedarach* with 42.5 cm. It statistically did not take place in the same group with the -control 45.2 cm. All the plants in the other applications were found out that they are under the +control (40.6 cm.) (F= 3.77; df:16.68; P<0.05) (Figure 1B).

Regarding the egg hatching, between the plant upper parts fresh weight and dry weight (Figure 1C, D) and root dry weight (Figure 1F) parameters no difference had been found statistically except the plant upper parts fresh weight and the fresh root weight in the larval toxicity trials (P>0.05).

The effect to the larval toxicity trials, the maximum effect about fresh root weight was found out for the 3, 6 and 12% concentrations of *M. azedarach* as 15.11, 15.74 and 15.76 g. Among these applications the minimum concentration application 3% is under –control (15.5 g). In all other applications, except the all concentrations of *X. strumarium* (13.79, 14.4 and 14.39 g) were under +control (13.34 g) (F=12.10; df:16.68; P<0.05) (Figure 1 E).

For the “egg hatching” point of view, the maximum effect (1.84 g) for the plant upper parts dry weight comes from the 12% concentration of *X. strumarium* applications which was higher than the -control (1.78 g). Except 12% *H. niger* and 3% *X. strumarium* applications (1.64 and 1.62 g) all the other applications were under +control (1.60 g) (F=4.84; df: 16.68; P<0.05) (Figure 1 F). When the trials were evaluated regarding the root weight terms; for the fresh root weight the maximum effect had come from 3% concentration of *X. strumarium* with 15.2 g. In the applications the –control had 14.3 g and the +control had 16.4 g root weight (F=2.55; df:16.68; P<0.05) (Figure 1 E). Regarding the root dry weight terms, although the two applications (for 12% concentration of *H. niger* it is 1.84 g and for 12% *X. strumarium* 1.86 g) had the maximum effect, they had been under the –control value of 2.08 g of root weight. All the other applications were under +control value of 1.78 g of root weight (F=4.24; df:16.68; P<0.05) (Figure 1 F).

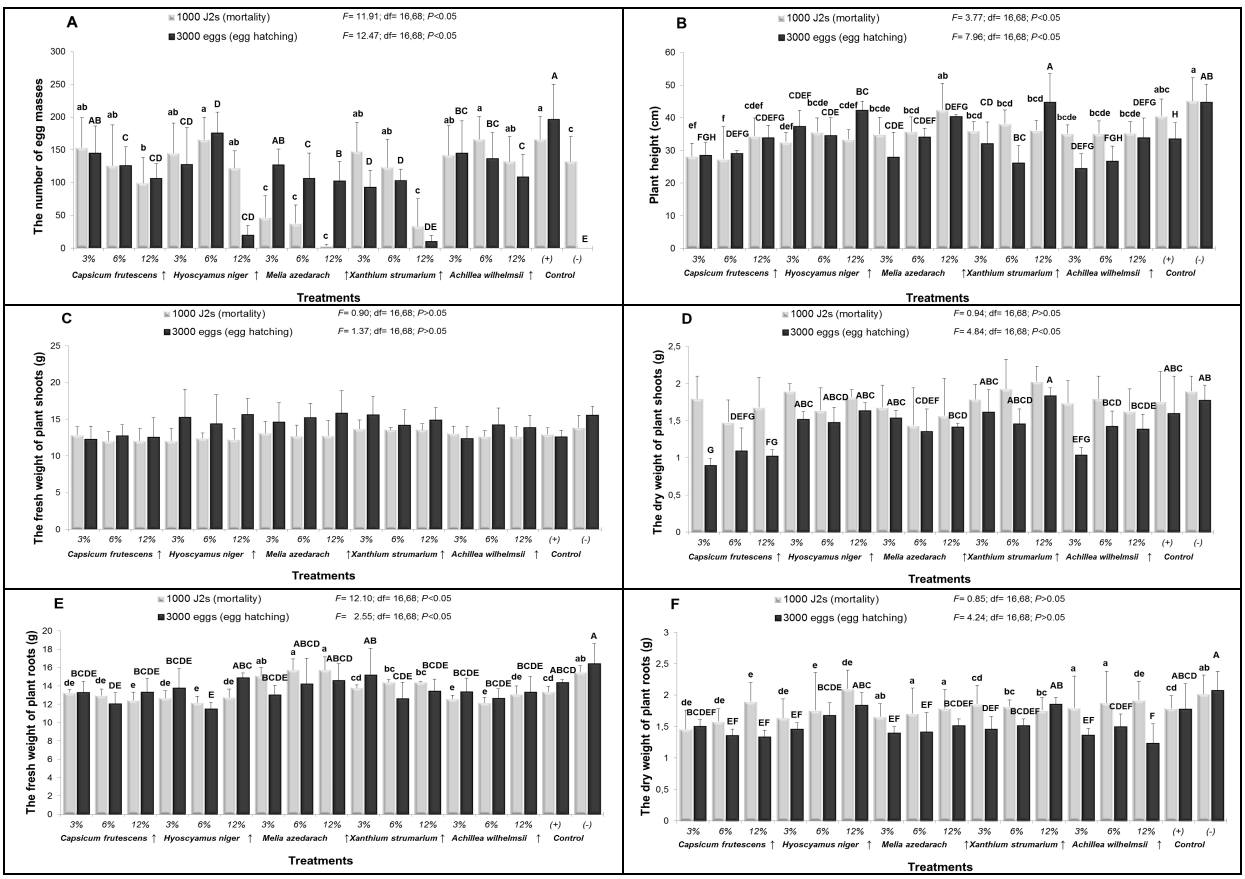

Figure 1. Effect of selected concentrations (3%, 6% and 12%) of five indigenous plant extracts (*Melia azedarach, Xanthium strumarium, Hyoscyamus niger, Achillea wilhelmsii* and *Capsicum frutescens*) on *Meloidogyne javanica* reproduction (egg hatching test, 3000 eggs were applied and mortality test, 1000 J2s were applied) under greenhouse tomatoes (SC-2121 variety) [negative (-) control, water only (no *M. javanica* eggs were applied); positive (+) control, (nematodes only, 3000 eggs or 1000 J2s)] [(A) total number of egg masses, (B) plant height, (C) fresh and (D) dry weight of the upper parts of plants and (E) fresh and (F) dry roots weight].

As a significant results of all greenhouse-pot experiments (both of all trials, egg hatching and larval toxicity); the number of egg masses found in the roots of tomato plants were evaluated; plant extracts of *X. strumarium* at 12% for egg hatching trials and *M. azedarach* at 12% for larval toxicity trials were reduced the nematode reproduction (10.4 and 3.6 egg masses per plant respectively) on tomato roots compared to control groups (196.2 and 166.2 egg masses per plant respectively).

Nature-greenhouse studies (*in vivo*): Nature-greenhouse trials were evaluated separately regarding the root index and efficiency. In the Fethiye trials (Figure 2A, B); the *M. azedarach*, which had

the maximum effect and which had the minimum root index or which caused the least galls on the tomatoes roots with a 2.97 root index was taken place statistically in the same group with a registered and +control [nematicide, *Quillaja saponaria* (QL Agri ®)]. *X. strumarium* and *H. niger* (7.35 and 7.53) had quite low effects and were taken place in the –control (8.01) groups (negative control was only applied water by drip irrigation) in the parcels-where no application were made and only watered- which were contaminated with the nematodes (mixed populations of *M. incognita* and *M. javanica* on tomatoes under natural greenhouse conditions) (F=17.307; df:4.19; P<0.05) (Figure 2 A). The *M. azedarach* applications had showed the maximum effect (62.54%) as being the closest to the value of +control (72.45%) root knot index effect (%). The applications that had high effects statistically were taken place in the same +control groups. The *H. niger* and *X. strumarium* applications (6.08 and 7.59%) that takes place in the same group with –control seems to have the quite low effect (F=21.823; df:4.19; P<0.05) (Figure 2 B). When the trials were evaluated regarding the efficiency terms, the maximum tomato efficiency (yields of tomatoes) were provided by the *M. azedarach* applications (3.90 g per plant). This application were statistically taken place in a different group and determined different than the +control (4.50 g per plant). The *H. niger* and *X. strumarium* applications (3.62 and 3.12 g per plant) had provided low efficiency and the *X. strumarium* from these applications were statistically taken place in the same group with the –control groups (2.95 g per plant) (F=8.331; df: 4.19; P<0.05) (Figure 2 A). When the trials were evaluated regarding the efficiency increase terms there were no applications found that could take place in the same group with +control (63.06%). The efficiency increase in the whole three applications were found low and they statistically were taken place in the same group (11.46, 31.30 and 39.41%) (F=1.697; df: 4.19; P<0.05) (Figure 2 B). When the Kepez (Antalya, Turkey) trials (Figure 2 C, D) were evaluated regarding the root index terms, the maximum effect from *M. azedarach* applications (3.56) and this *M. azedarach* applications were statistically taken place in the same group with +control (2.21). In the trials that were set up, the *X. strumarium* and *H. niger* (7.53 and 7.62) had quite low effect and were taken place in the same group with -control (8.40) (F=11.764; df: 4.19; P<0.05) (Figure 2 C). The *M. azedarach* application (45.05%) had the maximum effect and had the closest value to the +control (73.69) root knot index effect (%). The *H. niger* and *X. strumarium* applications (9.04 and 10.28%) determined to have the quite low effect (F=11.764; df:4.19; P<0.05) (Figure 2 D). When the trials evaluated regarding the efficiency, the highest tomato efficiency comes from the *M. azedarach* applications (2.94 kg per plant). This application had been statistically taken place in the same group with +control (2.95 kg per plant). Low efficiency was obtained from the *H. niger* and *X. strumarium* applications (2.39 and 2.65 kg per plant). In the –control group in this study 2.11 kg plant^{-1} efficiency was obtained (F=8.614; df:4.19; P<0.05) (Figure 2 C). When the trials were evaluated regarding the efficiency increase (%) terms, the *M. azedarach* applications had the highest efficiency increase with a value of 39.82% and had statistically taken place in the same group with the +control applications (40.19%) (F=4.042; df:4.19; P<0.05) (Figure 2 D).

The effect of *M. azedarach* on root galling was the highest in Fethiye compared to remaining *X. strumarium* and *H. niger* treatments in nature-greenhouse studies. Also, in Kepez *M. azedarach* sustained the highest effect and fall into the same group with + controls (nematicide). When the nature-greenhouse studies were evaluated together with the all trials those had been set up in Kepez and Fethiye; only *M. azedarach* had effect on the root knots and regarding the efficiency terms had effect on the root-knot nematodes (Figure 2).

Evaluated the +control parcels of the both greenhouses, the efficiency of the trials in Kepez were lower than the Fethiye. In Fethiye, sustained a quite high efficiency than those of in Kepez. In the control parcels of both greenhouses it had been determined 4.50 kg plant^{-1} for Fethiye and 2.95 kg plant^{-1} for Kepez. Besides the greenhouses were quite similar, regarding the root knot index [control without nematicide, only water used for (-control)] the tomato plant roots in Kepez had more knots than the other greenhouse (Fethiye). In the control parcels index values were found out as 8.01 for Fethiye and 8.40 for Kepez (Figure 2).

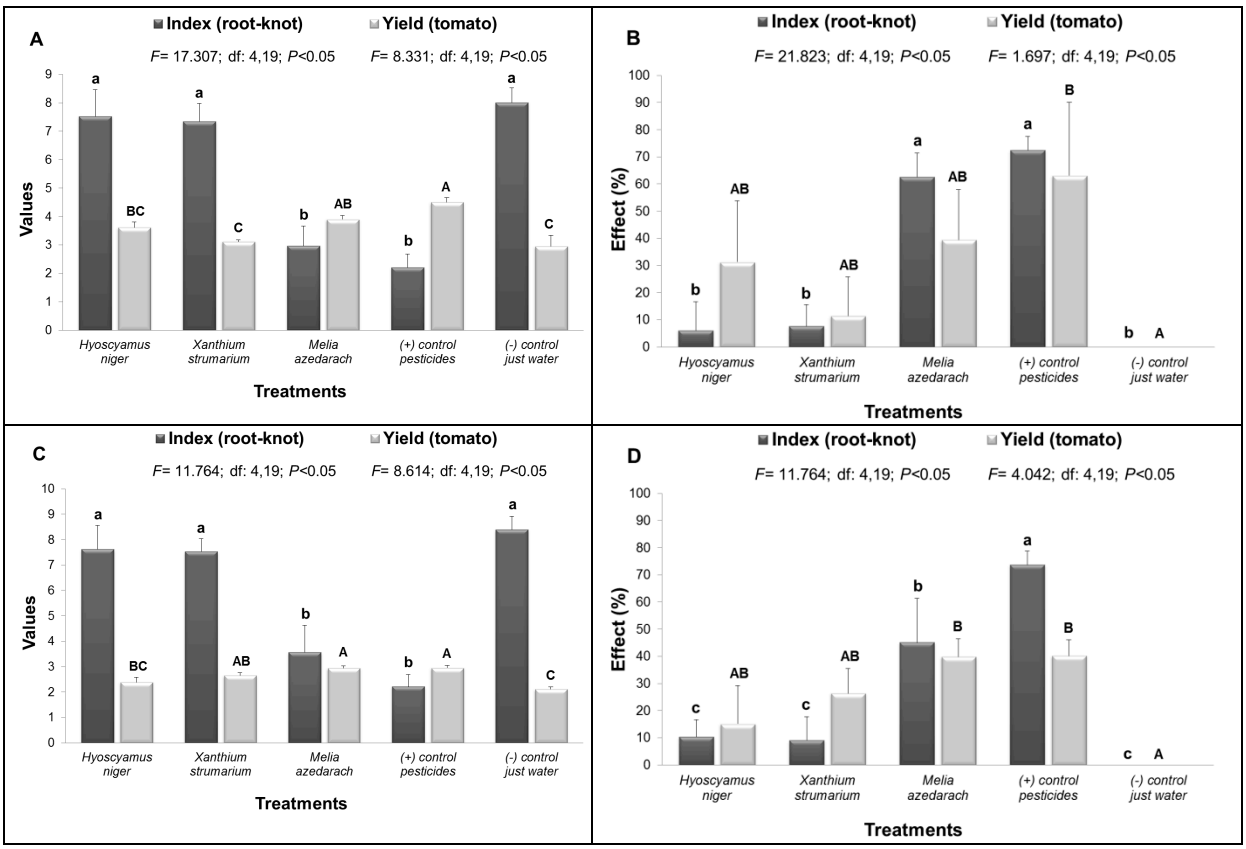

Figure 2. Effect of three indigenous plant extracts (*Melia azedarach, Xanthium strumarium* and *Hyoscyamus niger*) is naturally contaminated mixed populations of *Meloidogyne incognita* and *M. javanica* at Aegean (Fethiye-Muğla) (A, B) and Mediterranean (Kepez-Antalya) (C, D) regions where greenhouse products are widespread [negative (-) control, only periodically upkept and applied water by drip irrigation; positive (+) control, nematicide, *Quillaja saponaria* (QL Agri ®), had been applied to this extract as comparison pesticide] [the index (root knot) values, the index percentage effect (%), efficiency (yields) (kg plant^{-1}) and efficiency increase percentage (%)].

The result of this experiment showed that aquatic plant extract of *C. frutescens, H. niger, M. azedarach, X. strumarium* and *A. wilhelmsii* were toxic to *M. javanica in-vitro* conditions. Nematoxic effects were found even at the relatively low concentrations used in these experiments. Active ingredients of extract were effectively ensured to nematodes. Highest percentages of egg hatch and life activities of nematodes were monitored by control. *A. wilhelmsii* which is known poisonous plants use as an insecticides (Baytop, 1997; Çalmaşur et al*.,* 2006; Erdoğan et al., 2010; Khani & Asghari, 2012). Many studies were showed that *Achillea* sp. has antibacterial properties (Barel et al., 1991). *Achillea wilhelmsii* shows more effective nematicide effect then *Artemisia millefolium* L. (Asteraceae) (Dias et al., 2000; Ardekani et al., 2010). Ntallie et al. (2011) was studied some plant essential oils nematicidal activities against *M. incognita* so *A. millefolium* were not found to be nematicidal effect. Oka et al*.* (2000) showed that *A. fragrantissima* (Forssk.) Sch.Bip. (Asteraceae) was not effective against *M. javanica* on tomatoes plants*.* This plant extract was not demonstrated as a nematicide in our country. This study was showed that *A. wilhelmsii* has a low nematicide effect on root-knot nematodes. *H*. *niger*, a poisonous plant is used for medicinal purposes. Dried leaves of *H. niger* in enclose area used as a repellent against mice (Coffey, 1994). There were undesignated as nematicide in any study of *H. niger* was found to be effective in our experiment against *M. javanica*. Bead-tree is common in the Mediterranean region in Turkey. It was known that their leaves and fruits were used as pesticides in many years (Yelekçi et al., 1981; Erdoğan & Toros, 2005). *M. azedarach* was widely studied and successful results were obtained (Lee, 1990; Hasabo & Noweer, 2005; Maregiani et al., 2010; Rehman et al., 2012). In this study, the results have been promising. In particular has been demonstrated to be effective against the root-knot nematodes. Common

cocklebur is widely distributed all around the world. Many studies have been done with this plant in our country and other countries (Çetinsoy et al., 1998; Erdoğan & Toros, 2007). On this plant extracts have been done considerable nematological study so far and got effective results (Bala et al*.,* 1986; Nandal & Bhatti, 1986; Malik et al*.,* 1988; Shaukat & Siddiqui, 2001). Some study show that this plant extract effects egg hatch (Mennan et al., 2000). With this study, *X. strumarium* extract has been found to be successful inhibit egg hatching. *C. frutescens*, have been content many chemicals such as capsaicin, capsainoids and allyl isothiocyanate, are widely used as pesticide and their capsaicin and its analogues content have shown inhibitory activities towards the pests (Abbas et al., 2009; Mackeen et al., 1997). *C. frutescens* which is common used plant in Turkey has not been found any studies against nematodes. This study showed that *C. frutescens* plant extract didn't affect nematodes so much as similar effect show up *A. wilhlmsii*.

According to *X. strumarium, M. azedarach* and *H. niger* are a good inhibitor of nematode egg hatching and juvenile survival in this study. *X. strumarium, M. azedarach* and *H. niger* were found highly effective against RKNs. *H. niger* and *M. azedarach* extracts may be due to possessing ovicidal and larvicidal properties. *M. azedarach* is demonstrated more effective than other plant extracts and necessities has been arisen work on it and evaluate their results. This is a first time known that the effect of *H. niger* against nematode were revealed in the world. The use of *M. azedarach* extracts are suggested as a potential substitute for synthetic nematicides used in the management of RKNs in the greenhouse vegetable growing areas of the coastal regions.

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Orijinal araştırma (Original article)

On the subgenus *Eurysunius* Reitter in Turkey III. A new species from western Anatolia and additional records (Coleoptera: Staphylinidae, Paederinae, *Astenus*) [1]

Türkiye'deki *Eurysunius* Reitter türleri III. Batı Anadolu'dan yeni bir tür ve ek kayıtlar (Coleoptera: Staphylinidae: Paederinae, *Astenus*)

Sinan ANLAŞ[2]*

Summary

Astenus (*Eurysunius*) *ilgazi* sp. n. from Afyonkarahisar province is described, figured and distinguished from related species of the subgenus. Additional records of five species are also reported. Amongst them, *Astenus rhodicus* Assing, 2013 is recorded for the first time from Turkey.

Keywords: Coleoptera, Staphylinidae, Paederinae, *Astenus*, *Eurysunius*, Turkey, new species, additional records

Özet

Astenus (Eurysunius) ilgazi sp. n. türü Afyonkarahisar ilinden tanımlanmış, şekillendirilmiş ve bu altcinsin benzer türlerinden farklılıkları gösterilmiştir. Ayrıca, beş türe ait kayıtlar verilmiştir. Bunlardan, *Astenus rhodicus* Assing, 2013 türü Türkiye için yeni kayıttır.

Anahtar sözcükler: Coleoptera, Staphylinidae, Paederinae, *Astenus*, *Eurysunius*, Türkiye, yeni tür, ek kayıtlar

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Introduction

The subgenus *Eurysunius* Reitter comprises 58 species in the Palaearctic region, which are distributed mainly in the Mediterranean countries (Assing, 2015; Anlaş, 2015; Schülke & Smetana, 2015). Twelve of them are from Turkey and that represents more than 20 % of *Eurysunius* of the Palaearctic fauna. All these species seem to be endemic to Anatolia.

There is limited studies on the Paederinae of Turkey. Therefore, a research project on the diversity and biogeography of the Paederinae was carried out in the Aegean Region of Turkey. These research activities yielded new data on the subgenus *Eurysunius* in western Anatolia (Anlaş, 2014, 2015).

In this study, a new species and additional records are reported from Turkey, resulting 14 species of the subgenus known from the country. Amongst them, 13 species are endemic to Turkey.

Material and Methods

Insect samples were collected from fields in western Anatolia, in spring 2015. The terminology used in this paper follows Coiffait (1984) and Assing (2002). The study was conducted using a Stemi 2000-C microscope (Zeiss, Germany), combined with a digital camera (Zeiss Axiocam ERC5s) for the photographs. The reference specimens of this study are deposited in the collection of the Alaşehir Zoological Museum, Manisa (AZMM) of the Celal Bayar University.

Head length was measured from the anterior margin of the frons to the posterior margin of the head, length of pronotum was measured along the median line, elytral length was measured at the suture from the apex of the scutellum to the posterior margin of the elytra. The length of the median lobe of the aedeagus was measured from the apex of the ventral process to the base of the capsule.

Results

Taxonomy

Description of the new species

***Astenus* (*Eurysunius*) *ilgazi* sp. n.** (Figures 1A-G)

Type Material Holotype: TURKEY: ♂ "TR – Afyonkarahisar province, Ahır Mountains, Büyükhacet Hill, 1908 m, 38°40'30"N, 30°06'25"E, 02.V.2015, leg. Yağmur, Örgel & Altın / Holotypus ♂ *Astenus* (*Eurysunius*) *ilgazi* sp. n. det. S. Anlaş 2015" (AZMM). Paratypes: TURKEY: 1♂, 5♀, same data as holotype (AZMM); 3♂, 3♀, 02.V.2015, Afyonkarahisar province, Ahır Mountains, Büyükkavşak Hill, 38°43'08"N, 30°03'48"E, 1810 m, leg. Yağmur, Örgel & Altın (AZMM).

Etymology. The specific epithet honors Dr. Çetin Ilgaz, İzmir, a specialist on herpetology, who has carried out important zoological researches in Turkey.

Description. Habitus (Fig. 1A), body length 4.2-4.8 mm. Coloration: head and pronotum blackish or dark brown, anterior half of elytra blackish, with the posterior area reddish-yellow; abdomen blackish with narrow posterior margins of tergites and apex somewhat reddish, antennae rufous, legs reddish.

Head transverse, approximately 1.20 times as wide as long (Figs. 1A-B); dorsal surface convex, with very dense and average sized, but rather shallow punctures; interstices reduced to narrow ridges; pubescence short, yellowish. Eyes relatively small, in dorsal view distinctly shorter than postocular region. Antennae moderately slender, 1 mm long, antennomere III approximately 2.2 times as long as wide; antennomeres V–X elongate.

Pronotum slightly transverse, 1.18 times as wide as long (Figs. 1A-B), widest at anterior angles, slightly narrowed posteriorly; anterior and posterior angles each with a long setae of slightly more than half of length of lateral margin of pronotum; posterior margin convex; dorsal surface with well-defined impressions; microsculpture absent; punctation similar to that of head, but denser, surface somewhat glossier than that of head; pubescence of similar length as that of head, but more conspicuous.

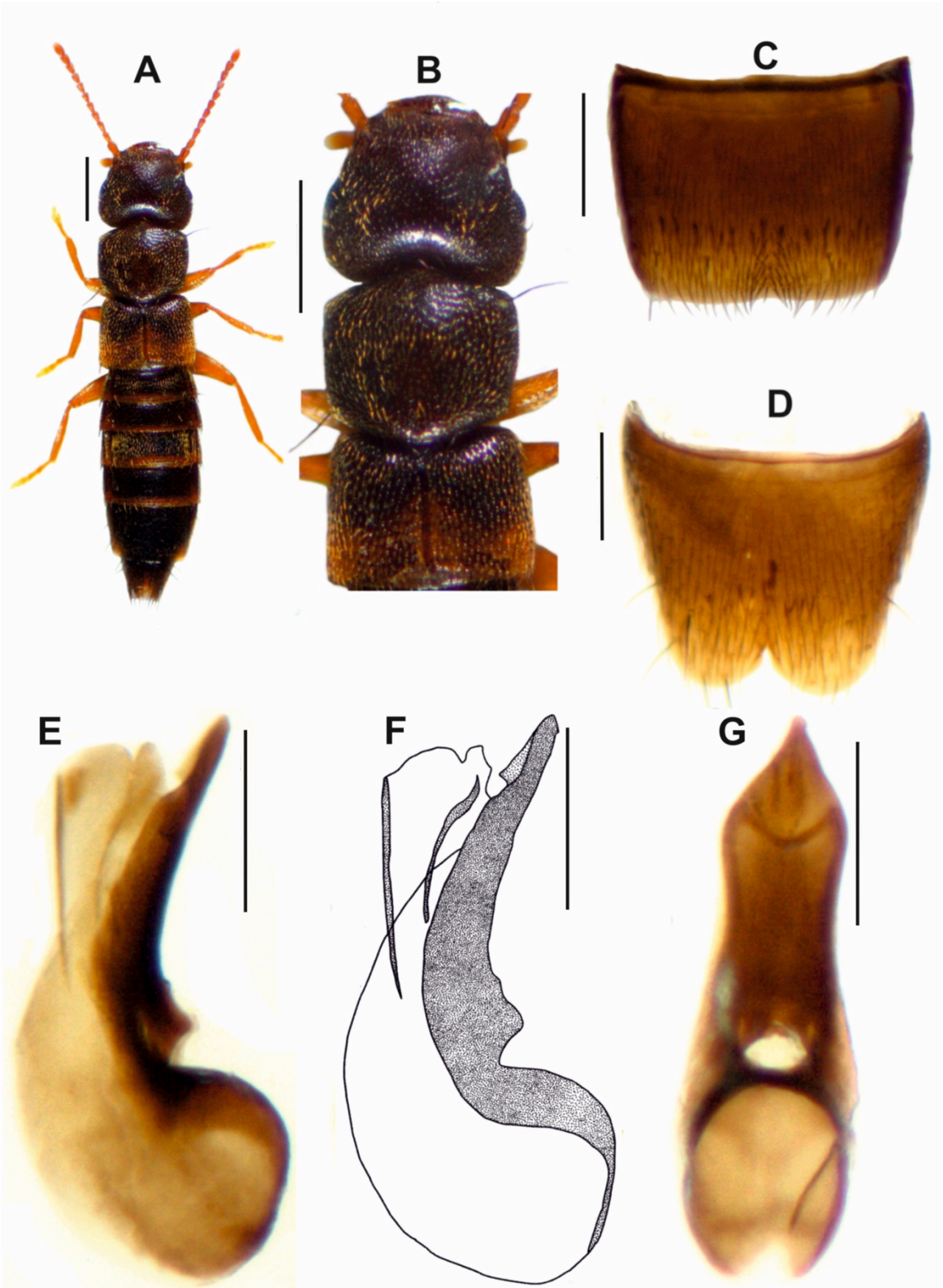

Figure 1. Details of A*stenus ilgazi* sp. n. (A)—habitus; (B)— forebody; (C)—male sternite VII; (D)—male sternite VIII; (E-F) — aedeagus, lateral view; (G)—aedeagus, ventral view; Scale bars: 1.0 mm (A); 0.5 mm (B); 0.2 mm (C-G).

Elytra transverse and short (Figs. 1A-B), approximately 1.60 times as wide as long and 0.70-0.75 times as long as pronotum; microsculpture absent; punctation very dense and distinctly granulose, distance between punctures slightly narrower than diameter of punctures; pubescence reddish-yellow, more distinct than that of head and pronotum; posterior margin of each elytron with 6-7 long black setae. Hind wings totally reduced.

Abdomen wider than elytra (Fig. 1B), approximately 1.15 times as wide as elytra, widest at segment V, segments III–VI moderately transverse, tergites III–V approximately twice as wide as long; punctation very dense and very fine; space bewteen puncures with distinct fine microsculpture; pubescence yellowish, sometimes reddish-yellow; posterior margin of tergite VII with palisade fringe.

♂: sternite VII in posterior median area slightly depressed and with some modified dark stout setae, posterior margin weakly concave (Fig. 1C); sternite VIII deeply and acutely incised posteriorly, pubescence unmodified (Fig. 1D); aedeagus approximately 0.65 mm long (Figs. 1E-G).

Comparative notes. The species is separated from all its consubgeners by the male sexual characters, especially by the ventral process of the aedeagus which is of different shape, particularly in lateral view. For illustrations of the male sexual characters of these species in Turkey see the figures in Assing (2002, 2007, 2010, 2011, 2015) and Anlaş (2014, 2015).

Based on the similar morphology of the male primary and secondary sexual characters, the new species is closely related to *Astenus sandiklicus* Anlaş, 2014 (Afyonkarahisar province) and *Astenus kumlutasi* Anlaş, 2015 (Afyonkarahisar and Uşak province), but distinguished as follows: from *A. sandiklicus* Anlaş by the different coloration (*A. sandiklicus*: head, pronotum and elytra reddish brown, abdomen blackish brown with the narrow posterior margins of the tergites and the apex somewhat paler), by the different shape of the apical portion of ventral process of the aedeagus in lateral process (*A. sandiklicus*: apical portion of ventral process shorter and weakly roundish shaped in lateral view); from *A. kumlutasi* Anlaş by the different coloration (in *A. kumlutasi*: forebody completely blackish, antennae rufous, legs reddish brown, with the femora slightly darker), by the much shorter antennae (in *A. kumlutasi*: antennae average 1.1 mm long), by the slightly narrower incision of the posterior margin of the male sternite VIII and by the different shape of the ventral process of the aedeagus in lateral view (in *A. kumlutasi*: apical portion of ventral process with more distinct protruding-shaped in lateral view). For descriptions and illustrations of the species are above (see Figures 2A, C) and the respective references.

Distribution and bionomics. The new species was collected in two localities in the Ahır Mountains, in Afyonkarahisar province, in grassland at an altitude of about 1800-1900 m. All specimens were collected in the nests of *Tetramorium* sp. (Hymenoptera: Formicidae: Myrmicinae). This species is most probably endemic to the Ahır Mountains.

Faunistic records

***Astenus* (*Eurysunius*) *sandiklicus* Anlaş, 2014** (Figure 2A)

Material examined: Afyonkarahisar: 3♀♀, 01.V.2015, Dinar, Karakuş Mountains, Şablalı Hill, 38°09'42"N, 30°26'11"E, 1960 m, leg. Yağmur, Örgel & Altın; 2♀♀, 31.V.2015, Karakuş Mountains, Şablalı Hill, 38°09'48"N, 30°26'19"E, 1990 m, leg. Yağmur & Örgel.

Distribution and Comments: The recently described species was known from Sandıklı Mountains, Afyonkarahisar province of Turkey (Anlaş, 2014, 2015). This species is recorded again from the surroundings of Sandıklı Mountains. Assing (2015) remarks that the “The illustrations provided in the original description do not reveal any differences whatsoever between the aedeagus of *Astenus sandiklicus* and that of the geographically close *Astenus sultanicus* Assing, 2010 suggesting that *A. sandiklicus* may represent a junior synonym”. *A. sandiklicus* seperated from *A. sultanicus* by the different coloration (in *A. sandiklicus*: head, pronotum and elytra reddish brown, abdomen blackish brown with the narrow posterior margins of the tergites and the apex somewhat paler; in *A. sultanicus*: head, pronotum, and abdomen blackish, elytra yellowish, with the anterior margin and the scutellar area narrowly, diffusely, and weakly infuscate), by the much longer and thinner body (in *A. sultanicus*: body broad and compact), more shorter antennae and by the different shape of the apical portion of ventral process of the aedeagus in lateral process (see Figs 2A-B).

Figure 2. Details of A*stenus sandiklicus* Anlaş (A), *A. sultanicus* Assing (B), *A. kumlutasi* Anlaş (C), *A. rhodicus* Assing (D-G): (A-D)— aedeagus, lateral view; (E)— aedeagus, ventral view; (F)— male sternite VII; (G)— male sternite VIII; Scale bars: 0.2 mm (A-G).

***Astenus* (*Eurysunius*) *sultanicus* Assing, 2010** (Figure 2B)

Material examined: Afyonkarahisar**:** 1♂, 4♀♀, 09.V.2015, Emir Mountains, 38°55'38"N, 31°12'43"E, 1586 m, leg. Yağmur & Örgel; 1♂, 09.V.2015, Emir Mountains, 38°54'59"N, 31°12'36"E, 1722 m, leg. Yağmur & Örgel.

Distribution: This species is known from Sultan Mountains in Konya province and Emir Mountains in Afyonkarahisar of Turkey (Assing, 2010; Anlaş, 2015).

***Astenus* (*Eurysunius*) *kumlutasi* Anlaş, 2015** (Figure 2C)

Material examined: Kütahya: 1♀, 11.V.2015, Emet, Eğrigöz Mountain, 39°22'57"N, 29°06'45"E, 1900 m, leg. Yağmur & Örgel. Uşak: 2♂♂, 3♀♀, 24.V.2015, Gediz, Murat Mountain, 38°56'58"N, 29°40'18"E, 2191 m, leg. Yağmur & Örgel.

Distribution. The very recently described species was reported in some localities from the Akdağlar and Murat Mountains, in the provinces of Kütahya and Uşak, central-western Anatolia. This species is recorded again from the surroundings of Akdağlar and Murat Mountains.

***Astenus* (*Eurysunius*) *rhodicus* Assing, 2013** (Figures 2D-G)

Material examined: Muğla: 2♂, 30.V.2015, Köyceğiz, Çiçekbaba Mountain, 37°03'13"N, 28°47'46"E, 1785 m, leg. Yağmur & Örgel.

Distribution: The recently described species was known only from the type locality on the Island Rhodos, Greece (Assing, 2013). New record for Turkey. For illustrations of the habitus and male sexual characters of this species see the figures 2D-G and Assing (2013: Figs. 7-13).

***Astenus* (*Eurysunius*) *honazicus* Anlaş, 2015**

Material examined: Denizli: 2♂, 3♀, 19.IV.2015, Ortaca Mountain, 37°41'48"N, 29°08'35"E, 1300 m, leg. Anlaş, Yağmur, Örgel & Altın.

Distribution. *A. honazicus* was known only from Honaz Dağı in Denizli province (Anlaş, 2015). This species is recorded again from the surroundings of the type locality. For illustrations of the species, see Anlaş (2015: Figs. 25-32 and 36).

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Orijinal araştırma (Original article)

Residual toxicity of Spinetoram against to bean weevil, *Acanthocelides obtectus* Say. (Coleoptera: Bruchidae) on bean

Spinetoram'ın fasulye üzerinde fasulye tohum böceği, *Acanthocelides obtectus* Say. (Coleoptera: Bruchidae)'a karşı rezidüel toksisitesi

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Summary

In present study, residual contact toxicity of spinetoram suspension applied to bean against *Acanthocelides obtectus* Say. (Coleoptera: Bruchidae) adults was investigated under laboratory conditions. In laboratory bioassays, *A. obtectus* adults were exposed to bean sprayed with spinetoram suspension at 0.1, 0.25, 0.5 and 1 ppm (mg active ingredient/kg commodity) at 26±1 ºC temperature, 65±5 % relative humidity and completely dark condition. Paralysis and mortality of the adults were recorded after 1, 3, 5 and 7 day of exposure and 40 day later the bean was examined for progeny production. Based on the results obtained from the biological tests, concentration of spinetoram suspension and the exposure period had a significant effect on paralysis and mortality rate of *A. obtectus* adults on bean. Spinetoram treatments at all concentrations after 1 day of exposure resulted in low mortality of *A. obtectus* adults. Mortality of *A. obtectus* adults increased after 1 day of exposure period. Spinetoram treatments at low concentrations (0.1 and 0.25 ppm), resulted in low mortality of paralysis or mortality of *A. obtectus* adults at all exposure times. However, spinetoram treatment at higher concentrations (0.5 and 1 ppm) after 3 day of exposure resulted in almost 100 % paralysis or mortality of *A. obtectus* adults. These results indicated that 1 ppm concentration of spinetoram is enough to obtain the complete mortality of *A. obtectus* for 3 day of exposure. Spinetoram treatments at 0.25, 0.5 and 1 ppm completely hindered its progeny production. In conclusion, based on mortality and progeny production results spinetoram would be potential to be used for control of *A. obtectus* on stored beans as an alternative protectant to the conventional insecticides.

Keywords: Spinetoram, *Acantocelides obtectus*, residual action, toxicity, bean

Özet

Laboratuvar koşullarında yürütülen bu çalışmada fasulye tanelerine uygulanmış Spinetoram'ın, Fasulye tohum böceği, *Acanthocelides obtectus* Say. (Coleoptera: Bruchidae) erginlerine karşı reziduel kontak toksisitesi araştırılmıştır. Laboratuvar denemelerinde, *A. obtectus* erginleri 26±1 ºC sıcaklık, 65±5 % nem koşullarında ve tamamen karanlık ortamda 0.1, 0.25, 0.5 ve 1 ppm (mg aktif madde/kg ürün) konsantrasyonlarındaki Spinetoram solüsyonu uygulanmış fasulye ile muamele edilmiştir. Uygulamadan 1, 3, 5 ve 7 gün sonra felç ve ölü ergin bireyler sayılmış ve 40 gün sonra yeni nesil ergin çıkışları gözlemlenmiştir. Biyolojik testlerden elde edilen sonuçlara göre fasulye üzerine uygulanan Sipenetoram konsantrasyonları ve uygulama süreleri, *A. obtectus* erginlerinin felç ve ölüm oranları üzerine önemli derecede etkiye sahip olduğu bulunmuştur. Spinetoram'ın tüm konsantrasyonları, 1 gün uygulama süresinde *A. obtectus* erginlerin düşük ölümlerine neden olmuştur. Bir günden sonraki uygulama sürelerinde *A. obtectus*'un ölüm oranlarında önemli artış görülmüştür. Spinetoram'ın düşük konsantrasyonları (0.1 ve 0.25 ppm) tüm uygulama sürelerinde, *A. obtectus* erginlerin düşük felç ve ölümüne neden olmuştur. Ancak, yüksek konsantrasyonlarda (0.5 ve 1 ppm) 3 gün uygulama süresinde *A. obtectus* erginlerin hemen hemen % 100 felç ya da ölümü görülmüştür. Elde edilen bu sonuçlar *A. obtectus* erginlerinin tamamını öldürmek için 1 ppm uygulama konsantrasyonu ve 3 günlük uygulama süresinin yeterli olduğunu ortaya koymuştur. Spinetoram'ın 0.25, 0.5 ve 1 ppm uygulama konsantrasyonları yeni nesil ergin çıkışlarını tamamen engellemiştir. Ölüm ve yeni nesil ergin sonuçları, Spinetoram'ın konvensiyonel insektisitlere bir alternatif koruyucu insektisit olarak depolanmış fasulyelerde zararlı *A. obtectus* mücadelesinde kullanılabilme potansiyeline sahip olabileceğini göstermiştir.

Anahtar sözcükler: Spinetoram, *Acantocelides obtectus*, rezidüel etki, toksisite, fasülye

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Introduction

Worldwide, the dry bean (*Phaseolus vulgaris* L.) (Fabaceae) is the most economically and nutritionally important legume for human consumption (Jones, 1999). Bean is one of the most commonly used vegetables in human nutrition particularly in developing countries (Jones et al., 2011; Lopes et al., 2015) and the common bean is estimated as the third-largest source of calories and the second-largest source of dietary protein (Hillocks et al., 2006). However, attack by bruchids (Coleoptera: Bruchidae) during storage compromises the quality and commercial value of beans. The bruchid, *Acanthoscelides obtectus* (Say) is one of the major insect pests affecting the common bean (Hagstrum & Subramanyam, 2009; Mutungi et al., 2015). Larvae developing within the grain cause the largest damage (Swella & Mushobozy, 2007), causing a reduction of dry matter and, hence, grain mass (Padin et al., 2002). Thus, besides the reduction in grain weight, the insects destroy the embryo while feeding, reducing the germination ability of the beans (Padin et al., 2002; Caneppele et al., 2003). Given the destructive power of bruchids, many farmers sell their entire crop of beans immediately after harvest when the prices in the market are still low, and they do not store seeds for the next sowing season or for their own consumption (Schmale et al., 2006; Lopes et al., 2015).

The control of *A. obtectus* relies mainly on the application of fumigation and synthetic insecticides on stored beans. Currently, phosphine (PH3) gas has been used for fumigation of stored beans infested by the insect pests. But resistance problems of phosphine has already reported in a number of countries, with very high levels of resistance in some parts of Asia and Africa (Mills, 1983; Taylor & Halliday, 1986; Taylor, 1989; Zettler, 1997; Sayaboc et al., 1998; Rajendran, 1999), Australia (Collins et al., 2001; Nayak et al., 2010; Emery et al., 2011) more recently in America (Opit et al., 2012; Saglam et al., 2015). The synthetic insecticides used against stored bean insects are primarily organophosphorus and pyrethroid compounds, and the residues from a single application can often prevent insects from establishing in stored beans. However, use of residual insecticides is becoming less desirable because of the resistance in major insects (Pimentel et al., 2007), regulatory restrictions on use of insecticides, awareness of environmental pollution, the increasing cost of storage insecticides, erratic supplies, worker safety and consumer desire for a pesticide-free product. All the above issues raise the need for the development of new active ingredients that pose fewer concerns for both humans and the environment and are more compatible with Integrated Pest Management (IPM) approaches in stored-grain protection.

Spinosyns group insecticides exhibit low mammalian toxicity and are considered harmless for the environment since they degrade to simpler fragments containing only carbon, oxygen, nitrogen, and hydrogen (Dripps et al., 2011). Spinosad is a naturally occurring mixture of spinosyns A (primary component) and D (minor component) (Sparks et al., 1999; Saldago & Sparks, 2005), Spinosad acts on the insect nervous system at a unique site on the nicotinic acetylcholine receptor, and is active through contact or ingestion (Dripps et al., 2011). Spinosad can be used effectively for organophosphate and pyrethroid resistant strains of several stored product insects (Daglish, 2008). Spinosad possesses a unique mode of action in insects and controls insect strains resistant to other grain protectants (Hertlein et al., 2011). Also, in comparison with OPs and pyrethroids, Pozidi-Metaxa & Athanassiou (2013), reported that spinosad was more effective than chlorpyriphos-methyl and equally effective as deltamethrin and pirimiphos-methyl against the larger grain borer, *Prostephanus truncates* (Horn) (Coleoptera: Bostrychidae).

Recently, spinetoram that is a mixture of two synthetically modified spinosyns (spinosyn J and spinosyn L), which are metabolites of the bacterium *Saccharopolyspora spinosa* Mertz and Yao (Bacteria: Actinobacteridae), was introduced as a new spinosyn insecticide with greater potency and faster speed of action in comparison with spinosad (Dripps et al., 2008; Sparks et al., 2008). Recently, spinetoram has been tested and found to be effective for the control of several stored grain beetle species (Vassilakos et al., 2012; Isikber et al., 2013) while its efficacy was practically not affected by temperature and relative humidity (RH) (Vassilakos & Athanassiou, 2013). Spinetoram has some surface treatment studies against all life stages of *Tribolium confusum* du Val. (Saglam et al., 2013). Vassiliakos & Athanassiou (2012) suggested that spinetoram is very effective against *R. dominica*, moderately effective against *S. oryzae*, and not very effective against *T. confusum.* Spinetoram is considered more active and more persistent than spinosad (Dripps et al., 2011).

In spite of the fact that there are several published studies for the efficacy and toxicity of Spinetoram against some stored grain insects (Vassilakos et al., 2012; Vassiliakos & Athanassiou, 2012; Vassiliakos & Athanassiou, 2013). However, to our knowledge, the efficacy of spinetoram against stored bean insects has not been tested so far. In the present work, residual toxicity of spinetoram against bean weevil, *A. obtectus* on beans was tested under the laboratory conditions.

Material and Methods

Test insect

The *A. obtectus* strain used in this study was obtained from laboratory culture that originated from bean seeds collected around Mersin Province, Turkey in October 2010. *A. obtectus* was reared on uninfested bean (*Phaseolus vulgaris*) at 1 l glass jars (8.6 cm in diameter and 17.5 cm in height) in incubators set at 65±5 % RH and 27±1 °C, under continuous darkness. New subcultures were established weekly, by removing approximately 100 beetles from each of the two oldest jars, the oldest then being discarded.

Commodity

Uninfested and untreated bean (*Phoselus vulgaris* L. var. Elbistan) with 8±0.5 % of moisture content was used for the bioassays and insect rearing. The bean was placed a freezer at -18 °C for one week to destroy any remaining insects before the seed was used for insect rearing and laboratory trials. Moisture content of the bean was measured by using by using KETT-Pm-600 moisture meter (Kett Electric Laboratory, Japan).

Insecticide and insecticide treatment

A water dispersible granule (WG) formulation of spinetoram (Delegate 250 WG) that contained 250 g of active ingredient (AI) per liter and was supplied by Dow AgroSciences, UK was used for bioassays. One kg of bean was sprayed with spinetoram to create four concentration levels: 0 (control), 0.1, 0.25, 0.5 and 1 ppm (mg AI/kg of bean). The spinetoram WG formulation was suspended with distilled water to prepare each concentration and 1 ml of the appropriate suspension was sprayed in each lot. The insecticide application was made by using HSENG Airbrush AS18 model (Ningbo Haosheng Pnömatik Machinery Co., Zhejiong, China). To achieve even distribution of the insecticide, the bean was spread into a plastic tray (48 x 33 x 8 cm) providing a thin mono layer as a spraying surface. Then, the sprayed bean lots in the plastic tray were shaken manually for approx. 1 min to enhance the insecticide distribution. Additional lots of 1 kg of beans were sprayed with distilled water as a control treatment. After application, commodities were left one day for drying under laboratory conditions

Bioassays

Cylindrical glass vials with 450 ml of capacity were used as the experimental units for bioassays. For each spinetoram concentration, five samples, each of 200 g, were taken from each jar of treated bean and placed in vials. Then, 25 one to two-day old and mixed sex adults of *A. obtectus* were introduced into each vial (separate vials for each concentration). All these vials were placed in incubators set at 25±1 °C, 65±5 % RH and continuous darkness. Dead (no motion) and paralysis (only moving antenna and legs) of the exposed individuals were recorded after 1, 3, 5 and 7 day of exposure in the treated and untreated substrate. After the 7 day of exposure, all adults (dead or alive) were removed and the jars returned at the experiment conditions. Forty days later (Rees, 2004), adult progeny emergences (F_1) were counted in the vials.

Data analysis

For each count, mortality rate, paralysis rate and morality + paralysis rate of *A. obtectus* adults were calculated. Control mortality was generally low, so no correction was considered necessary. Adult mortality rate, paralysis rate and morality + paralysis rate were analyzed separately for each species using the MANOVA Fit Repeated Measures Procedure with Wilk's lambda estimate of JMP software (Sall et al., 2001), with dose rate as main effects, and time as the repeated variable. Arcsine transformation was applied to mortality and paralysis data that were subjected by one-way ANOVA (Factor: concentration). For progeny production, one-way ANOVA was performed, by using the same software,

with number of progeny as the response variable, and concentration as main effect. In this case, the number of progeny in the control vials was also included in the analysis. The means were separated using Duncan test at the 5% level (Proc CM: One-way ANOVA, SPSS Statics 18, 2009).

Results

Regarding to mortality counts, all main effects and their interactions were significant as repeated measures MANOVA parameters (Table 1). Generally, mortality of *A. obtectus* adults increased with increasing of concentration at each exposure time, apart from first day. In all exposure times, except first day, spinetoram treatments at concentration of 1 ppm resulted in significantly higher mortality than those at the other concentrations. The lowest mortalities were achieved at 0.1 ppm concentration at each exposure time. After 7 day of exposure, 48.8%, 66.4%, 93.6% and 100% of mortality of *A. obtectus* were obtained at 0.1, 0.25, 0.5 and 1 ppm concentration of spinetoram respectively. Thus, the complete mortality was achieved only at 1 ppm for 5 and 7 day of exposure (Fig. 1).

Table 1. Repeated measures MANOVA parameters for mortality, paralysis and mortality + paralysis counts of the bean weevil (in all cases, error df=20)

	df	Mortality	Paralysis	Mortality + Paralysis	*P*
		F	F	F	
All between	4	106.4055	80.6058	190.5743	<0.0001
Intercept	1	1178.7295	948.0423	1959.0035	<0.0001
Dose	4	106.4055	80.6058	190.5743	<0.0001
All within	12	9.9648	13.7511	9.1045	<0.0001
Time	3	401.5952	44.5557	181.5643	<0.0001
Time*Dose	12	9.9648	13.7511	9.1045	<0.0001

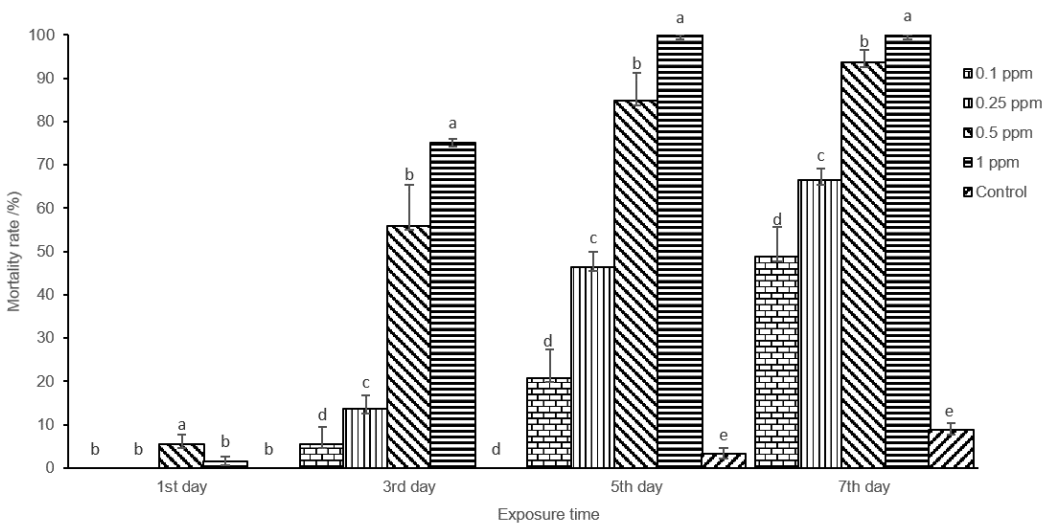

Fig. 1. Mean mortality (%±SE) of *Acanthocelides obtectus* on beans treated by spinetoram at the different concentrations for 1, 3, 5 and 7 day of exposure time (Means followed by the same lower case letter at each concentration are not significantly different; Duncan test at 0.05; Errors bars on the graph indicate the standard error of mean mortality of each treatment).

Regarding to paralysis levels, all main effects and their interactions were significant (Table 1). After 7 day of exposure, 0%, 12.8%, 60.8% and 95.2% of mortality of *A. obtectus* were obtained at 0.1, 0.25, 0.5 and 1 ppm respectively. Spinetoram treatments at 0.5 and 1 ppm after 1 day of exposure had significantly higher paralysis levels of *A. obtectus* than those at 0.1 and 0.25 ppm. However, very low paralysis levels were obtained at same concentrations after 5 and 7 day of exposure. Paralysis data indicated that adults of *A. obtectus* exposed on beans exposed spinetoram at high concentrations (0.5 and 1 ppm) were highly paralyzed just after 1 day of exposure (Fig. 2).

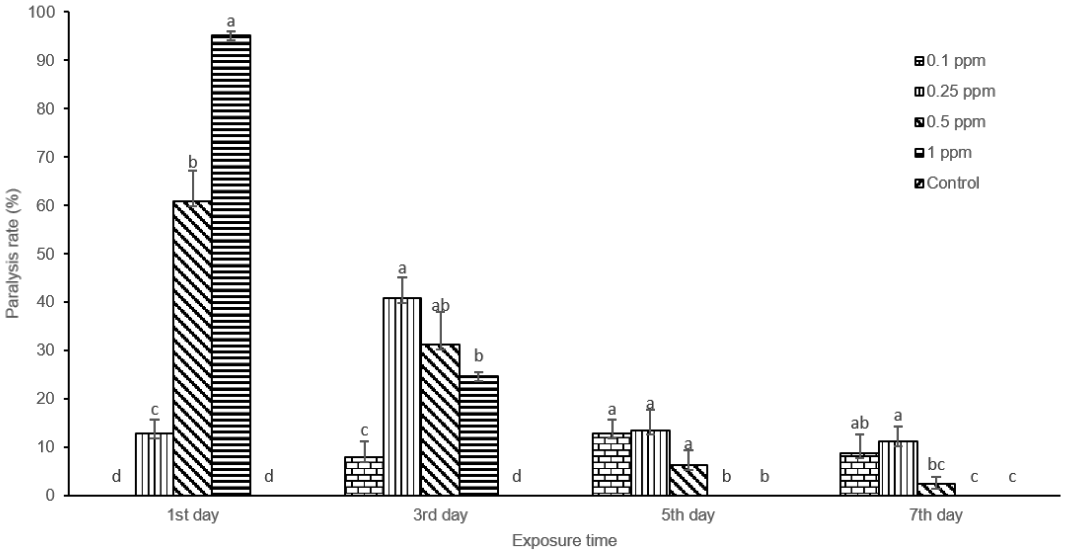

Fig. 2. Mean paralysis levels (%±SE) of *Acanthocelides obtectus* adults on beans treated with spinetoram at the different concentrations for 1, 3, 5 and 7 day of exposure (Means followed by the same lower case letter at each concentration are not significantly different; Duncan test at 0.05; Errors bars on the graph indicate the standard error of mean mortality of each treatment).

Analysis of mortality+paralysis data indicated that all main effects and their interactions were also significant (Table 1). Generally, mortality+paralysis level of *A. obtectus* adults increased with increasing of concentration at all exposure times, apart from 7-day of exposure. Mortality+paralysis levels of *A. obtectus* adults at 1 ppm were higher than those at the other concentrations for 1, 3 and 5 day of exposure. However, after 7 day of exposure, mortality+paralysis levels at 0.5 and 1 ppm were statistically similar whilst they were higher than those at 0.1 and 0.25 ppm. 100% or nearly 100% mortality+paralysis level of *A. obtectus* adults was achieved at 1 ppm after 1, 3 and 5 day of exposure, whilst it was obtained at 0.5 and 1 ppm after 7 day of exposure.

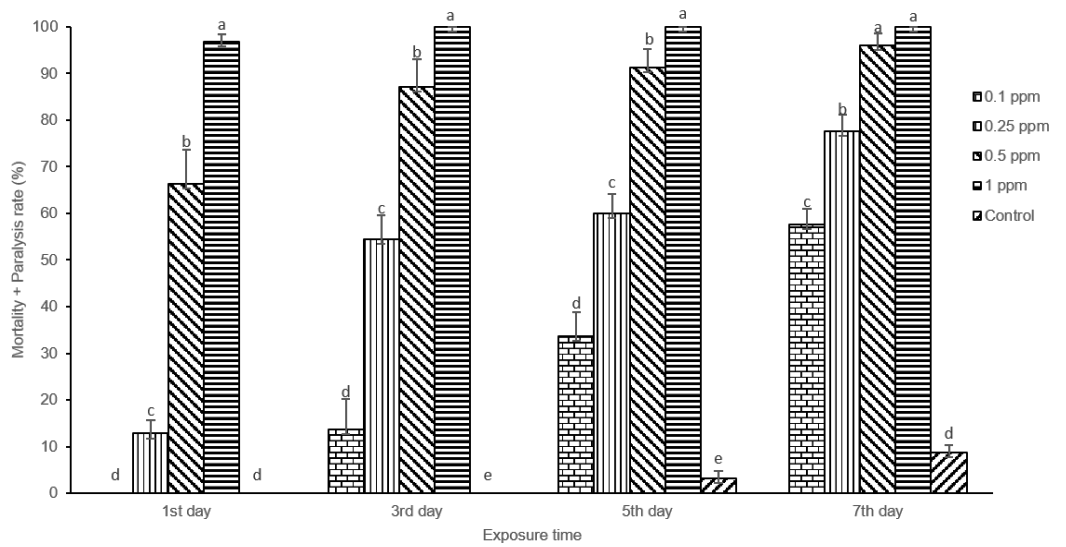

Fig. 3. Mean mortality + paralysis levels (%±SE) of *Acanthocelides obtectus* on beans treated with spinetoram at the different concentrations for 1, 3, 5 and 7 day of exposure (Means followed by the same lower case letter at each concentration are not significantly different; Duncan test at 0.05; Errors bars on the graph indicate the standard error of mean mortality of each treatment).

Progeny production (F_1)

There were significant differences in progeny production between spinetoram concentrations and control treatment ($F_{3,20}$=433.9, P<0.0001). At 0.25, 0.5 and 1 ppm, no progeny of *A. obtectus* was produced, whilst progeny production was observed at the lowest concentration (0.1 ppm). However, progeny production at 0.1 ppm was significantly lower than that at control treatment. These results indicated that spinetoram treatment at 0.25, 0.5 and 1 ppm would completely suppress the progeny production of *A. obtectus* (Fig. 4).

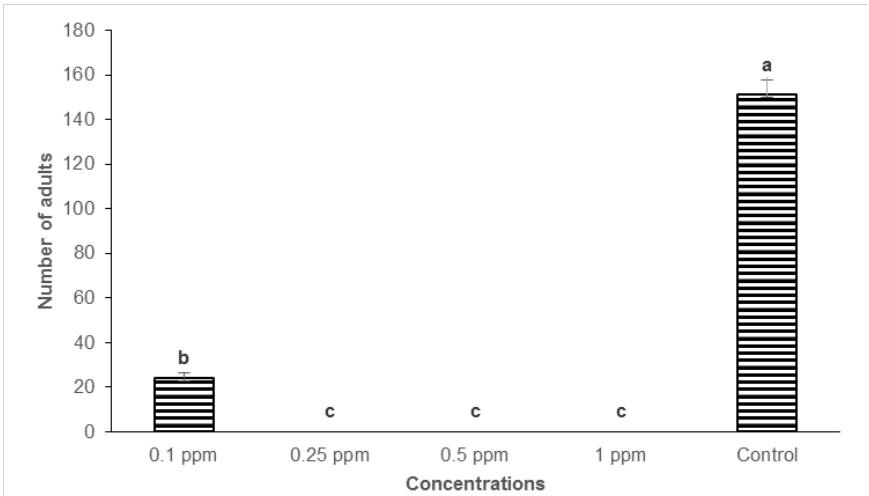

Fig.4. Mean number of adult progeny of *Acanthocelides obtectus* on beans treated by spinetoram at the different concentrations (Means followed by the same upper case letter at each concentration are not significantly different; Duncan test at 0.05; Error bars on the graph indicate the standard error of mean number of adult progeny of each treatment).

Discussion

Several grain protectants can provide long-term protection against a wide range of stored-product beetle species. However, despite the fact that persistence is a desirable characteristic of a given grain protectant, the use of an insecticide that is toxic to mammals in conjunction with high residues on the products, is not permitted in stored product protection. Therefore, the use of an insecticide of very low mammalian toxicity, such as spinetoram (Rat oral LD_{50} > 5000 mg/kg of body weight) can be considered as a safe solution in this respect.

Based on the results obtained from the biological tests, the concentration of spinetoram suspension and the exposure period had a significant effect on paralysis and mortality rate of *A. obtectus* adults on beans. The mortality of *A. obtectus* significantly increased with increasing the concentration of spinetoram and exposure time. Spinetoram treatments at all concentrations after 1 day of exposure resulted in low mortality of *A. obtectus* adults. Mortality of *A. obtectus* adults increased after 1 day of exposure period. Spinetoram treatments at low concentrations (0.1 and 0.25 ppm), resulted in low mortality of paralysis or mortality of *A. obtectus* adults at all exposure times. However, spinetoram treatment at higher concentrations (0.5 and 1 ppm) after 3 day of exposure resulted in almost 100 % paralysis or mortality of *A. obtectus* adults. These results indicated that 1 ppm concentration of spinetoram is enough to obtain the complete mortality of *A. obtectus* for 3 day of exposure. At 0.25, 0.5 and 1 ppm, no progeny of *A. obtectus* was produced, whilst progeny production was observed at the lowest concentration (0.1 ppm). These results indicated that spinetoram treatments at 0.25, 0.5 and 1 ppm completely hindered its progeny production.

Currently, no studies for efficacy of spinetoram against *A. obtectus* on beans have been published in literature. However, there are some studies published about efficacy of spinetoram against several stored grain insects. Vassilakos et al. (2012) found that spinetoram was effective against *Tribolium confusum* du Val. (Coleoptera: Tenebrionidae) only in the high doses of 5 and 10 ppm (mg of AI/kg of grain) and ineffective at 2 ppm after 21 days of exposure in treated wheat. *Tribolium confusum* young larvae are susceptible to both spinosad and spinetoram (Vayias et al., 2009; Saglam et al., 2013). *Rhyzopertha dominica* F. (Coleoptera: Bostrichidae) was the most susceptible among the species, while concentrations of 0.5 and 1 ppm were needed to control *Sitophilus granarius* L. (Coleoptera: Curculionidae) and *Sitophilus oryzae* L. (Coleoptera: Curculionidae) adults, respectively. These findings for the concentration of spinetoram required to obtain the complete mortality of *R. dominica, S. granarius* and *S. oryzae* on wheat stand in accordance with the results obtained in present study for *A. obtectus* on beans. However, compared with the findings for *T. confusum* on wheat, reported by Vassilakos et al. (2012), the concentration of spinetoram required to obtain the complete mortality of *A. obtectus* in present study is much lower than that for *T. confusum.* This discrepancy can be due to the difference in insect species and commodity tested. Likewise, previous studies document that the insecticidal efficacy of spinosad and spinetoram is affected by several biotic or abiotic factors, such as the target species, the type of commodity, the exposure interval and the type of surface that spinosad is applied to (Fang et al., 2002; Subramanyam et al., 2003; Toews & Subramanyam 2003; Toews et al., 2003; Nayak et al., 2005; Daglish & Nayak, 2006; Subramanyam, 2006; Subramanyam et al., 2007; Vassilakos et al., 2015).

In this study, spinetoram proved to be effective against *A. obtectus* on bean. Based on mortality and progeny production results, at 1 ppm, spinetoram was found to result in the complete mortality of *A. obtectus* and completely prevent its progeny production. In conclusion, present study indicated that spinetoram would be potential to be used for control of *A. obtectus* on bean as an alternative protectant to the conventional insecticides. However, further research is needed to obtain data on its persistence on beans, its toxicity for other stored bean insects under laboratory and field conditions.

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Orijinal araştırma (Original article)

Karyotype analysis of *Zygoribatula cognata* (Oudemans) (Acari: Oribatida: Oribatulidae)

Zygoribatula cognata (Oudemans)'nın (Acari: Oribatida: Oribatulidae) karyotip analizi

Halil Erhan EROĞLU[1*] **Sedat PER[1]**

Summary

Karyotypic characters, mitotic metaphase chromosomes, monoploid ideogram and karyogram of *Zygoribatula cognata* (Oudemans) (Acari: Oribatida: Oribatulidae) were investigated for the first time. The chromosome number of *Zygoribatula cognata* was 2n = 30. The chromosomes were holocentric characterized with lack a localized centromere. No satellite was observed in the chromosomes. The sex chromosomes and sex determination of *Zygoribatula cognata* could not be determined.

Keywords: *Zygoribatula cognate*, oribatid mite, karyotype, holocentric chromosome

Özet

Bu çalışmada, *Zygoribatula cognata*'nın (Oudemans) (Acari: Oribatida: Oribatulidae) karyotipik karakterleri, mitotik metafaz kromozomları, monoploid idiyogramı ve karyogramı ilk kez araştırılmıştır. *Zygoribatula cognata*'nın kromozom sayısı 2n = 30'dur. Kromozomlarının lokalize bir sentromer eksikliği ile karakterize olan holosentrik yapıda olduğu saptanmıştır. Kromozomlarda satellit gözlenmemiştir. *Zygoribatula cognata*'nın cinsiyet kromozomları ve cinsiyet tayini belirlenememiştir.

Anahtar sözcükler: *Zygoribatula cognata*, oribatid akar, karyotip, holosentrik kromozom

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Introduction

Oribatid mites are placed in the order Oribatida Dugès in the subclass Acari Leach. The oribatid mites often called “moss mites” or “beetle mites”, are associated with organic matter in most terrestrial ecosystems. They found throughout the soil profile, in surface litter, on grasses, herbs and low-growing shrubs, the bark, twigs and leaves of trees, and in aquatic, semi-aquatic and coastal habitats (Behan-Pelletier, 1999). Orbatids comprise more than 10000 species representing 172 families (Gan, 2013).

The genus *Zygoribatula* Berlese placed in the family Oribatulidae Thor is distinguished from *Oribatula* Berlese with the presence of a strong translamella (Fritz, 1982). This genus has a cosmopolitan distribution and consists of at least 91 species and 6 subspecies are known (Subías, 2004; Bayartogtokh & Smelyansky, 2008).

The species *Zygoribatula cognata* (Oudemans) is distributed in the Palaearctic region (Subías, 2004). There are many studies on distribution, habitats and morphology of this species (Fritz, 1982; Ayyıldız, 1988; Behan-Pelletier, 1999; Subías, 2004; Bayartogtokh & Smelyansky, 2008; Gan, 2013).

Although there are many studies on *Z. cognata*, there is no information about the chromosome number and karyotype analysis in the literature. The aim of this study is to investigate the chromosome number, karyotype, ideogram and other detailed chromosomal measurements of *Z. cognata*.

Materials and Methods

The samples collected from natural habitats by Sedat Per. The collecting data is: Turkey: Kayseri, Erciyes Mountain, 38° 35.988' N, 35° 30.575' E, 1944 m, in moss under *Quercus pubescens* Willd. (Fagaceae), 11.XI.2001. 8 samples were used for chromosomal preparations.

Chromosomal preparations were obtained from the technique developed by Imai et al. (1988) and substantially modified by Gokhman & Quicke (1995). The samples were crushed in 1% hypotonic sodium citrate solution containing 0.005% colchicine (Sigma-Aldrich, Taufkirchen, Germany). Material was incubated with a fresh hypotonic solution for 20 min. Then the material was treated with solutions of fixative 1 (glacial acetic acid: absolute ethanol: distilled water 3:3:4), fixative 2 (glacial acetic acid: absolute ethanol 1:1), and fixative 3 (glacial acetic acid). After fixation process, the material was transferred onto pre-cleaned glass slides. The preparation was dried for 30 min, it was stained with Giemsa (Sigma-Aldrich, Taufkirchen, Germany).

10 metaphase plates were obtained. A qualified metaphase plate was selected and photographed with Olympus BX53 microscope (Figure 1). The chromosomes were measured in micrometers (µm) using the Bs200ProP image processing and analysis system. The karyogram and ideogram were drawn based on length of chromosome size (arranged large to small). In Figure 2 the karyogram derived from the metaphase plate in Figure 1. In Figure 1, the chromosomes were grown and separated with Adobe Photoshop CS5. The karyogram was formed. The sharpness was given to the karyogram.

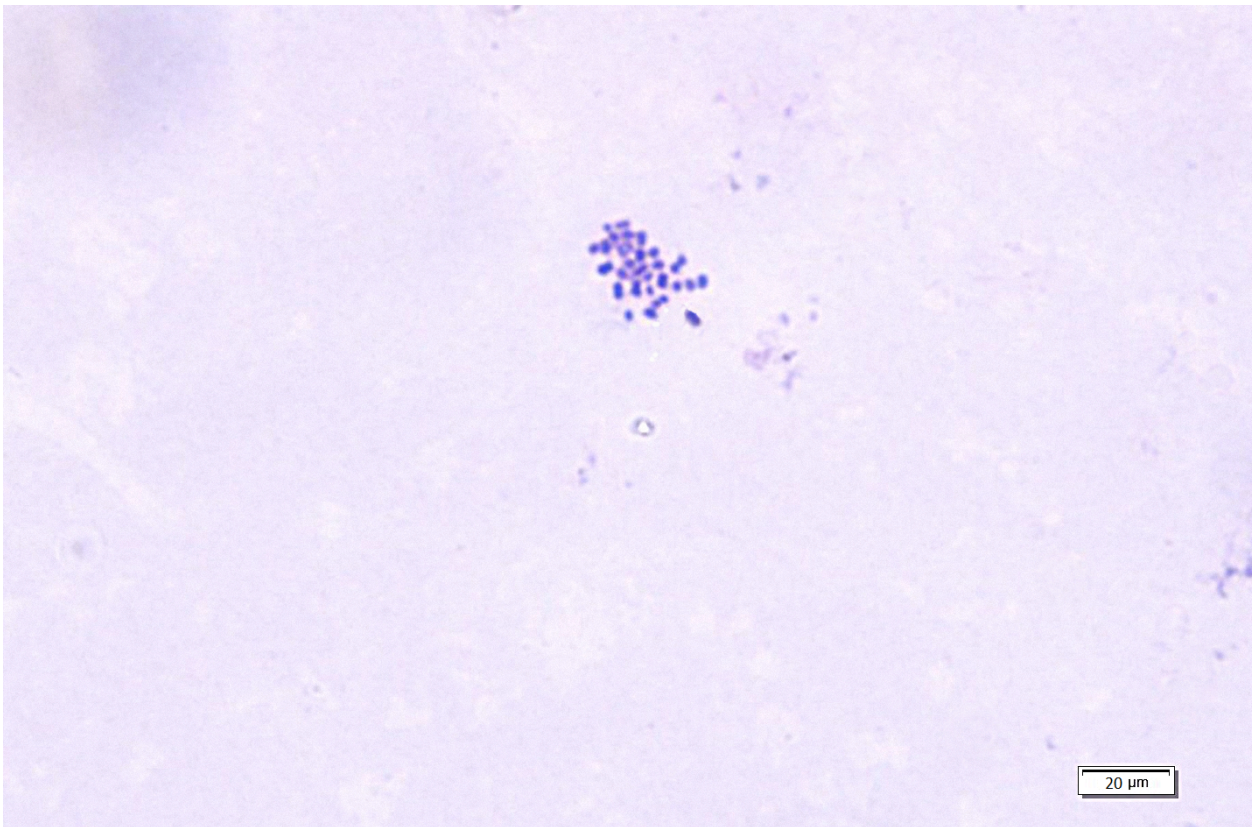

Figure 1. Somatic metaphase chromosomes of *Zygoribatula cognata*.

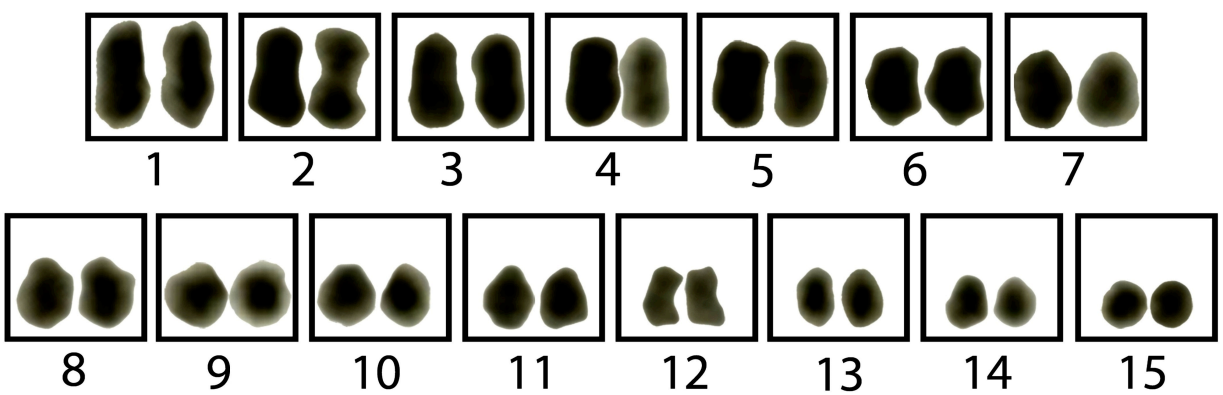

Figure 2. The karyogram of *Zygoribatula cognata*.

Results

Mitotic metaphase chromosomes, karyotype and monoploid ideogram of *Z. cognata* shown in Figures 1-3, respectively. Analysis of somatic metaphases shown that the chromosome number of the species was 2n = 30. Karyotype formula could not be given because the chromosomes were holocentric. No satellite was observed in the chromosomes.

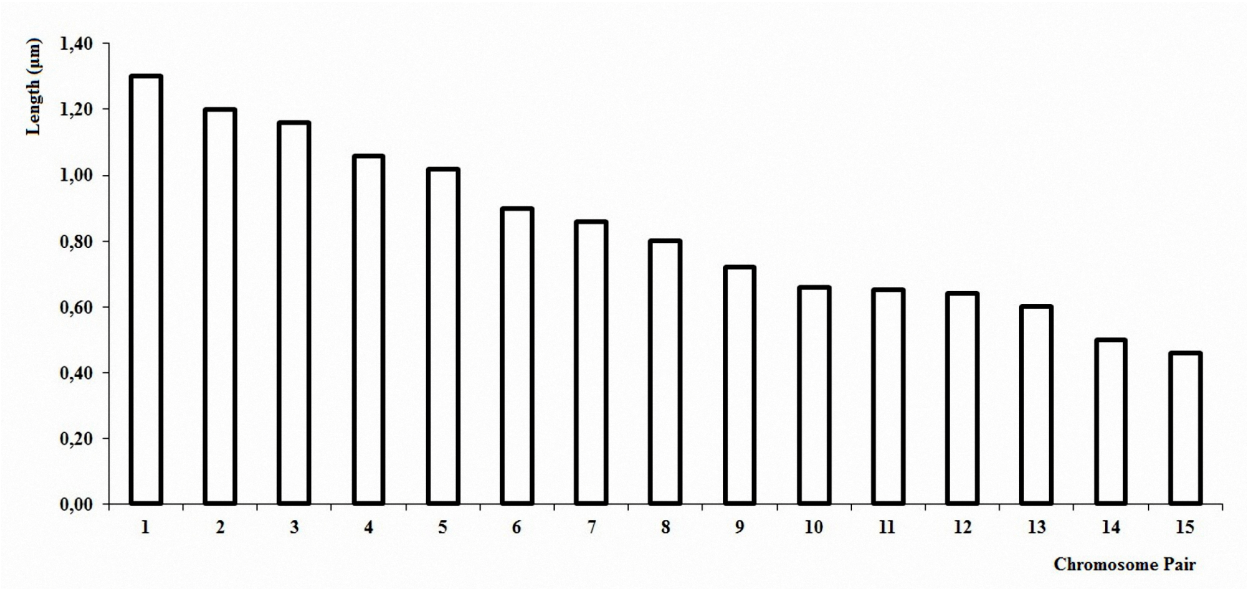

Figure 3. The ideogram of *Zygoribatula cognata*.

The measurement data of the chromosomes were given (Table 1). The length of chromosomes varied from 0.46 to 1.30 µm, and the average length of chromosomes was 0.84 ± 0.26 µm. The total haploid length was 12.53 µm.

Table 1. The measurement data of the chromosomes of *Zygoribatula cognata*

Chromosome Pair	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15
Length (µm)	1.30	1.20	1.16	1.06	1.02	0.90	0.86	0.80	0.72	0.66	0.65	0.64	0.60	0.50	0.46

Discussion

The chromosome number of *Z. cognata* is 2n = 30. The situation is uncommon. Although the chromosome numbers in mites and ticks vary from 2 to 36 (Oliver Jr., 1977), the oribatid karyotypes usually have much smaller chromosome numbers in the range of 14-18 (Norton et al., 1993; Heethoff et al., 2006). A comparable situation was reported from two sibling species of muntjak which are Indian muntjac (*Muntiacus muntjak*, Zimmermann) and Chinese muntjac (*Muntiacus reevesi*, Ogilby) (Yang et al., 1995). Chromosome numbers of *Muntiacus* are extremely variable, ranging from 6 in Indian muntjac to the relatively high number of 46 in Chinese muntjac (Wang & Lan, 2000).

The chromosomes are holocentric or holokinetic chromosomes. The holocentric chromosomes are characterized with lack a localized centromere. In contrast, monocentric chromosomes are characterized with a single localized centromere in many organisms (Schvarzstein et al., 2010). Chromosome break is a diagnostic feature of holocentric chromosomes. In addition, if a holocentric chromosome is fragmented, each fragment retains centromere activity. The holocentric chromosomes, owning to their diffuse kinetochor activity, do at least theoretically pose no restriction to the multiplication of chromosomes via multiple breakages, as the recovery of all fragments after chromosome break (Melters et al., 2012). The holocentric chromosomes have been described in arthropods such as Acari, Odonata, Hemiptera and Lepidoptera (White, 1973; Heethoff et al., 2006). The small holocentric chromosomes are approximately

0.5–2 µm length (Wrensch et al., 1994). Mitotic figures of metaphase plates of *Z. cognata* have small holocentric chromosomes between 0.46–1.30 µm. Wrensch et al. (1994) reported that there are holocentric chromosomes in the oribatid mites and superorder Acariformes. Many mites such as *Platynothrus* (Berlese) (Camisiidae) (Taberly, 1987; Palmer & Norton, 1992), *Trhypochthonius tectorum* (Berlese) (Trhypochthoniidae) (Taberly, 1987; Palmer & Norton, 1992) *Tetranychus urticae* (Koch) (Tetranychidae) (Wrensch et al., 1994), *Hemisarcoptes coccophagus* (Meyer) (Hemisarcoptidae) (Izraylevich et al., 1995) and *Archegozetes longisetosus* (Aoki) (Trhypochthoniidae) (Heethoff et al., 2006) have holocentric chromosomes.

The sex chromosomes of *Z. cognata* could not be determined. In addition, the sex determination of *Z. cognata* is quite difficult according to morphological features. Gender of the samples could not be determined because their genital opening are covered with plates and very small. There are diplodiploidy in the Acari and their ancestors (Norton et al., 1993; Wrensch et al., 1994). In diplodiploidy, sex determination is often performed with sex chromosomes and sex ratio is 1:1, approximately (Fisher, 1930). Despite diplodiploidy, the sex determination is unknown in order Oribatida with the lack of sex chromosomes (Sokolov, 1954; Norton et al., 1993; Wrensch et al., 1994; Heethoff et al., 2006). Therefore, the males and females of oribatid mites have similar karyotypes with the equal number and type of chromosomes (Sokolov, 1954). However, the sex rate is close to unity in sexual oribatid mites. Beside that males are rare and sterile in parthenogenetic species (Taberly, 1987).

The chromosome number, chromosome type and chromosome symmetry/asymmetry are important cytotaxonomic characters. The karyotype symmetry/asymmetry index formula was reported for calculation of karyotype asymmetry in animal organisms (Eroğlu, 2015). The formula includes monocentric chromosomal type and centromeric position. It is not possible to calculate the karyotype symmetry/asymmetry in animal organisms have holocentric chromosomes. Therefore, the karyotype asymmetry of *Z. cognata* can not be determined.

In this study, the chromosome number, karyotype and ideogram of *Z. cognata* were determined. The chromosome number was firstly reported.

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Orijinal araştırma (Original article)

Natural cellular immunity in field-collected insects from Hatay province by assessing nodulation[1]

Hatay yöresinden toplanan böceklerde oluşan hücresel bağışıklığın nodülasyon testi ile tespiti

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Summary

Natural microbial infections to insects collected from agrarian fields surrounding Hatay Province,Turkey were determined by assessing nodulaiton which is one of insect cellular immunity. After identifying insect specimens, the insects were dissected to assess numbers of nodules. Nodulation is one of the predominant cellular immune reactions to microbial infections and the nodules are permanently attached to internal surfaces of the insects. We collected about 660 insect specimens for nodulation and found nodules in 99 % out of all the examined specimens. Appearance of examined insects was healthy. Number of nodules in each insect ranged from 1 to 118. Our results indicated that insects are regularly challenged by microbial infections in nature and insect immune systems can limit the host range and effectiveness of microbial agents deployed in biological control programs. Therefore, undertsanding insect immune systems is important for the efficacy and use of microbial pesticides in biological control of insects.

Keywords: Insect immunology, naturally occurring infections, nodulation

Özet

Bu çalışma Hatay yöresinden toplanan böceklerde doğada mikrobiyal hastalıklara karşı oluşan hücresel bağışıklığı oratya koymak için yürütülmüştür. Böcek türleri teşhis edildikten sonra doğal mikrobiyal enfeksiyonlara karşı oluşan hücresel bağışıklıklardan nodülasyon testi için böcekler buz üzerinde bayıltılarak mikroskop altında vücutları kesilerek (dissect) açılmıştır. Böceklerde nodülasyon mikrobiyal enfeksiyonlara karşı oluşturalan hücresel bağışıklardan birisi olup böceğin iç organlarında görülmektedir. Çalışmada yaklaşık 660 böcek bireyi nodülasyon reaksiyonu için test edilmiş ve test edilen böceklerde % 99 oranında nodüle rastlanmıştır. Böcek bireylerinde nodül sayısı 1 ile 118 arasında değişmiştir. Bu sonuçlar doğada böceklerin mikrobiyal enfeksiyonlarla karşı karşıya olduğunu, böceklerin bu enfeksiyonların üstesinden gelebildiğini ve doğada böcek bağışıklığını anlamanın zararlı böceklerle mücadelede kullanılacak mikrobiyal pestisitlerin etkinliğinin ve kullanımının artırılması bakımından önemli olduğunu ortaya çıkarmıştır.

Anahtar sözcükler: Böcek bağışıklığı, doğal oluşan enfeksiyon, nodülasyon

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Introduction

Insects are regularly infected by entomopathogens including viruses, fungi and bacteria in nature. These entomopathogenic microbes can regulate insect populations in nature (Lacey et al., 2001). Insect diseases and the possibilities of using insect disease agents in biological control of insects have been known since 1957 (Steinhaus, 1957; Tanada, 1959). Currently entomopathogens including viruses, fungi, bacteria, protozoans, parasitoids, nematodes and predators are commercially used in biocontrol of insect pests, weeds and plant diseases.

Lomer (1999) mentioned that the success and failure of biocontrol of insects mostly depend on various factors, including costs, the context of comprehensive IPM programs, education of users, government activities, as well as political and environmental concerns. However, biological issues are important for successful biocontrol of insects. These biological issues include the ecological level of microbe-host population dynamics and the molecular and cell biology of host defense mechanisms, such as, insect immunity, one of crucial barriers to the success of insect biocontrol programs.

Insect immunity is comprised of a number of systems. These are physical barriers, cellular and humoral immunity. Integument and alimentary canal of insects protect them from microbial invasions as physical barriers. Once microbes pass the physical barriers surrounding insect bodies, insect cellular immunity, including phagocytosis and nodule formation, takes action against microbial invaders (Lavine & Strand, 2002; Stanley & Miller, 2006). Finally, the humoral immune system of insect, which involves anti-microbial peptides, takes some hours to activate (Lemaitre & Hoffmann, 2007). Insects also express behavioral fevers following infection. The combined arsenal of immune effector mechanisms allows insects to either stifle infections at their onset or to overcome invasions and infections.

We cannot figure out which insect immunity functions protect insects from microbial infections in nature and we do not know how insect immunity can influence biocontrol programs. However, Ouedraogo et al. (2004) mentioned that insect febrile reactions alone may affect the effectiveness of fungal biocontrol agents in laboratory and field experiments. Therefore, insect defense mechanisms can limit the effectiveness of microbes deployed for biocontrol of insect. Now, we have also known that most insects in agrarian habitats of Kahramanmaraş and Adana/Turkey experience naturally occurring infections and the insects recover from invading microbes with fast-acting cellular defense actions, including nodule formation (Tunaz & Stanley, 2009; Tunaz et al., 2015). Their work also showed that insect cellular defense mechanism, nodulation, was affected by various factors including location, season, altitude, taxonomic position, and biological stage of insects collected. Hence, to broaden this area, in this paper we investigated natural microbial infections to field-collected insects from Hatay Province, another different geographic area, by assessing nodulation, which is one of the insect cellular immune reactions. For this purpose, we hypothesized that most insects in nature experienced microbial infections and they overcome the infections. If the hyporhesis is true, insect immunity can limit the effectiveness of microbial-based biocontrol programs. Here is the report of our research results to test the hypothesis.

Materials and Methods

Organisms

Insects were collected from Hatay Province of Turkey from April, 2011 until September, 2013. Insects were collected either by hand or a net. We identified and recorded the collected insect species and their biological stages, the collection sites and site altitudes. We transferred the insects to the laboratory (20 ± 1 ^{o}C, 60 ± 5 % RH) at Kahramanmaras Sütçü İmam University. The insects were further identified to mostly species level. Voucher insect specimens were kept in the Entomology Collection, Kahramanmaras Sütçü İmam University.

Assessing nodulation

For nodulation assay, identified live insects were anesthetized by chilling on ice and then their hemocoels were exposed. We counted nodules under a stereo microscope at 45x. The determination of nodules and level of cellular immune response are based on Miller & Stanley (1998), who identified nodules as distinct, melanized, brownish-black color nodules and the number of nodules reflected the extent of cellular immune response to infections. The internal tissues including gonads, fat bodies and others were carefully probed for previously unseen nodules.

Statistical analysis

Data on nodulation were analyzed using the General Linear Models procedure, and mean comparisons were made using Duncan test (SAS Institute Inc., 1989).

Results

A total of 66 different insect species collected during winter, spring, summer and fall of 2011, 2012 and 2013 were checked for nodulation (Tables 1-3). Thirteen different insect species belonging to Lepidoptera, Hemiptera, Coleoptera, Orthoptera and Phasmida in 2011, fourthy-four different insect species belonging to Lepidoptera, Hemiptera, Coleoptera, Orthoptera, Diptera, Hymenoptera, Odonata and Phasmida in 2012, fifty-six different insect species belonging to Lepidoptera, Hemiptera, Coleoptera, Orthoptera, Diptera, Hymenoptera, Odonata, Dermaptera and Phasmida in 2013 were collected from various plants and soil and were checked for nodulation (Tables 1-3). We saw nodules in 99 % of the 660 specimens examined, although the range of nodules/specimen (from 1 nodule/insect to >118 nodules/insect) was quite wide.

Generally, more nodules were seen in the insects associated with soil than in those collected from plants (Table 4). Specifically, we observed higher number of nodules in orthopteran specimens and sunn pest adults. There were more nodules in orthopteran specimens and sunn pest adults associated with soil (Tables 1- 3). It was also noted that the overwintered generations of *Ostrinia nubilalis, Sesamia nonagrioides* and *Eurygaster integriceps* had much more nodules compared to new genaration larvae and adults (Table 1- 3). Examining insect orders for nodulation assay, significantly more nodules were recorded from orthopteran species than lepidopteran, hemipteran and coleopteran species (Table 6), which is logical because orthopteran species were mostly collected from soil. There were no nodulation differences in number among biological stages of insects. It was recorded statistically similar numbers of nodules in larvae, nymphs and adults of insect species (Table 5). While the insects collected at lower altidutes had more nodules than the insects collected at higher altitudes in 2011, there were no nodulation number differences in collecting altitudes of insects in 2012 and 2013 (Table 7). Putting together, insect orders in contact with soil are probably the main associations with higher number of nodules. However, the actual occurrence of natural infection may be a random event with no predominant patterns. On the other hand, the data indicate virtually all insects had experienced infection(s) in nature.

Table 1. Average numbers of nodules in insects collected from various sources in Hatay Province in 2011. Values indicate numbers of discrete nodules ± SEM. Collection dates are dd/m/yr

	Average number of nodules	Sources	Developmental stages	Collection dates, altitudes
Lepidoptera				
Pieris brassicae	59.4 ± 18.6	Weeds	Larvae	28/04/11, 350m
Pieris brassicae	12.3 ± 3.6	Cabbage	Larvae	21/10/11, 420m
Pieris rapae	2.7 ± 0.92	Cabbage	Larvae	21/10/11, 420m
Ostrinia nubilalis	3.6 ± 0.94	Corn stalk	Larvae	21/10/11, 420m
Sesamia nonagrioides	6.1 ± 1.84	Corn stalk	Larvae	21/10/11, 420m
Heliothis armigera	12.2 ± 5.22	Cabbage	Larvae	21/10/11,420m
Hemiptera				
Eurygaster integriceps	11.8 ± 10.4	Wheat	New generation adults	01/06/11,150m
Eurygaster integriceps	117.5 ± 38.2	Wheat	Wintered adults	28/04/11,150m
Nezara viridula	5.7±1.19	Beans	Adults	21/10/11, 420m
Eurydema ornatum	2.7±0.49	Radish	Nymphs	21/10/11, 420m
Eurydema ornatum	3.4±0.78	Radish	Adults	21/10/11, 420m
Coleoptera				
Capnodis spp.	11.1 ± 3.4	Apricot	Adults	23/04/11, 500m
Coccinella semptempunctata	2.5±0.88	Weeds	Adults	21/10/11, 420m
Orthoptera				
Acrididae	6 ± 2.57	Weeds	Adults	21/10/11 420 m
Gryllus assimilis	20.8 ± 5.14	Soil	Adults	21/10/11, 420m
Phasmida				
Gratidia sp.	28.5 ± 7.90	Soil	Adults	21/10/11 420 m

Table 2. Average numbers of nodules in insects collected from various sources in Hatay Province in 2012. Values indicate numbers of discrete nodules ± SEM. Collection dates are dd/m/yr

	Average number of nodules	Sources	Developmental stages	Collection dates, altitudes
Lepidoptera				
Arctia sp.	13.9 ± 1.9	Weeds	Larvae	26/03/12, 420m
Pieris brassicae	48.6 ± 17.3	Weeds	Larvae	23/04/12, 300m
Pieris brassicae	9.7 ± 1.64	Cabbage	Larvae	19/11/12, 411m
Pieris brassicae	20.5 ± 2.4	Cabbage	Larvae	24/12/12, 410m
Papilio machaon	20.7 ± 2.6	Weeds	Larvae	20/06/12,,270m
Thaumetopoea pityocampa	14.2± 4.3	Pine	Larvae	20/6/12,700m
Colias croceus	1.44 ± 0.41	Weeds	Adults	12/07/12,,310m
Ostrinia nubilalis	5.61.6	Corn stalk	Larvae	19/11/12, 334m
Sesamia nonagrioides	8.6 ± 3.52	Corn stalk	Larvae	19/11/12, 334m
Pieris rapae	1.2 ± 0.37	Cabbage	Adults	22/10/12, 380m
Pieris rapae	11.7 ± 2.1	Cabbage	Larvae	19/11/12, 411m
Pieris rapae	22.5 ± 4.5	Cabbage	Larvae	24/12/12, 410m
Pieris rapae	5.5 ± 1.5	Cabbage	Larvae	21/01/13, 410m
Pieris rapae	6.7 ±0.73	Cabbage	Adults	18/02/13, 387m
Helicoverpa armigera	5.42 ± 1.13	Cabbage	Larvae	22/10/12, 350m
Helicoverpa armigera	36.33 ± 5.6	Alfalfa	Larvae	19/11/12,164m
Helicoverpa armigera	25 ± 1.7	Alfalfa	Larvae	24/12/12, 164m
Acronicta spp.	17 ± 1.15	Alfalfa	Larvae	19/11/12, 164m
Coleoptera				
Cantharis sp.	5 ± 1.14	Wheat	Adults	26/3/12, 100m
Cantharis sp.	3.4 ± 0.75	Wheat	Adults	23/04/12,100m
Cantharis sp.	3.1±0.72	Wheat	Adults	18/02/13, 360m
Carabidae	7.5 ± 1.25	Weeds	Adults	28/05/12,420m
Carabidae	17.25 ± 2.91	Weeds	Adults	05/06/12,320m
Coccinella semptempunctata	3.2±0.6	Weeds	Adults	15/05/12, 420m
Coccinella semptempunctata	4.66±1.62	Weeds	Adults	13/06/12, 400m
Coccinella semptempunctata	2.7±0.78	Weeds	Adults	22/10/12, 389m
Coccinella semptempunctata	0.66±0.33	Weeds,	Larvae	19/11/12, 164m
Coccinella semptempunctata	2.6±0.68	Weeds	Adults	19/11/12, 164m
Coccinella semptempunctata	2.5±1.7	Weeds	Adults	24/12/12, 180m
Coccinella semptempunctata	0.9±0.31	Wheat	Adults	18/02/13, 361m
Larinus latus	9.7±1.37	Weeds	Adults	20/06/12, 400m
Oxythyrea cinctella	11.5±0.5	Weeds	Adults	13/06/12, 400m
Hippodemia variegata	0.428±0.2	Weeds	Adults	29/06/12, 270m
Coccinella bipunctata	0.57±0.29	Weeds	Adults	12/07/12, 310m
Hypera variabilis	0.5±0.5	Alfalfa	Adults	24/12/12, 164m
Staphylinidae	17.13±6.24	Soil	Adults	21/01/12, 138m
Hemiptera				
Dolycoris baccarum	5.87 ± 0.87	Weeds	Adults	08/06/12,400m
Dolycoris baccarum	1.6 ± 0.4	Weeds	Nymphs	28/05/12, 420m
Dolycoris baccarum	22.4 ± 6.24	Weeds	Adults	15/05/12,420m
Carpocoris mediterraneus	18 ± 5.56	Weeds	Adults	15/05/12, 420m
Carpocoris mediterraneus	30 ± 6	Weeds	Adults	08/06/12, 40 m
Eurydema ornatum	4.7 ± 1.35	Corn	Adults	26/03/12, 420m
Eurydema ornatum	4.37 ± 0.82	Weeds	Nymphs	8/06/12, 400m
Eurydema ornatum	3.5 ± 0.80	Weeds	Nymphs	12/07/12, 400 m
Eurydema ornatum	3.5±0.5	Alfalfa	Adults	19/11/12, 164m
Cercopidae	0.7 ± 0.26	Weeds	Adults	26/03/12, 100 m
Eurygaster integriceps	105.1 ± 14.078	Soil	Wintered adults	23/04/12, 90m
Eurygaster integriceps	12 ± 1.53	Wheat	New generation adults	20/06/12, 390m
Miridae	5.1 ± 1.7	Weeds	Adults	15/05/12, 320m
Miridae	6.6 ± 4.17	Weeds	Adults	13/06/12, 390m
Aelia rostrata	7.5 ± 2.5	Wheat	New generation adults	20/06/12,390m
Ancyrosoma leucogrammes	10.66 ± 3.71	Weeds	Adults	08/06/12, 381m
Ancyrosoma leucogrammes	2 ± 0.57	Weeds	Adults	12/07/12, 400m
Cicadidae	6.6 ± 1.77	Weeds	Adults	13/06/12, 400 m
Nezara viridula	5.5±0.94	Weeds	Adults	20/6/12, 230m
Rhynocoris sp	10 ± 1.73	Weeds	Adults	13/06/12, 39 m

Table 2. (Continued)

	Average number of nodules	Sources	Developmental stages	Collection dates, altitudes
Orthoptera				
Acrididae	18 ± 3.57	Weeds	Adults	28/05/12 420m
Acrididae	37 ± 1.6	Weeds	Adults	13/06/12 400m
Acrididae	30.5± 5.139	Weeds	Adults	12/07/12 428m
Acrididae	23.87 ± 2.71	Weeds	Adults	22/10/12, 389m
Acrididae	52.3 ± 3.6	Soil	Adults	19/11/12, 175m
Acrididae	54 ± 9.6	Soil	Adults	24/12/12, 385m
Acrididae	39 ± 6.8	Weeds	Adults	21/01/12, 138m
Poecilimon spp.(Tettigoniidae)	38 ± 11.48	Weeds	Nymphs	13/06/12, 400m
Poecilimon spp.(Tettigoniidae)	29.75 ± 6.58	Weeds	Nymphs	29/6/12, 428m
Gryllus bimaculatus	15.2 ± 11	Weeds	Adults	21/01/12, 140m
Gryllus bimaculatus	28.32 ± 3.8	Soil	Adults	18/02/12, 351m
Diptera				
Eristalis tenax	12.5 ± 2.65	Weeds	Adults	04/04/12, 420m
Hymenoptera				
Cephus pygmaeus	4.3 ± 0.66	Weeds	Larvae	28/5/12 420m
Apidae	20.7 ± 2.6	Weeds	Adults	13/06/12,270m
Apis mellifera	5.1 ± 0.79	Weeds	Adults	29/06/12 383m
Bombus sp.	3 ± 0.36	Weeds	Adults	12/7/12 420m
Xylocopidae	13.3 ± 4.5	Weeds	Adults	18/02/13, 380m
Odonata				
Libellulidae	28.5 ± 1.5	Weeds	Adults	29/06/12 400m
Libellula depressa	27.5 ± 3.5	Weeds	Adults	22/10/12, 389m
Libellula depressa	67.26 ± 13.14	Weeds	Adults	19/11/12, 164m
Anax imperator	55.12 ± 16.14	Weeds	Adults	19/11/12, 164m
Phasmida				
Gratidia sp.	9.7 ± 2.78	Weeds	Adults	12/7/12 270m

Table 3. Average numbers of nodules in insects collected from various sources in Hatay Province in 2013. Values indicate numbers of discrete nodules ± SEM. Collection dates are dd/m/yr

	Average number of nodules	Sources	Developmental stages	Collection dates, altitudes
Lepidoptera				
Ostrinia nubilalis	14.8±2.1	Corn stalk	Wintered larvae	21/01/13, 338m
Ostrinia nubilalis	28.6±1.91	Corn stalk	Wintered larvae	18/02/13, 105m
Sesamia nonagrioides	17 ± 3.2	Corn stalk	Wintered larvae	21/01/13, 105m
Sesamia nonagrioides	36 ± 2	Corn stalk	Wintered larvae	18/02/13, 338m
Sesemia nanogrioides	23.2±2.33	Corn	Adults	24/05/13, 90m
Papilio machaon	23±3.1	Weeds	Larvae	21/06/13, 350m
Thaumetopoea pityocampa	41.2±9.3.	Pine	Larvae	28/06/13, 600m
Arctia sp.	14.2±2.1	Weeds	Larvae	24/03/13, 420m
Vanessa cardui	7.2±1.3	Weeds	Adults	12/04/13, 95m
Pieris brassicae	26.1 ± 1.9	Cabbage	Larvae	21/01/13, 410m
Pieris brassicae	38.4 ± 12.3	Weeds	Larvae	19/04/13, 400m
Pieris brassicae	40.2 ± 7.3	Weeds	Larvae	21/06/13, 450m
Pieris brassicae	12.3 ± 4.12	Weeds	Adults	11/07/13, 95m
Pieris brassicae	1.75 ± 0.47	Alfalfa	Adults	23/08/13, 65m
Pieris rapae	5.3 ± 1.2	Cabbage	Adults	5/04/13, 90m
Pieris rapae	8.2 ± 2.1	Cabbage	Adults	12/04/13, 85m
Pieris rapae	11.1 ± 2.03	Alfalfa	Adults	19/07/13, 67m
Pieris rapae	11.2 ± 5.2	Cabbage	Larvae	26/07/13, 470m
Geometridae	10.2±2.3	Alfalfa	Larvae	5/07/13, 100m
Colias crocea	2.1±0.41	Weeds	Adults	11/07/13, 300m
Colias crocea	17.3±4.05	Alfalfa	Adults	19/07/13, 70m
Colias crocea	16.3±3.9	Alfalfa	Adults	2/08/13, 370m
Colias crocea	1.8±0.98	Alfalfa	Adults	16/08/13, 60m
Colias crocea	2.6±0.76	Alfalfa	Adults	23/08/13, 65m
Pontia sp.	9.33±2.02	Alfalfa	Adults	19/07/13, 60m
Aspitates sp.	20.5±7.2	Weeds	Adults	17/05/13, 100m
Helicoverpa armigera	38.2 ± 5.4	Alfalfa	Larvae	8/06/13, 100m
Helicoverpa armigera	8.2 ± 1.17	Alfalfa	Adults	16/08/13, 60m
Helicoverpa armigera	20.16 ± 3.88	Alfalfa	Larvae	23/08/13, 65m
Polyommatus sp.	6 ± 2.3	Alfalfa	Adults	19/07/13, 70m

Table 3. (Continued)

	Average number of nodules	Sources	Developmental stages	Collection dates, altitudes
Coleoptera				
Oxythyrea cinctella	11.14±1.29	Weeds	Adults	3/05/13, 73m
Oxythyrea cinctella	13.1±.4.7	Weeds	Adults	14/06/13, 350m
Coccinella semptempunctata	0.7±0.4	Weeds	Adults	24/03/13 100m
Coccinella semptempunctata	1.9±1.28	Weeds	Adults	26/04/13, 85m
Coccinella semptempunctata	0.6±0.22	Weeds	Adults	3/05/13, 80m
Coccinella semptempunctata	0.37±0.18	Weeds	Adults	10/05/13, 68m
Coccinella semptempunctata	1±0.5	Weeds	Adults	24/05/13, 90m
Coccinella semptempunctata	2.1±0.34	Weeds	Adults	8/06/13, 100m
Coccinella semptempunctata	1.2±0.62	Alfalfa	Adults	26/07/13, 470m
Coccinella semptempunctata	1.2±0.86	Alfalfa	Adults	16/08/13, 420m
Coccinella semptempunctata	1.5±1.5	Alfalfa	Adults	30/08/13, 110m
Larinus latus	9.2±2.8	Weeds	Adults	5/04/13, 100m
Larinus latus	28.66±2.02	Weeds	Adults	10/05/13, 68m
Larinus latus	22.5±2.5	Weeds	Adults	10/05/13, 68m
Larinus latus	10.2±1.9	Weeds	Adults	28/06/13, 410m
Larinus onopordi	31±2.32	Weeds	Adults	3/05/13, 73m
Lixus sp.	17.3±6.4	Weeds	Adults	26/04/13, 85m
Lixus sp.	8.5±5.5	Weeds	Adults	19/07/13, 65m
Cantharis spp.	5.8±2.2	Wheat	Adults	24/03/13 100m
Cantharis spp.	6.4±2.1	Weeds	Adults	12/04/13, 85m
Cantharis spp.	4.8±0.92	Wheat	Adults	19/04/13, 100m
Cantharis spp.	9±1.28	Weeds	Adults	26/04/13, 85 m
Cantharis spp.	3.85±0.76	Weeds	Adults	3/05/13, 73m
Cantharis spp.	22±7	Weeds	Adults	24/05/13, 90m
Phyllopertha horticola	4.2±0.77	Weeds	Adults	26/04/13, 85m
Phyllopertha horticola	20.5±2.06	Weeds	Adults	3/05/13, 73m
Phyllopertha horticola	8.8±0.86	Weeds	Adults	10/05/13, 68m
Gonioctena fornicata	5.3±1.9	Alfalfa	Adults	21/06/13, 450m
Hypera variabilis	3.2±1.2	Alfalfa	Adults	24/05/13, 90m
Hippodemia variegata	12.2±6.7	Weeds	Adults	28/06/13, 600m
Hippodemia variegata	0.62±0.23	Weeds	Adults	5/07/13, 410m
Adalia decempuncatata	0.9±0.4	Weeds	Adults	5/07/13, 410m
Coccinella decemlinata	0.28±0.18	Weeds	Adults	24/05/13, 90m
Coccinella decemlinata	0.6±0.4	Alfalfa	Adults	30/08/13, 110m
Adelia bipunctata	0.71±0.28	Weeds	Adults	24/05/13, 90m
Adelia bipunctata	1.62±0.63	Alfalfa	Adults	11/07/13, 95m
Adelia bipunctata	0.76±0.26	Alfalfa	Adults	16/08/13, 420m
Coccinella undecipunctata	0.4±0.16	Alfalfa	Adults	19/07/13, 67m
Coccinella undecipunctata	0.6±0.21	Alfalfa	Adults	2/08/13, 370m
Hemiptera				
Membracidae	1.1 ± 0.7	Weeds	Adults	28/06/13, 600m
Ancyrosoma leucogrammes	9.9± 3	Weeds	Adults	14/06/13, 350m
Ancyrosoma leucogrammes	8± 2.3	Weeds	Adults	19/07/13, 65m
Ancyrosoma leucogrammes	9± 3.4	Weeds	Adults	2/08/13, 400m
Eurygaster integriceps	91.7 ± 4.65	Wheat	Wintered adults	24/03/13, 75m
Eurygaster integriceps	84.3 ± 9.4	Wheat	Wintered adults	12/04/13, 90m
Eurygaster integriceps	101.2 ± 12.8	Wheat	Wintered adults	19/04/13, 400m
Eurygaster integriceps	89.2 ± 5.13	Wheat	Wintered adults	26/04/13, 85m
Eurygaster integriceps	95.7 ± 5.08	Wheat	Wintered adults	3/05/13, 73m
Eurygaster integriceps	8.8 ± 7.3	Wheat	New generation adults	31/05/13, 73m
Nezara viridula	9±2	Weeds	Adults	8/06/13, 400m
Nezara viridula	12.1±3.8	Alfalfa	Adults	11/07/13, 95m
Nezara viridula	3.2±1.2	Alfalfa	Nymphs	26/07/13, 480m
Nezara viridula	9.1±2.3	Alfalfa	Adults	2/08/13, 60m
Nezara viridula	0.4±0.24	Alfalfa	Nymphs	23/08/13, 65m
Nezara viridula	1.25±0.94	Alfalfa	Nymphs	30/08/13, 110m
Cercopidae	1.2±0.92	Weeds	Adults	24/03/13 90m
Eurydema ornatum	3.1 ± 0.9	Weeds	Adults	24/03/13 100m
Eurydema ornatum	8.1 ± 1.9	Weeds	Adults	12/04/13, 90m
Eurydema ornatum	4.3 ± 0.9	Weeds,	Nymphs	17/05/13, 100m
Eurydema ornatum	5.1 ± 1.2	Weeds	Nymphs	8/06/13, 410m
Eurydema ornatum	4.1 ± 0.9	Weeds	Nymphs	11/07/13,350m
Eurydema ornatum	6.2 ± 1.9	Alfalfa	Nymphs	26/07/13, 480m

Table 3. (Continued)

	Average number of nodules	Sources	Developmental stages	Collection dates, altitudes
Eurydema ornatum	5.9 ± 0.68	Weeds	Adults	16/08/13, 48m
Eurydema ornatum	0.66 ± 0.66	Weeds	Nymphs	30/08/13, 110m
Aelia rostrata	4.7 ± 2.8	Wheat	New generation adults	28/06/13, 600m
Cicadidae	6.2 ± 2.1	Weeds	Adults	5/07/13,100m
Rhyncoris sp.	11.3 ± 2.1	Weeds	Adults	14/06/13, 350m
Rhyncoris sp.	7.9 ± 1.9	Weeds	Adults	26/07/13, 410m
Dolycoris baccarum	13.2 ± 0.92	Weeds	Adults	5/04/13, 90m
Dolycoris baccarum	2.1 ± 1.1	Weeds	Nymphs	17/05/13, 400m
Dolycoris baccarum	2.1 ± 0.9	Weeds	Nymphs	31/05/13, 410m
Carpocoris mediterranus	21.2 ± 4.2	Weeds	Adults	17/05/13, 400m
Carpocoris mediterranus	28.32 ± 11.2	Weeds	Adults	8/06/13, 400m
Carpocoris mediterranus	6.2 ± 2,3	Weeds	Adults	30/08/13, 110m
Carpocoris sp.	7.24 ± 3.18	Alfalfa	Adults	11/07/13, 95m
Carpocoris sp.	8.46 ± 3.1	Alfalfa	Adults	2/08/13, 65m
Lygaeidae	18.2 ± 3.2	Soil	Adults	5/07/13,100m
Miridae	0.85 ± 0.34	Weeds	Adults	30/08/13,110m
Orthoptera				
Acrididae	11.3 ± 3.4	Weeds	Nymphs	19/04/13, 100m
Acrididae	9.42± 0.94	Weeds	Nymphs	3/05/13, 73m
Acrididae	20.33± 4.2	Weeds	Adults	31/05/13, 410m
Acrididae	22.3 ± 5.2	Weeds	Nymphs	8/06/13, 100m
Acrididae	31.2 ± 9.3	Weeds	Adults	11/07/13,350m
Acrididae	32.6 ± 6.26	Weeds	Adults	19/07/13, 65m
Acrididae	82.8 ± 11.2	Soil	Adults	2/08/13, 60m
Acrididae	52.8 ± 6.63	Alfalfa	Adults	16/08/13, 60m
Acrididae	57.12 ± 7.02	Alfalfa	Adults	23/08/13, 65m
Acrididae	56 ± 10.39	Soil	Adults	30/08/13, 110m
Poecilimon spp. (Tettigoniidae)	58.32 ± 7.2	Soil	Nymphs	5/04/13, 90m
Poecilimon spp. (Tettigoniidae)	38.2 ± 3.8	Weeds	Nymphs	26/04/13, 85m
Poecilimon spp. (Tettigoniidae)	6.55 ± 0.88	Weeds	Nymphs	3/05/13, 73m
Poecilimon spp. (Tettigoniidae)	30 ± 3	Weeds	Nymphs	10/05/13, 68m
Poecilimon spp. (Tettigoniidae)	32.3 ± 7.4	Weeds	Nymphs	8/06/13, 100m
Poecilimon spp. (Tettigoniidae)	31.2 ± 7.3	Weeds	Nymphs	28/06/13, 410m
Poecilimon spp. (Tettigoniidae)	22.6 ± 3.28	Weeds	Nymphs	30/08/13, 110m
Gryllotalpa gryllotalpa	67.3 ± 10.2	Soil	Adults	24/05/13, 90m
Diptera				
Eristalis arbustorum	19.5 ± 1.5	Weeds	Adults	18/02/13, 360m
Eristalis tenax	16.3 ± 1.7	Weeds	Adults	18/02/13, 361m
Eristalis tenax	11.8 ± 3.1	Weeds	Adults	24/03/13, 420m
Eristalis tenax	8.2 ± 1.3	Weeds	Adults	17/05/13, 400m
Eristalis tenax	7.1 ± 1.06	Weeds	Adults	21/06/13, 450m
Phasmida				
Phasmidae	6 ± 1.23	Weeds	Adults	21/01/13, 138m
Gratidia sp.	8.6 ± 2	Weeds	Nymphs	14/06/13, 350m
Gratidia sp.	13.8 ± 3.26	Weeds	Adults	11/07/13, 350m
Dermaptera				
Dermaptera	26.75 ± 1.49	Soil	Adults	18/02/13, 321m
Hymenoptera				
Cephus pygmaeus	3.9 ± 0.99	Wheat	Larvae	31/05/13, 410m
Apidae	8.1 ± 3.1	Weeds	Adults	21/06/13, 350m
Apis mellifera	4.9 ± 0.59	Weeds	Adults	28/06/13, 410m
Vespidae	4 ± 2	Weeds	Adults	30/08/13, 110m
Odanata				
Libellulidae	30.1 ± 2.8	Near the water	Adults	21/06/13, 350m
Libellulidae	30.5 ± 3.5	Near the water	Adults	19/07/13, 65m
Libellula depressa	8.5 ± 3.5	Near the water	Adults	30/08/13, 110m

Table 4. A single-factor ANOVA across species for collection sources differences

Year	Collection sources	Nodules /insect[a]	Number of individuals
2011	Plant material	18.3±8.5[a]	140
	Soil	24.65±3.8[a]	20
2012	Plant material	14.7±2.0[b]	740
	Soil	37.9±9.0[a]	40
2013	Plant material	15.5±1.6[b]	1370
	Soil	58.2±9.1[a]	50

[a]Mean number of nodules in a column followed by different letters are significantly different for each year {($F_{(1,14)}$ = 0.07, P = 0.7915 for 2011), ($F_{(1,76)}$ =6.72, P <0.05 for 2012) and ($F_{(1,140)}$ = 23.86, P < 0.0001 for 2013)}

Table 5. A single-factor ANOVA across species for developmental stage differences

Year	Developmental stages	Nodules /insect	Number of individuals
2011	Adults	23.0±12.1[a]	90
	Larvae	16.05±8.8[a]	60
	Nymphs	2.70±0.4[a]	10
2012	Adults	16.0±2.6[a]	560
	Larvae	15.9±3.0[a]	170
	Nymphs	15.44±7.6[a]	50
2013	Adults	16.2±2.1[a]	1060
	Larvae	24.2±3.1[a]	150
	Nymphs	14.2±3.4[a]	210

No significant differences were detected for each year{($F_{(2,13)}$ = 0.23, P=0.7957 for 2011), ($F_{(2,75)}$ = 0.00, P=0.9973 for 2012) and ($F_{(2,139)}$ = 1.19, P=0.3084 for 2013)}

Table 6. A single-factor ANOVA across species for insect order differences

Year	Insect orders	Nodules /insect[a]	Number of individuals
2011	Lepidoptera	16.0±8.8[a]	60
	Hemiptera	28.2±22.39[a]	50
	Coleoptera	6.8±4.3[a]	20
	Orthoptera	13.4±7.4[a]	20
2012	Lepidoptera	12.2±2.9[b]	180
	Hemiptera	13.2±5.1[b]	200
	Coleoptera	5.1±1.2[b]	180
	Orthoptera	33.2±3.7[a]	110
	Hymenoptera	9.2±3.3[b]	50
	Odanata	44.5±9.8[a]	40
2013	Lepidoptera	17.0±2.2b[a]	300
	Hemiptera	18.2±4.7b[a]	390
	Coleoptera	6.9±1.3b	380
	Orthoptera	36.7±5[a]	180
	Hymenoptera	5.2±0.9[b]	40
	Odanata	23±7.2b[a]	30
	Diptera	12.5±2.3[b]	50

[a]Mean number of nodules in a column followed by different letters are significantly different for each year {($F_{(3,11)}$ = 0.24, P=0.8634 for 2011), ($F_{(5,70)}$ = 7.93, P<0.0001 for 2012) and ($F_{(6,131)}$ = 5.33, P<0.0001 for 2013)

Table 7. A single-factor ANOVA across species for altitude differences

Year	Altitudes	Nodules /insect[a]	Number of individuals
2011	0-150 m	64.6±52.8[a]	20
	151- 300 m	12.6±4[b]	140
2012	0-150 m	25.0±10.8[a]	90
	151- 300 m	21.6±5.3[a]	170
	301-450m	12.8±1.6[a]	510
2013	0-150 m	17.6±2.3[a]	90
	151- 300 m	2.1±0.4[a]	10
	301-450m	16.1±2.6[a]	430
	450m- …	10.1±4.6[a]	80

[a]Mean number of nodules in a column followed by different letters are significantly different for each year {($F_{(1,14)}$ = 7.62, P<0.05 for 2011), ($F_{(2,74)}$ = 3.05, P=0.0536 for 2012) and ($F_{(3,138)}$ = 0.51, P=0.6771 for 2013)}

Discussion

Insects pysiologically produce two categories of defense responses to microbial infections, humoral and hemocytic defence reactions (Dunn, 1986; Gupta, 1991). Humoral reactions take several hours for their full expression, and involve induced synthesis of antibacterial proteins, such as cecropins, attacins, diptericins, and defensins (Dunn, 1986). In the presence of these proteins, bacteria lose their cellular integrity because of the detergent properties of peptides. Insects also synthesize lysozymes, which enzymatically attack bacteria by hydrolyzing their peptidoglycan cell walls (Dunn, 1986; Russell & Dunn, 1996).

Hemocytic reactions involve direct cellular interactions between circulating hemocytes and bacteria. In contrast to humoral defense reactions, hemocytic responses are very quick, typically occur within minutes of an infection cycle. Specific cellular defense mechanisms include phagocytosis, nodulation and encapsulation (Gupta, 1991).

Nodulation reaction is one of insect cellular or hemocytic defense actions. Dunn and Drake (1983) indicated that following an injection of bacterial cells into tobacco hornworm *Manduca sexta*, the insects were capable to clear most bacterial cells from their hemolymph circulation by nodulation in the first 2 h following the artificial infection. Occurence of nodulation involves more than one steps. First, insect granulocytes attache to infecting microbe cells, second, the granulocytes are degranuted that causes attraction of insect plasmatocytes to the growing nodule, and the spreading of plasmatocytes around the nodule (Dean et al., 2004). Finally, the darkened, melanized nodules attach to an internal organ or body wall, where they remain through the life of the insect.

Nodules are not easilly moved away from insect hemocoels when insect has experienced a microbial infection. The presence of nodules in insect hemocoels indicates that the insect was infected with microbes in the past or has a past microbial infection. Many researchers report nodulation reactions in insects following infections of insects with microbes including bacteria, fungal spores and some viruses (Miller et al., 1994; Dean et al., 2002; Lord et al., 2002; Büyükgüzel et al., 2007; Durmuş et al., 2008). Howard et al. (1998) also reported that some bacterial species evoked far more nodules than similar infections with other species.

In this paper, we obtained results which support the hypothesis that insects mostly have experienced microbial infections in nature, they recovered and continued living. The results of all experiments support this hypothesis. Nodules were seen in virtually all examined insect specimens,

altough we recorded various numbers of nodules in different insect specimens, which indicate that depending on conditions, insect may have small number of invaders or large number of invaders in nature. Moreover, when we tested major insect orders, including Coleoptera, Lepidoptera, Hemiptera, and Orthoptera for nodulation, the nodules were seen in all tested specimens from which we infer the finding that apply to most insect species. Finally more nodules were seen in the insects collected from soil, a site of significant microbial challenge, than in the insect collected from other sites. These results showed that insects may face significant microbial infections during their lives but survive because their immune systems are capable of overcoming the microbial infections in nature.

As referred above, far more nodules observed in insects associated with soil than in insects collected from plant materials, such as the orthopterans and sunn pest adults. This result is important because previous studies showed that using imidacloprid and entomopathogenic fungi together will lead to increasing mortality of soil pests (Boucias et al., 1996; Quintella & McCoy, 1997). The result also showed that overwintered insect generations, *Ostrinia nubilalis, Sesamia nonagrioides* and *Eurygaster integriceps,* had much more nodules as compared to new generation larvae of *O. nubilalis, S. nonagrioides* and adults of *E. integriceps.* Similarly, Tunaz & Stanley (2009) and Tunaz et al. (2015) reported that the new generation of sunn pests had very few nodules as compared to older, overwintered adults collected from Kahramanmaraş and Adana provinces of Turkey. We recorded no nodulation number differences at different developmental stages and altitutes of insects collected. However, in 2011, the insects collected at lower altitudes showed more nodules than the insects collected at higher altitudes Our data also indicated that significantly more nodules were seen in the orthopteran species than in the lepidopteran, hemipteran, and coleopteran species, which is reasonable since orthopteran species were mostly collected from soil, which support the findings of Tunaz & Stanley (2009) and Tunaz et al. (2015). Drawing together, main issues that cause higher numbers of nodules in insects are insect orders and soil contact of insects. From these results, we can speculate that all insects are exposed to possible infection, and the actual occurrence of a natural infection is a random event. Collected specimens in our study were healty in the field. These observations mean that the insects had been infected by microbes, and by the time of our collections they had either checked the invasion or had recovered from the infections.

Pests cause crop losses about 30–40% per year, depending on the particular crop, a large proportion of which is due to insects (Oerke & Dehne, 2004). Therefore understanding of insect immunity and pathogens on insect immunity are very important for controlling pest insects in agriculture, in which microbial insecticides are used. Insects have the ability to recover from infections in nature which is important for biological and agricultural implications. On the other hand, biologically, many microbes have the ability to overcome insect immune systems. Wang & St. Leger (2006) reported that the fungal insect pathogen, *Metarhizium anisopliae* produces a 60.4-kDa gene product, which effectively hides the hyphal bodies from immune surveillance of insects. Similarly, Stanley & Miller (2006) indicated that the bacterium *Xenorhabdus nematophila* secretes factors that inhibit the eicosanoid signaling, which is crucial to launching cellular immune reactions. Therefore, we need to understand evolutionary mechanisms of insect immunity and inhibitory actions of insect pathogens for getting effective result on insect pest management. It is known that insect immunity sytems may limit use of biopesticides, which are environmentally friendly insecticide. Hence, in this paper, we tried to understand insect immune reactions in nature in which the insects were collected from Hatay Province of Turkey.

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Orijinal araştırma (Original article)

Temperature-dependent development of the tomato leaf miner, *Tuta absoluta* (Meyrick) (Lepidoptera: Gelechiidae) on tomato plant *Lycopersicon esculentum* Mill. (Solanaceae)[1]

Tuta absoluta (Meyrick) (Lepidoptera: Gelechiidae)'nın domates bitkisi, *Lycopersicon esculentum* Mill. üzerinde sıcaklığa bağlı gelişmesi

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Summary

Laboratory studies on the temperature-dependent development of *Tuta absoluta* (Meyrick) (Lepidoptera: Gelechiidae) were performed at 10 constant temperatures ranging from 15 to 34±1°C. The duration of total development was measured for every temperature. Tayfun F1 tomato variety was used for larval feeding and all experiments were carried out at climatic cabinets where had long daylight period (16:8) and 65±5% constant humidity for every temperature. According to obtained data, developmental threshold (C) and thermal constant (K) were calculated by using linear regression, and lower (T_{min}), optimum (T_{opt}) and upper (T_{max}) temperature thresholds for total developmental period of pest were calculated by using Polynomial (4^{th}), Logan 6, Logan 10, Lactin 1, Briere 1 nonlinear regresyon models. Development time decreased with increasing temperature ranging from 78.17 days to 21.39 days within the range 15-29°C. Developmental threshold and thermal constant for total development of tomato leaf miner were estimated as 8.94°C and 419.46 degree-days respectively. Lower, optimum and upper temperature requests were estimated with different models and results obtained werein the range 8.9-12.5, 31.00-31.07 and 35.9-38.5, respectively.

Keywords: *Tuta absoluta*, temperature-dependent development, developmental threshold, thermal constant, optimum developmental

Özet

Tuta absoluta (Meyrick) (Lepidoptera: Gelechiidae)'nın sıcaklığa bağlı gelişmesi laboratuvar koşullarında 15-34±1°C arasında değişen 10 farklı sabit sıcaklıkta incelenmiştir. Her bir sıcaklıkta toplam gelişme süresi belirlenmiştir. Larvaların beslenmesi için Tayfun F1 domates çeşidi kullanılmıştır ve denemeler her bir sıcaklık için uzun gün aydınlatmalı (16:8), %65±5 orantılı nem koşullarına sahip iklim kabinlerinde yürütülmüştür. Elde edilen verilere göre zararlının gelişme eşiği (C) ve sıcaklık sabiti (K), doğrusal regresyon yöntemiyle ve en düşük, en iyi ve en yüksek sıcaklık eşikleri doğrusal olmayan regresyon modelleri Polynomial (4^{th}), Logan 6, Logan 10, Lactin 1, Briere 1 yardımıyla hesaplanmıştır. Gelişme süresi artan sıcaklığa bağlı olarak15-29°C sıcaklık aralığında 78.17 günden 21.39 güne azalmıştır. Domates güvesi'nin toplam gelişme süresi için gelişme eşiği 8.94°C ve sıcaklık sabitesi 419.46 gün-derece olarak tahmin edilmiştir. En düşük, en iyi ve en yüksek sıcaklık istekleri farklı modeller yardımıyla sırasıyla 8.90-12.50, 31.00-31.07 and 35.90-38.50 aralığında tahmin edilmiştir.

Anahtar sözcükler: *Tuta absoluta*, sıcaklığa bağlı gelişme, gelişme eşiği, sıcaklık sabiti, en iyi gelişme

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Introduction

The tomato leaf miner, *Tuta absloluta* (Meyrick) (Lepidoptera: Gelechiidae), originated in South America and feeds on many cultivars and weeds, which belong to the families of Solanacae and Fabacea. So far, 26 different plant species have been specified as host plants. It feeds mainly on tomatoes (*Lycopersicon esculentum* Mill.), potatoes (*Solanum tuberosum* L.), aubergines (*Solanum melongena* L.), peppers (*Capsicum annum* L.), some weeds (*Datura stramonium* L.*, Lycium chilense* Coralillo*, Solanum nigrum* L., and *Nicotiana glauca* Graham) (Solanaceae) and beans (*Phaseolus vulgaris* L.) (Fabaceae) (Harizanova et al., 2009; Abdul-Rassoul, 2014). Besides leaves of hosts, it also attacks the stalk, stem, fruit, and even flowers, and by this way it can cause damage of approximately 100% (Souza et al., 1992).

The pest was first reported to have been transmitted from Chile to Argentina in 1964, and then it was occurred in Valencia, Spain in 2007 (Vieira, 2008), in Cosenza, Italy in 2008 spring, and then in autumn of the same year in the south France (EPPO, 2009). It has appeared in Turkey since 2009 and caused considerable damage by spreading rapidly over suitable areas. It was occurred in North Africa, in the Middle East, and in some Asian countries in the same year and the following years. It became one of the greatest troubles of tomato growers in a short time by spreading rapidly all over the areas where tomato was grown. Besides host suitability, suitable temperature is one of the most important reasons why the pest spreads and causes damage. Temperature is one of the most important abiotic factors affecting the biology of insects (Chapman, 1998). Determining the appropriate temperature conditions for the pest is essential in terms of the studies of population dynamics. One of the main reasons why this pest, which is of Neotropical origin, can mainly be seen more at northern latitudes and why it causes damage is that these regions have the appropriate temperature zone for the pest. This study was conducted in order to determine the temperature ranges in which tomato leaf minercan grow best, and in which it can endure most. The most appropriate growth curves were determined in relation to the developmentrates obtainedat various constant temperatures. Thus, the temperature values having the potential for the pest to spread and create an epidemic, best developmental temperature, lower and upper temperature requests, and thermal constant were all obtained for total developmental period.

Material and Methods

Tomato leaf miner adults were collected from tomato fields in Antalya and reared on potted Tayfun F1 tomato variety, *Lycopersicon esculentum*, within sealed Plexiglas cages covered with tulle (40 cm x 50 cm x 50 cm) at 25ºC temperature, 65±5% relative humidity and day light period (16:8 h). Experiments were initiated with adults of tomato leaf miner taken from stock culture. Three potted plants were put in each cage and 10 adults oftomato leaf miner were released for one day to lay eggs on seedlings. Then, potted plants having eggs were taken to climate cabinets whose temperature, humidity, and light had been adjusted before. In the study,10 different experimental groups, which had ranged 10 and 79 larvae, were created in the climate cabinets adjusted to 15, 20, 23, 25, 27.5, 29, 30, 31, 33 and 34ºC (Table 1). Relative humidity was 65±5% and day light period was16:8 h for each temperature. Developmental and survival data were recorded daily. Generation numbers of the pest in the most important tomato growing areas of Turkey were calculatedtheoretically based on obtained development threshold and temperature constant with average daily temperature.

Table 1. The development periods (days) of *Tuta absoluta* at ten different temperature conditions

Temperatures(°C)	n	Development periods (days) ± Std. Dev
15	10	78.17±4.222
20	25	40.24±1.022
23	67	29.24±0.474
25	79	26.75±0.297
27.5	18	22.67±0.642
29	38	21.39±0.398
30	50	21.48±0.276
31	38	20.49±0.344
33	70	21.39±0.216
34	15	23.93±0.372

Statistical analyses

A linear and six non-linear regression models were used for data analysis. The developmental threshold (C) and the thermal constant (K) of tomato leaf minerin relation to total developmental time were calculated by means of linear regression, and lower (T_{min}), optimum (T_{opt}) and upper (T_{max}) temperature thresholds for total developmental period of tomato leaf miner were calculated theoretically by means of nonlinear models; Polynominal (4^{th}), Logan 6, Logan 10, Lactin 1, and Briere 1. The curves were fitted with nonlinear regression using by CurveExpert Pro, SPSS (v.20), and MS Excel software.

Linear regression model

The thermal constant (K) and developmental threshold (C) can be estimated only by the linear equation (Campbell et al., 1974; Obrycki & Tauber, 1982; Kontodimas et al., 2004).

$$d(T) = a + b.t \qquad K = \frac{1}{b} \qquad C = \frac{-a}{b}$$

Where $d(T)$ is the rate of development at temperature T (°C) (days-1), and *a*and *b* are constants. Constant parameters were calculated based on the values obtained between 15 °C and 29 °C where developmentrate increased linearly. Outside of this range, the relationship between developmental time and temperature was nonlinear (Mills, 1981). The linear relationship between development time and temperature was used for degree-days model:K: thermal constant (day-temperature); C: developmental threshold (°C) (Wigglesworth, 1953; Campbell et al., 1974; Mills, 1981).

Non-linear regression models

Polynomial model (4th)

$$y = a + b.x + c.x^2 + d.x^3 + e.x^4$$

Logan 6 model

$d(T) = \psi.\left[e^{\rho.T} - e^{(\rho.T_{max} - \frac{T_{max}-T}{\Delta}}\right]$ (Logan et al., 1976; Logan, 1988)

Where $d(T)$ is the rate of development at temperature T (°C) (days^{-1}), ψ is the maximum development rate, ρ is a constant defining the rate of optimal temperature, T_{max} is the high temperature threshold, and Δ is the temperature range over which physiological breakdown becomes the overriding influence.

Optimum temperature for development (T_{opt}) was calculated by the equations of Logan et al. (1976).

$$T_{opt} = T_{max}.\left(1 + \varepsilon.\frac{ln\,(\varepsilon.b_0)}{1 - \varepsilon.b_0}\right)$$

Where $\varepsilon = \frac{\Delta T}{T_{max}}$ and $b_0 = \rho.T_{max}$ (Palyvos, 2009).

Logan10 model

$d(T) = \alpha.\left[\frac{1}{1+k.e^{-\rho.T}} - e^{(\frac{T_{max}-T}{\Delta}}\right]$ (Logan et al., 1976; Logan, 1988)

Whereα and k are the empirical constants, and ρ, T_{max} and Δ are as in Logan 6 model.

Janish model

$d(T) = \frac{T_{min}}{2}.\left[e^{k.(T-T_{opt})} + e^{-\lambda.(T-T_{opt})}\right]$ (Janisch, 1932; Analytis, 1981)

Where T_{min} is the lower temperature and T_{opt} is optimum temperature, λ and k are the empirical constants.

Lactin1 model

$d(T) = e^{\rho.T} - e^{(\rho.T_{max} - \frac{T_{max}-T}{\Delta})} + \lambda$ (Brie`re & Pracros, 1998; Tsai & Liu, 1998)

Where ρ, T_{max} and Δ are as in Logan 6 model and λ forces the curve to intercept the *y*-axis at *a* value below zero and thus allow estimation of a low temperature threshold.

Briere1 model

$d(T) = \alpha.T.(T - T_{min}).\sqrt{T_{max} - T}$ (Brie`re et al., 1999)

Where T is the rearing temperature (°C), a is an empirical constant, T_{min} is the low temperature development threshold and T_{max} is the lethal temperature threshold.

Results and Discussion

Development time and survival

All experimental cohorts of tomato leaf miner were able to complete their developmentin every temperatures conditions, except 35°C. Total development period from egg hatching toadult exclusionsharply decreased ranging from 78.17 days at 15 °C to 21.39 days at 29°C, and then smoothly increased ranging from 21.48 days at 30°C to 23.93 days at 34°C (Table 1). Like most ectothermic organisms, temperature affects developmental time of tomato leaf miner significantly. Findings obtained in this study are consistent with results ofother insects including tomato leaf miner (Estay, 2000; Uygun & Atlıhan, 2000; Atlıhan & Özgökçe, 2002; Pereyra et al., 2006; Andrew et al., 2013; Mahdi & Doumandji, 2013).

Survival rate from egg to adult of tomato leaf miner obtained at different temperaturesare shown in Figure 1. The survival rates obtained at temperatures ranged 23 - 27.5°C were higher than those of other temperatures tested, andit was the lowest at 15 and 34°C. Survival rates of insects are under effects of temperature as stated in different studies (Aldyhim & Khalil, 1993; Kersting et al., 1999; Atlıhan & Chi, 2008; Andreadis et al., 2013).

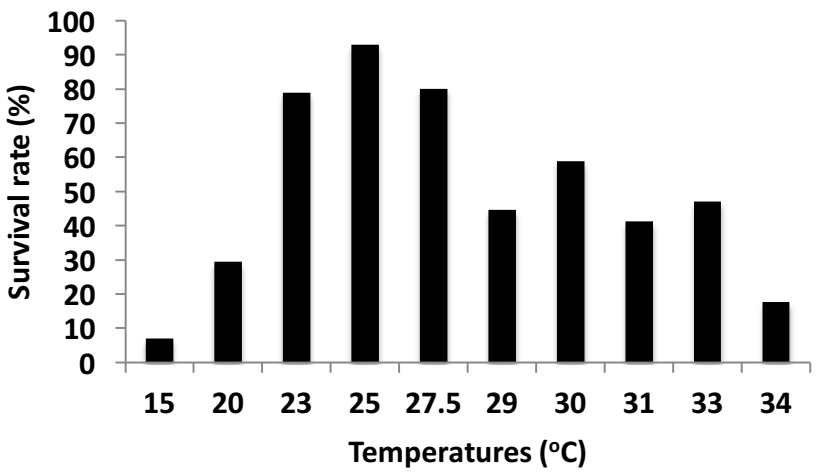

Fig. 1. Survival rate from egg to adult of tomato leaf miner, *Tuta absoluta* at different temperatures.

The developmental threshold, thermal constant, optimum development, lower and upper development requests

The developmental threshold (C), thermal constant (K), optimum (T_{opt}) development temperature, lower (T_{min}) and upper (T_{max}) temperature requests for total immature development of tomato leaf miner are presented in Table 2 and Figure 2. The development rate increased linearly with increasing temperature from 15 to 29 °C withthe highestcoefficient of determination (r^2) of 0.851. The developmental

threshold and thermal constant were found 8.94°C and 419.46 Degree-days, respectively. The development threshold of tomato leaf miner was reported as 9.8 °C by Mahdi & Doumandji (2013) and 8.1 °C by Estay (2000). Our result is between these two results mentioned. Differences from literature might be due to host plants used for experiments.

Table 2. Values of the fitted coefficients and measurable parameters, the adjusted coefficient of determination of linear and five nonlinear models for describing total development of *Tuta absoluta*

Models		Parameters± Std. Dev.
Linear	a	-2.1E-02±2.4E-03
	b	2.4E-03±9.1E-05
	K	419.460±
	C	8.940
	r	0.851
	SE	4.5E-03
Polynomial (4^{th})	a	-2.7E-01±1.3E-01
	b	4.9E-02±2.3E-02
	c	-3.1E-03±1.4E-03
	d	9.4E-05±3.9E-05
	e	-1.0E-06±3.8E-07
	r	0.867
	T_{min}	12.500
	T_{opt}	31.000
	T_{max}	38.500
	SE	4.3E-03
Logan 6	ψ	8.1E-02±6.5E+02
	ρ	1.4E-01±4.4E+00
	T_{opt}	31.070
	T_{max}	38.173±6.4E-01
	Δ	7.1E+00±2.2E+02
	r	0.865
	SE	0.004
Logan 10	α	5.6E-02±2.9E-03
	k	5.7E+01±2.2E+01
	ρ	1.9E-01±2.2E-02
	T_{opt}	31.070
	T_{max}	35.891±5.3E-01
	Δ	1.1E+00±3.1E-01
	r	0.869
	SE	4.3E-03
Lactin 1	ρ	2.4E-03±1.4E-04
	T_{min}	9.500
	T_{opt}	31.000
	T_{max}	38.000±1.7E+00
	Δ	2.4E+00±4.4E-01
	λ	-1.0E+00±3.4E-03
	r	0.868
	SE	4.3E-03
Briere 1	a	2.7E-05±1.5E-06
	T_{min}	8.893±7.3E-01
	T_{opt}	31.000
	T_{max}	37.597±2.9E-01
	r	0.866
	SE	4.3E-03

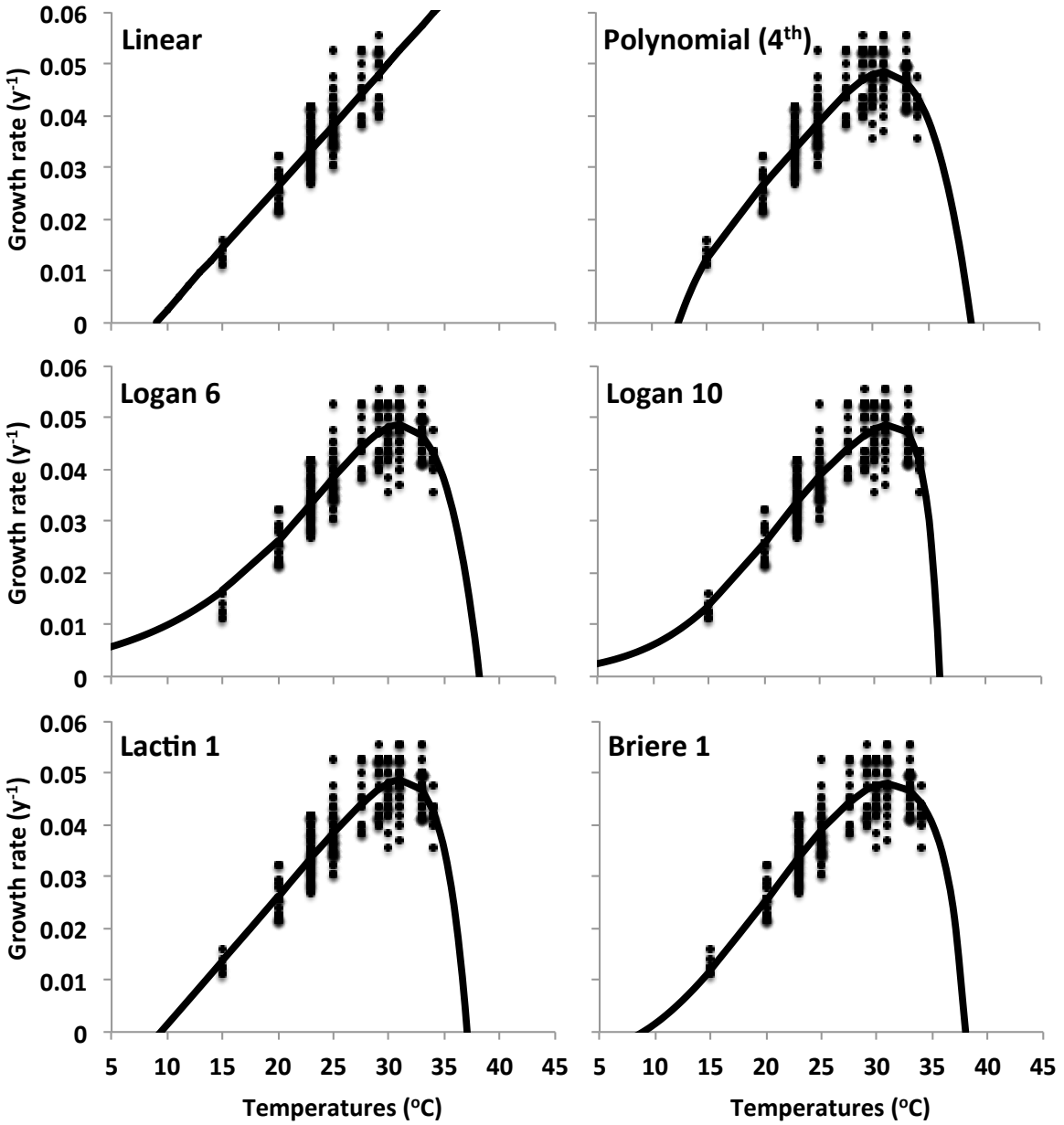

Fig 2. Fitting a linear and five nonlinear models, relationship of development rate of *Tuta absoluta* with temperature described by the total preadult period.

Results of parameter estimation of Polynomial (*4th*), Logan 6, Logan 10, Lactin 1 and Briere 1 models for development rates of tomato leaf miner are presented in Table 2. At all models, r^2 was calculated as around 0.87. While Logan 6 and Logan 10 models provide optimum (T_{opt}) and upper (T_{max}) temperatures, Polynomial (*4th*), Lactin 1 and Briere 1 models provide lower (T_{min}), temperatures, additionally. The lower temperatures for development were estimated as 8.89, 9.50 and 12.50°C with Briere 1, Lactin 1 and Poynomial (*4th*), respectively. First two results are more close to the developmental threshold. The optimum temperature of the pest was estimated as 31 °C withall models. The upper temperature of pest was estimated very close to eachother with models used, except Logan 10, and results are as follows; 38.5 °C (Polynomial *4th*), 38.17 °C (Logan 6), 38,00 °C (Lactin 1), 37.60 °C (Briere 1) and 35.89 °C (Logan 10).

As an invasive species, potential of tomato leafminer to spread to more extensive areas within the years to come is very high due to atmospheric movements or other reasons. Thus, knowing well the environmental conditions affecting the pest's life would be essential in terms of the measures that could be taken. The minimum and maximum temperature requests of the pest would allow us to predict the probable areas that it would spread as well as the periods when the pest would start causing damage. The number of generations of any insect can be estimated theoretically by using development threshold and temperature constant with average daily temperature. Based on parameters mentioned above, theoretically calculated generation number of the pest in Turkey was presented in Table 3. Mersin and Antalya were located in Mediterranean coast but the number of generations of the pest in Mersin is higher than that of Antalya. Our results indicated that generation number of tomato leaf miner could change even in the same region because of climatic differencesas mentioned in literature (Mahdi & Doumandji, 2013).

Table 3. Estimated generation numbers of *Tuta absoluta* in the most important tomato produced areas in Turkey after infestation

	Years				
	2010	2011	2012	2013	2014
Ankara	6.12	4.75	6.23	5.43	5.42
Antalya	8.51	7.55	8.62	8.16	8.06
Bursa	7.01	5.46	6.83	6.43	6.64
Çanakkale	7.27	5.95	7.32	6.83	6.69
İzmir	9.59	7.96	9.01	8.66	8.66
Manisa	8.74	7.06	8.51	7.95	7.79
Mersin	10.87	9.59	9.96	10.32	10.28
Muğla	6.84	6.06	7.08	6.56	6.14
Samsun	6.98	5.29	6.51	6.19	6.39
Şanlıurfa	10.38	8.47	9.65	9.34	9.59
Tokat	6.21	4.92	5.81	5.17	5.66

Biology and the development periods of tomato leaf minerat different temperatures were examined in previous studies (Haji et al., 1988; Coelho & France, 1987; Estay, 2000; Pereyra et al., 2006; Andrew et al., 2013; Mahdi & Doumandji, 2013). However, they studied at temperatures that development rate increased linearly. Temperatures at which development rate stopped and declined were not examined yet. In this study, the best temperature values for development of tomato leaf miner and maximum temperature for the pest to resist were also calculated. The temperature range for tomato leaf miner to grow best was 29.5-32.0°C, and maximum temperature range for it to resist was 34.18-40.5°C.

Results obtained here can be used to estimate population development, and the regions where the pest can spread. Additionally, greenhouse conditions can be arranged based on results obtained in this study to control the pest.

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Orijinal araştırma (Original article)

Pest status of western flower thrips, *Frankliniella occidentalis* (Pergande) (Thysanoptera: Thripidae), in tunnel-grown strawberry

Örtü altında yetiştirilen çileklerde Batı çiçek thripsi, *Frankliniella occidentalis* (Pergande) (Thysanoptera: Thripidae)'nin zarar durumunun araştırılması

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Summary

The western flower thrips, *Frankliniella occidentalis* (Pergande) (Thysanoptera: Thripidae) is often recognized as a serious pest of strawberries worldwide. Although *F. occidentalis* is known as the dominant species among thrips infesting strawberry plants, limited information is available about comparable data of treated and untreated plots of tunnel-grown strawberry in Turkey. In this study, significantly more larvae and adults of *F. occidentalis* were collected from the flowers, when compared with red or green fruits during the year 2011-2012 ($P<0.05$). Nearly 60% of flowers were infested with 10 or more adults, which is the current economic threshold level (ETL; 10 thrips per flower) in this crop. Furthermore, about 6% of red fruits were found to be infested with one or more adults and larval thrips. However, it was observed that population density i.e. 0.4-0.6 and 15 adults per green or red fruit and flowers, respectively, did not cause any damage to the flowers and fruiting parts. No significant difference was observed in the yields of treated and untreated strawberry plots during 2011-2012. It was concluded that population density i.e. 15 *F. occidentalis* individuals per flower may not cause visible damage. Furthermore, economic threshold level for *F. occidentalis* (ETL; 10 thrips per flower), appears to be too low. It is suggested that the ETL of *F. occidentalis* in strawberry needs to be re-evaluated in Turkey.

Keywords: Western flower thrips, strawberry, plastic tunnel, damage

Özet

Batı çiçek thripsi, *Frankliniella occidentalis* (Pergande) (Thysanoptera: Thripidae) çileğin önemli bir zararlısı olarak bilinmektedir. *F. occidentalis* Türkiye'de çileklerde ana thrips türü olarak bilinmesine karşın, örtü altında ilaçlı ve ilaçsız parsellerinde bitkiye olan zararına ilişkin karşılaştırmalı veriler bulunmamaktadır. 2011-2012 yıllarında, yeşil ve kırmızı meyveler ile karşılaştırıldığında, önemli sayıda *F. occidentalis* larvaları ve erginleri çiçeklerde bulunmuştur ($P<0.05$). Ekonomik zarar eşiği (EZE) değerine (10 thrips /çiçek) göre çiçeklerin %60'ı 10 veya daha fazla thrips ile bulaşık olmuştur. Ayrıca, kırmızı meyvelerin yaklaşık %6'sının 1 veya daha fazla sayıda thripsle bulaşık olduğu da saptanmıştır. Onbeş ergin thrips /çiçek ve ayrıca yeşil veya kırmızı meyvelerde 0.40 veya 0.60 ergin thrips /meyve ile en yüksek thrips yoğunlukları zarara neden olmamıştır. 2011 ve 2012 yıllarında ilaçlı ve ilaçsız parseller arasında verim yönünden önemli farklılıklar bulunmamıştır. Mevcut ekonomik zarar eşiğinin *F. occidentalis* için oldukça düşük olduğu, çiçek başına 15 *F. occidentalis* bireyinin bile çiçeklerde görünen zarara neden olmadığı kaydedilmiştir. Elde edilen sonuçlara dayanarak, *F. occidentalis*'in EZE'nin yeniden değerlendirilmesi önerilmektedir.

Anahtar sözcükler: Batı çiçek thripsi, çilek, örtü altı, zarar

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Introduction

Turkey is the fourth main strawberry (*Fragaria ananassa* and Rosaceae) producer among the European countries (Anonymous, 2014). Marmara, Aegean and Mediterranean are among the potential strawberry growing regions of Turkey. Mersin province located in the eastern Mediterranean region is the main strawberry producing region in Turkey.

Frankliniella occidentalis (Pergande) (Thysanoptera: Thripidae) is a serious pest infesting a wide range of crops worldwide (Lewis, 1997). It is often considered as a major pest of open field and tunnel-grown strawberries in some countries (Allen & Gaede, 1963; Argaman et al., 1989; Tommasini & Maini, 1995; Linder et al., 1998; Steiner, 2003). *F. occidentalis* which has a sucking-piercing mouth cone, scars plant tissues and finally causes damages of flower abortion, fruit bronzing, and fruit deformations of strawberries. *F. occidentalis* has a habit of visiting many different plant flowers. This issue is more likely due to flowers providing essential resources of nectars and pollens especially for female thrips to produce eggs (Trichilo & Leigh, 1988) and mating site for thrips (Rosenheim et al., 1990). Several species of thrips are having serious pest status in strawberry (Sterk, 1990; Steiner, 2002; Steiner & Goodwin, 2005).

Frankliniella occidentalis which was recorded for the first time in Turkey in 1993 (Tunç & Göçmen, 1994). Recently, it has been reported as the dominant pest species in strawberry fields of the eastern Mediterranean region of Turkey (Atakan, 2008). This thrips has not been reported previously as a pest of strawberry grown in open fields in the southeastern Mediterranean region of Turkey (Şekeroğlu et al., 1998).

Generally, it is difficult to manage thrips species due to their wide geographical distribution, high reproductive capacity, as well as large numbers of host plants and feeding on flowers, buds and other plant organs (German et al., 1992). Additionally, insecticides are not capable to control cryptic stages (i.e. their eggs being in the plant tissues and pre-adult stages, pre-pupa and pupa present in the soil or in hidden sites) leading to reduce apparent efficacy of pesticides against *F. occidentalis* (Immaraju et al., 1992). Chemical control is commonly used against pest thrips species, but frequent use of insecticides may induce problems such as destruction of natural enemies and resurgence of insecticide resistance (Desneux et al., 2007; Nauen & Denholm, 2005)

The *F. occidentalis* damage results in flower abortion, fruit bronzing, and fruit deformation on strawberries. Flowers may provide the all the essential elements for life, such as nutrition (Trichilo & Leigh, 1988) and mating site (Rosenheim et al., 1990). Therefore, some observations associated with thrips on strawberry suggest that *F. occidentalis* infestation affects mainly the development of strawberry flowers and fruits. In Turkey, economic threshold level of flower inhabiting thrips in strawberry is 10 individuals (adult or larval thrips) per flower. According to Atakan (2008), over 15 adults of *F. occidentalis* per flowers had caused slight necrotic spots around petals or beneath the calyx of fresh strawberry flowers (Atakan, 2008). Despite of these well-known facts, strawberry growers in the Çukurova region generally rely on pesticidies for the management of this pest (Şekeroğlu et al., 1998; Atakan, 2011). Furthermore, comparable data associated with a described relationship between thrips infestation levels in flowers or on fruits and the yield and quality of strawberry in treated and untreated plots of tunnel-grown strawberry crop have still been lacking.

Main objective of this study is to determine abundance of thrips on various plant parts and population dynamics of larval and adult flower thrips in insecticide-treated and untreated plots and thus to confirm whether insecticide application done against the thrips on strawberry is realistic at the current economic damage level.

Material and Methods

Study site

The experiments were conducted in high plastic tunnels at the Yaltır Agricultural Produce Corporation (Adana province, Turkey) during the years 2011 and 2012. The total area of the experiment was nearly 0.25 ha. Plot size was 62.5 m^2 (10 m length x 5 row x 1.25 m in between rows). The middle

two rows were selected as sampling unit in each plot. Half of the plots were treated with active ingredient spinosad 480 SC (Laser 480 SC, Dow AgroSciences, Turkey) 200 ml ha^{-1} when *F. occidentalis* infestation reached to the economic threshold level (10 thrips per flower), the other half served as control (untreated). There were two main treatments: insecticide treated-plot and untreated plot. Each treatment was replicated for four times. All plots were treated with acaricides such as bifenazate (Floramite 240 SC, Hektaş, Turkey) 60 ml per 100 l water and spiromesifen (Oberon 240 SC, Bayer Crop Science, Turkey) 50 ml per 100 l water against red spider mites *Tetranychus cinnabarinus* (Boisd.) (Acarina: Tetranychidae). All plots were also treated with fungicide pyraclostrobin + boscalid WG 12.8 + 25.2% (Signum WG, BASF Türk, Turkey) 150 g per 100 l water to prevent grey mould (*Botrytis*) infection. A knapsack sprayer with a 15 l tank was used to apply all of the pesticide treatments in the experiment. Spinosad were used against thrips on 29 March and 24 May in 2011 and on 16 May in 2012 in treated plot. Both of treated and untreated plots were sprayed with spiromesifen (12 April and 12 May in 2011, and 11 April and 9 May in 2012), bifenazate (19 April in 2011 and 25 April in 2012) and pyraclostrobin + boscalid (12 April in 2011 and 11 April in 2012). Experimental plots (treated and untreated) were irrigated by the drip irrigation system. Magnesium sulphate 16% (50 kg ha^{-1}), zinc sulphate 23% (25 kg ha^{-1}), iron 6% (2000 gr ha^{-1}), nitrogen (200 kg ha^{-1}), fumic acid (10 l ha^{-1}) and phosphoric acid 85% (45 l ha^{-1}) were applied to all plots in March, April and May.

Sampling and identifying of thrips species

To determine the abundance of *F. occidentalis* and other arthropods in the flowers, five flowers from each sampling unit were randomly selected, yielding 20 flowers each treatment on each sampling date. Flowers were gently removed and placed in plastic tubes (50 cc) individually. Five red or green fruits in each plot were also randomly selected, yielding 20 green or red fruits from each treatment. Fruits were also gently removed and immediately inspected for the presence of thrips by aid of a hand lens. Flower samples were stored in tubes in an ice-chest and transported to the laboratory for further processing. For extraction of insects, only flowers were submerged into 60% ethanol and agitated for 25 sec. Flowers were dissected carefully to remove any remaining thrips and rinsed in 60% ethanol for 25 sec. Thrips collected from the flowers were put into small plastic vials (2 ml) containing 60% ethanol for slide-mounted processing. Each empty collection tube was washed into a Petri dish with ethanol (60%) for two times in the same way to get any remaining thrips. Adult thrips were slide-mounted and identified to species. Thrips adults were counted under the stereomicroscope with 45x magnifications, and immature thrips (thrips larvae) were pooled into a single category.

Distribution of *Frankliniella occidentalis* on plant parts

To determine the preference of adults and larvae on various plant parts (fresh and fully opened flowers, young green fruits and mature fruits) of strawberry, were sampled throughout the sampling dates in 2011. A total of five plants were randomly selected on each sampling date. One flower or fruit (green and red fruits) from each plant was taken. Fruits were removed and immediately inspected for the presence of thrips by aid of a hand lens. Flowers were picked into plastic tubes and stored in an ice-box. *F. occidentalis* were removed by rinsing the samples with ethanol in the laboratory to determine the number of larvae and adults in the collected samples. Collected *F. occidentalis* and other thrips species were identified using above mentioned method. Thrips collected at each sampling date were divided by the number of examined fruiting parts i.e. flowers, red and green fruits sampled to obtain an average number of thrips per sampling unit. The mean numbers of adults or larvae on fruiting parts at each sampling date were pooled over a month because there were few numbers of thrips on red and green fruits. Effects of plant parts on the abundance of adults and larvae (monthly mean number of larvae and adults) were analysed.

Yield

All plants of strawberry in each experimental plot were harvested by hand picking on 19 April, 17 May, 21 May and 4 June in 2011, and 15 May and 19 May in 2012. The harvested fruits were classified as first and second degree, and mature fruits were weighted.

Statistical analysis

Means of thrips (larvae and adult) numbers on various plant organs were compared by Tukey's honest significance test at $P<0.05$. Total number of thrips in flowers was plotted against percentage of flowers infested with 10 or more thrips in 2011 and 2012. Linear regression analysis at $P<0.05$ was completed to describe the correlation between these two variables. A total of 15 data points per year were used to determine linear correlation between the above mentioned two variables as well as between the percentage of red fruits infested with 1 or more thrips and total numbers of thrips in flowers. Densities of thrips in treated and untreated plots as well as yield of strawberry in treated and untreated plots were compared by Student t-test at $P<0.05$ (independent two-tailed test). All analyses were performed by using the statistical program SPSS 15.0. (SPSS, 2006).

Results

Distribution of *Frankliniella occidentalis* on plant parts

Distribution of larvae and adult *F. occidentalis* on fruiting bodies of strawberry is presented in Figure 1. Significantly more larvae (March: $F= 12.957$, df= 2,57, $P<0.0001$; April: $F= 46.901$, df= 2,57, $P<0.0001$; May: $F= 75.507$, df= 2,57, $P<0.0001$) and adults (March: $F= 94.2957$, df= 2,57, $P<0.0001$; April: $F= 98.8101$, df= 2,57, $P<0.0001$; May: $F= 179.286$, df= 2,57, $P<0.0001$) were collected from the flowers in sampling months in treated experimental plot during 2011. Similarly significantly more numbers of larvae (March: $F= 24.223$, df= 2,57, $P<0.0001$; April: $F= 39.643$, df= 2,57, $P<0.0001$; May: $F= 105.281$, df= 2,57, $P<0.001$) and adult (March: $F= 95.234$, df= 2,57, $P<0.001$; April: $F= 32.023$, df= 2,57, $P<0.0001$; May: $F= 245.771$, df= 2,57, $P<0.0001$) of *F. occidentalis* were detected in flowers in untreated experimental plot during 2011. A relatively low number of *F. occidentalis* was recorded on green or red fruits compared to the flowers. Red fruits hosted more larvae or adults than immature green fruits but these differences were not statistically significant ($P>0.05$). Significantly more numbers of adult *F. occidentalis* in flowers were recorded in May for each of the treatment.

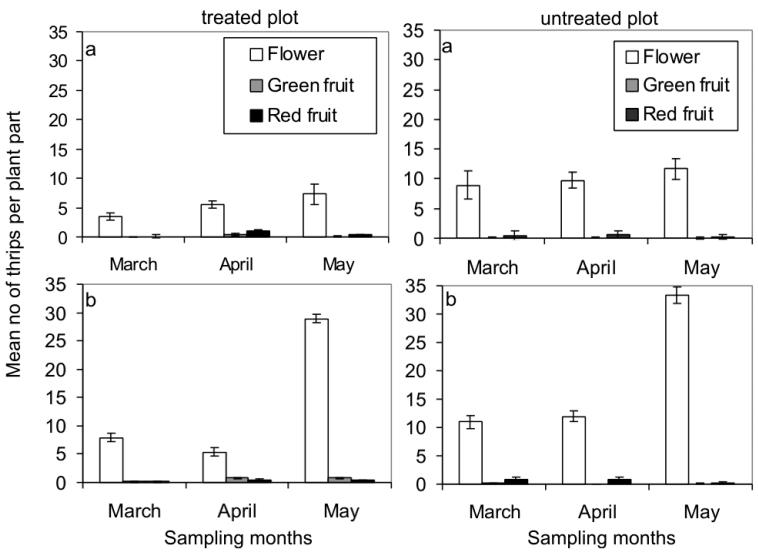

Figure 1. Distribution of *Frankliniella occidentalis* on various plant parts of strawberry in larvae (a) and adult (b) in treated and untreated plots during 2011.

Population dynamics of *Frankliniella occidentalis* in flowers

Mean numbers of *F. occidentalis* in flowers of treated and untreated plots in 2011 is given in Figure 2. In treated plots minimum larval (0.45 per flower) and adult populations (0.08 per flower) were recorded on 22 March and 8 March, respectively. The highest larval (5.05 per flower) and adult (11.75 per flower)

densities were recorded on 29 March and 24 May, respectively. The number of adults and larvae peaked in flowers (5.05 larvae and 5.98 adult thrips per flower) on 29 March. Thrips population density (total density) exceeded the action threshold level on that sampling date. The total number of thrips slowly but steadily increased after the treatment. The abundance of adults had a second peak with 11.75 adults per flower on 24 May. The abundance of adults sharply declined to low level after 31 May. In mid-June very few larval and adult *F. occidentalis* were extracted from the flowers.

Although population trends of larvae and adults in untreated plots were in general similar to that of in treated plots, there were some significant differences on some sampling dates (Figure 2). In untreated, minimum larval (0.25 per flower) and adult population (0.12 per flower) was recorded on 7 June and 15 March, respectively in 2011. The highest larval (6.30 per flower) and adult (9.00 per flower) densities were recorded on 24 March and 24 May, respectively. Abundance of larvae and adults in untreated flowers peaked on 29 March and 24 May. The highest recorded abundance of total thrips was 11.2 thrips per flower on 24 May.

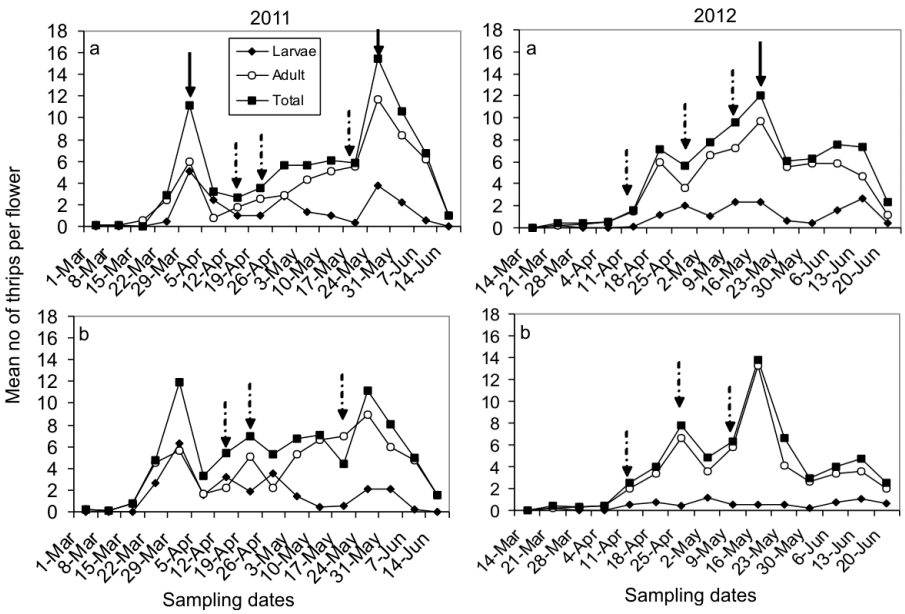

Figure 2. Mean numbers of *Frankliniella occidentalis* in flowers in treated (a) and untreated plots (b) in 2011 and 2012. Solid arrows (insecticide) and dashed arrows (fungicides and/or acaricides) indicate applications [Spinosad were applied against thrips on 29 March and 24 May in 2011 and on 16 May in 2012. Both of treated and untreated plots were sprayed with spiromesifen (12 April and 12 May in 2011, and 11 April and 9 May in 2012) and bifenazate (19 April in 2011 and 25 April in 2012) against spider mites and pyraclostrobin + boscalid (12 April in 2011 and 11 April in 2012) against *Botrytis*].

Total mean numbers of *F. occidentalis* were significantly higher on 22 March *(t*= 3.741, df= 38, *P*<0.001), 19 April (*t*= 3.168, df= 38, *P*<0.001), 24 May (*t*= 2.382, df= 38, *P*<0.05) and 31 May (*t*= 5.139, df= 38, *P*<0.0001) than those found numbers in untreated plots.

Mean numbers of *F. occidentalis* in flowers of treated and untreated plots in 2012 is given in Figure 2. In treated plots minimum larval (0.10 per flower) and adult population (0.20 per flower) were recorded on 11 April and 21 March of 2012, respectively. The highest larval (2.35 per flower) and adult (9.65 per flower) densities were recorded on 16 May. Abundance of larvae and adults in treated plots started to increase in the second week of April (Figure 2). Peak abundance of adults with a mean of 9.65 thrips per flower and larval thrips with a mean of 2.32 thrips per flower was recorded on 16 May in 2012. The abundance of thrips fluctuated between 6 and 7 thrips per flower from late May until mid-July. There were a few adults and larvae of *F. occidentalis* in late-June (20 June). In untreated plots, minimum larval (0.20 per flower) and adult population (0.20 per flower) was recorded on 21 March of 2012. The highest larval

(1.20 per flower) and adult (13.30 per flower) densities were recorded on 2 May and 16 May, respectively. The abundance of thrips fluctuated until 16 May. The observed peak abundance on this date was 13.3 adults per flower. After the peak the population density of adults abruptly declined to a very low infestation level. The mean number of larvae throughout the entire sampling period remained low compared to that of in treated plots. Total mean numbers of *F. occidentalis* were significantly higher on 18 April (t= 3.911, df=38, P<0.001), 9 May (t= 5.223, df= 38, P<0.0001), 30 May (t= 6.764, df= 38, P<0.0001), 6 June (t= 6.693, df= 38, P<0.0001) and 31 May (t= 5.364, df= 38, P<0.0001) than those found in untreated plots.

Correlation between *Frankliniella occidentalis* abundance and flower density

Linear correlation between number of *F.occidentalis* per flower and number of flowers per plant in treated and untreated plots are given in Figure 3. Thrips abundance in any treatment was not correlated to flower density (Figure 3; P>0.05). *F. occidentalis* abundance was relatively low when loads of the flowers on plants peaked. In other words, thrips abundance did not follow flower density on strawberry plants.

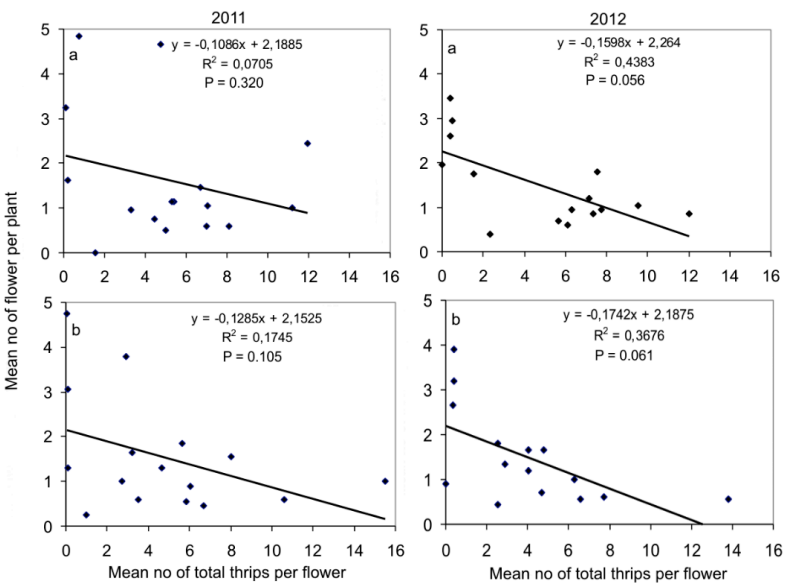

Figure 3. Linear correlation between the mean number of *Frankliniella occidentalis* per flower and the mean number of flowers per plant in treated (a) and untreated plots (b) in 2011 and 2012.

Correlation between damage threshold and *Frankliniella occidentalis* abundance in flowers and red fruits

Linear correlation between number of thrips per flower (x) and percentage of strawberry (y) flowers with a minimum of 10 thrips are reported in Figure 4 and Table 1. Negative correlation was observed between these two variables in treated and untreated plots during both years. Percentage of flowers with 10 or more adults *F. occidentalis* in treated plots in 2011 and 2012 were 63% and 52%, respectively. Percentage of flowers with 10 or more adults *F. occidentalis* in untreated plots in 2011 and 2012 were 56% and 60%, respectively. Percentage of flowers with 10 or more adults + larvae of thrips in treated and untreated plots was equated to 3%.

The percentage of red fruits with 1 or more thrips and the number of adult thrips per flower are plotted in Figure 5. The correlation between these two variables was significantly positive in treated and untreated plots in 2011 (treated plot: F= 10.342, df= 1,15, P= 0.0006, R^2 = 0.42, Y= 1.8184x + 4.005; untreated: F= 7.304, df= 1,15, P= 0.017, R^2= 0.34, Y= 2.314x + 4.674).

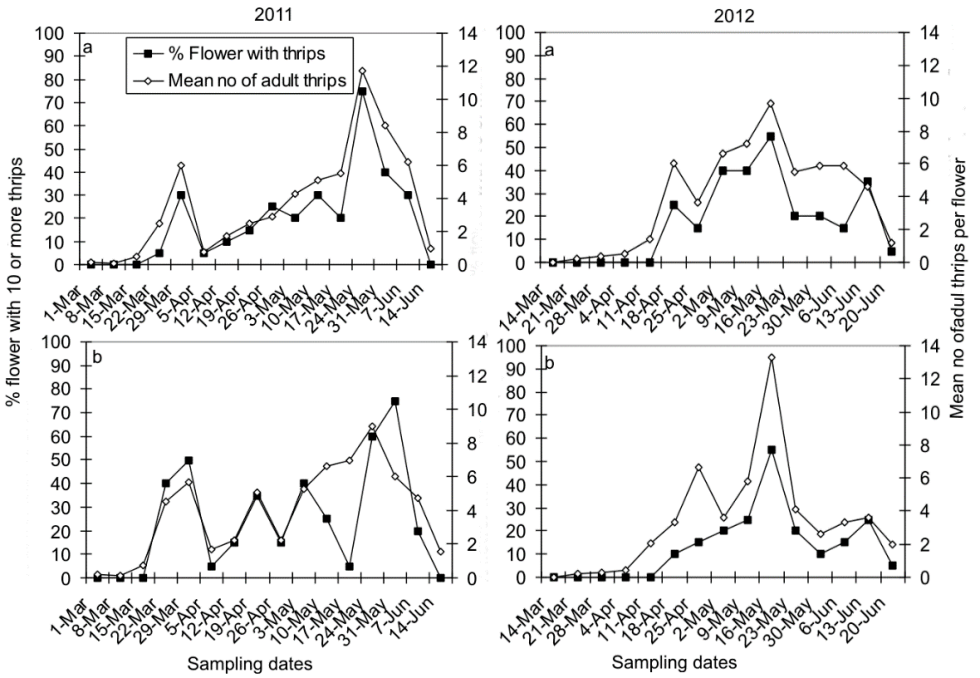

Figure 4. Percentage of flowers with 10 or more thrips and the mean number of adult *Frankliniella occidentalis* per flower in treated (a) and untreated plots (b) in 2011 and 2012.

Table 1. Linear correlation between the percentage of strawberry flowers with a minimum of 10 *Frankliniella occidentalis* (y) and the mean number of thrips per flower sampled (x). The action threshold is calculated from the equation

Year	Plot type	Stage(s)	Damage threshold Used	Linear equation	R^2	Action threshold for minimum number per unit
2011	Treated	Adult	10	6.512x-1.473	0.54	63%
		Adult+larvae	10	0.126x+2.138	0.71	3%
	Untreated	Adult	10	5.844x-2.212	0.92	56%
		Adult+larvae	10	0.205x+0.979	0.95	3%
2012	Treated	Adult	10	5.363x-3.039	0.85	52%
		Adult+larvae	10	0.201+1.353	0.89	3%
	Untreated	Adult	10	6.137x-1.368	0.91	60%
		Adult+larvae	10	0.234x+1.340	0.83	3%

Yield

Strawberry yield in the treated and untreated plots are given in Table 2. There was a significant difference between treated and untreated plots only on 21 May, 2011 *(t*= 4.243, df= 1,6, *P*= 0.005) and no difference in strawberry fruit yield between treated and untreated plots on other harvesting dates was found in both years (*P*>0.05)

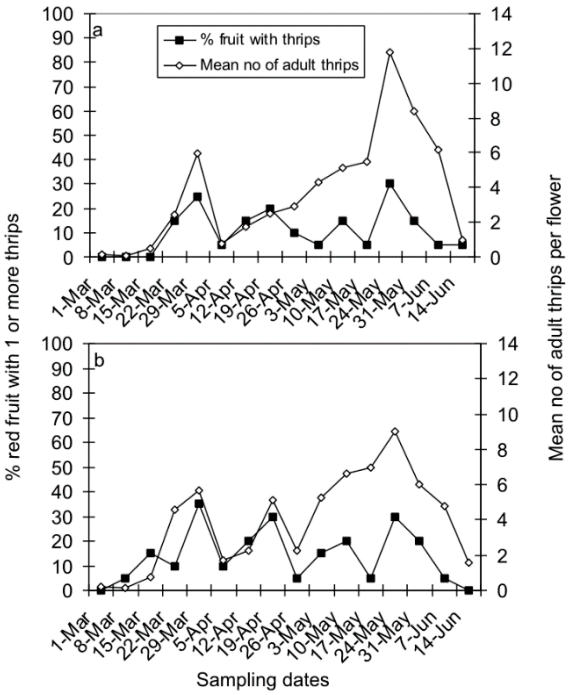

Figure 5. Percentage of red fruits with 1 or more thrips and the mean number of adult *Frankliniella occidentalis* per flower in treated (a) and untreated plots (b) in 2011.

Table 2. Strawberry yield (kg ha^{-1}) in treated and untreated plots in Adana province, Turkey in 2011 and 2012

Year		First class		Second class	
	Sampling dates	Treated*	Untreated	Treated	Untreated
2011	19 Apr	2440.00±17.73a	2400.00±61.27a	640.00±10.20a	400.00±13.65a
	17 May	1440.00±20.65a	1280.00±22.40a	440.00±12.00a	400.00±13.85a
	21 May	1200.00±8.24a	720.00±8.01b	220.00±14.00a	240.00±15.87a
	4 Jun	2050.00±13.06a	1902.00±8.24a	400.00±13.85a	520.00±12.00a
	Total	6400.83±47.10a	6800.00±52.86a	1700.00±35.53a	1540.00±26.60a
2012	15 May	1200.00±20.13a	1160.00±7.65a	480.00±9.23a	440.00±12.75a
	19 May	1440.00±2.13a	1200.00±8.50a	1040.00±15.31a	800.00±16.00a
	Total	2600.00±16.49a	2440.00±23.88a	1520.00±15.45a	1240.00±24.78a

* Means with same letters in rows are not statistically significant according to Student t-test at $P<0.05$.

Discussion

Frankliniella occidentalis were collected mainly from flowers and relatively low numbers of this thrips were recorded on green and red fruits of strawberry. Therefore, it is suggested that it is more appropriate to sample flowers in order to estimate thrips abundance in strawberry. Our results are in agreement with the findings of Steiner & Goodwin (2005) reported that the majority of *F. occidentalis* were detected in flowers in Australian strawberry fields. Furthermore, immature green and red fruits can be used for sampling when flowers are not available in adequate numbers for monitoring of *F. occidentalis.*

During late season relatively high population of *F. occidentalis* was recorded in strawberry flowers, this might be due to migration of the *F. occidentalis* from the surrounding wild vegetation to the strawberry

flowers being in less numbers in that period. *F. occidentalis* (mainly adults) has been noted on the majority of the flowering weeds grown nearby the experimental area. This thrips left the spring annual weedy plants when these host plants lost their flowers or nutritional contents in late spring period (May) in Çukurova region (Adana province) (Atakan & Uygur, 2005). Abundance of *F.occidentalis* in general and abundance of larvae in particular was significantly greater in flowers of treated than untreated plots on some sampling dates (Figure 2). Reason of this issue remained unknown.

Abundance of *F. occidentalis* recorded in every treatment was below the action threshold level of 10 thrips per flower at most sampling dates in both years. *F. occidentalis* abandoned strawberry plants after May when the number of flowers per plant was much less than those in April. Yıldırım & Başpınar (2013) also observed that *F. occidentalis* migrated from strawberry plants after early summer (June) in Aydın province, Turkey. In the current study, *F. occidentalis* exceeded action threshold level only once or twice in treated and untreated plots. Nearly 60% of flowers were infested with 10 or more adults of *F. occidentalis*. Additionally, nearly 6% of red fruit was found infested with 1 or more thrips (adults + larvae). However, there was no apparent damage of *F. occidentalis* to flowers even in untreated plots. Our results seem to be in conflict with previous studies carried out in various countries. For example, while Gremo et al. (1997) stated that 10 thrips individuals should be considered as an action threshold, Laudonia et al. (2000) suggested 15 *F. occidentalis* per flower as an action threshold level in Italy. These differences among the studies may be due to different ecological conditions such as climate, different variety of strawberry or different strain of *F. occidentalis*. However, findings of the current study are agreed with findings of Stefania et al. (1999), who reported that 15-20 mobile forms of *F. occidentalis* on strawberry crop grown in plastic tunnels in Italy is economic threshold level. Coll et al. (2006) also suggested that 25 *F. occidentalis* individuals per flower in spring time cause economic damage to strawberries grown in open field in Israel.

Although, there was a weak but statistically significant correlation between the percentage of red fruit with the mean number of adult thrips in flowers, no typical damage (bronzed or scarred fruits) was observed on sampled red or green fruits. At the peak abundance of thrips in flowers (15 total thrips per flower) the mean number of thrips on red or green fruits was very few. In our previous study carried out in a strawberry field outdoors (Atakan, 2008), *F. occidentalis* densities with at least two times higher than the ETL was reported to cause damage in the form of withered stigmas, anthers and slight necrotic spots on petals of some flowers. Additionally, at the peak abundance of thrips in flowers (22-24 thrips per flower), less numbers of the thrips adults or larvae were observed on fruits and their feeding damage resulted in the occurrence of slightly bronzed fruit surface beneath the calyx of red fruits. Contrary to our previous work, Coll et al. (2006) reported that when thrips abundance increased to an infestation level of 25 *F. occidentalis* adults per flower, typical thrips damage with silvering and bronzing of the fruits were encountered. These differences might be due to different strain of *F. occidentalis* and different strawberry variety. However, Steiner (2002) reported that important bronzing damage due to larval and adult *F. occidentalis* feeding upon green or red fruit was crucial when *F. occidentalis* abundance was over 10 thrips per fruit, and relative humidity and temperature were both high. Sampson & Kirk (2012) also reported that there was a good correlation between abundance of larval *F. occidentalis* and strawberry fruit damage in the Midlands, UK.

In conclusion, *F. occidentalis* infestation rarely exceeds the action threshold level and at this threshold level no typical thrips damage was detected on flowers or fruits. Although *F. occidentalis* infests mainly strawberry flowers, no association was found between thrips abundance and the density of flowers on plants. In this study *F. occidentalis* did not affect strawberry yield at all. *F. occidentalis* is the dominant thrips species in strawberry crops cultivated in the South-eastern Mediterranean region of Turkey. However, *F. occidentalis* did not seem to be an economically important pest of strawberry even if they cause damage to the fruiting parts in late-season as previously reported (Atakan, 2008). The registered

insecticide active ingredient spinosad is widely used to control thrips in strawberry in Turkey but the residual effect of this insecticide against *F. occidentalis* lasts for less than 10 days. In the Mediterranean region, strawberry growers have used this insecticide frequently in thrips management. Use of the same insecticide at frequent intervals may lead to the selection of a resistant strain in the target pest and cause negative side-effects such as killing of beneficial insects commonly found in strawberry.

Based on our field results, it appears that population rarely exceeds the economic threshold and only for a short period of time, therefore, no control measure should be applied against thrips in strawberry. The current ETL for *F. occidentalis* appears to be too low, and it appears that even 15 thrips per flower may not cause typical damage to flowers. Based on our results, it is concluded that ETL of *F. occidentalis* needs to be re-evaluated in strawberry fields in Turkey.

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Orijinal araştırma (Original article)

Contributions to the Turkish oribatid mite fauna (Acari: Oribatida)

Türkiye oribatid akar faunasına katkılar (Acari: Oribatida)

Nusret AYYILDIZ[1] **Ayşe TOLUK**[1*]

Summary

Seven known oribatid mite species *viz. Nothrus silvestris* Nicolet, *Hermanniella multipora* Sitnikova, *Licnobelba caesarea* (Berlese), *Jacotella frondeus* (Kulijev), *Adoristes* (*Gordeeviella*) *krivolutskyi* Shtanchaeva, Subías & Arillo, *Autogneta* (*Rhaphigneta*) *flagellata* (Mahunka) and *Phauloppia lucorum* (Koch), are recorded for the first time from Turkey. The morphological features of species mentioned here have been presented with SEM photographs, and their zoogeographical distributions are given. Key to the species for each genus is also given.

Keywords: *Nothrus*, *Hermanniella, Licnobelba, Jacotella, Adoristes, Autogneta, Phauloppia,* new records.

Özet

Yedi oribatid akar türü, isim olarak *Nothrus silvestris* Nicolet*, Hermanniella multipora* Sitnikova, *Licnobelba caesarea* (Berlese), *Jacotella frondeus* (Kulijev), *Adoristes (Gordeeviella) krivolutskyi* Shtanchaeva, Subías & Arillo, *Autogneta (Rhaphigneta) flagellata* (Mahunka) ve *Phauloppia lucorum* (Koch), Türkiye'den ilk kayıttır. Adı geçen türlere ilişkin morfolojik özellikler tarama elektron mikroskobu fotoğrafları ile birlikte sunulmuş ve zoocoğrafik dağılımları verilmiştir. Aynı zamanda her bir cins için türlere teşhis anahtarı verilmiştir.

Anahtar sözcükler: *Nothrus*, *Hermanniella, Licnobelba, Jacotella, Adoristes, Autogneta, Phauloppia,* yeni kayıtlar.

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Introduction

Oribatid mites (Acari) are often the dominant microarthropod group in forest soil-litter habitats. They are actively involved in decomposition of organic matter, in nutrient cycling and soil formation (Behan-Pelletier, 1999). Oribatid mites consist of 10.826 described species and subspecies worldwide (Subías, 2004, updated 2016) and the total species number is estimated to be up to 100000 (Schatz, 2002). The highest number of species is known from the Palaearctic region, followed by the Oriental and Neotropical regions (Schatz, 2004). The beginning of taxonomic works on oribatid mites in Turkey goes back to the 1980s (Ayyildiz, 1988 a-e, 1989; Ayyildiz & Luxton, 1989 a,b; Ayyildiz & Özkan, 1988). To date, the number of oribatid mite species and genera known from Turkey is about 200 and 90, respectively (Özkan et al., 1994; Bayartogtokh et al., 2000; Bayartogtokh et al., 2002; Grobler et al., 2003, 2004; Erman et al., 2007; Toluk & Ayyildiz, 2008). So, it is expected that continued researches on the Turkish oribatid mite fauna will significantly augment the known species diversity for the country.

In the present paper, seven known species belong to the genera *Nothrus* Koch, *Hermanniella* Berlese, *Licnobelba* Grandjean, *Jacotella* Banks, *Adoristes* Hull, *Autogneta* Hull, and *Phauloppia* Berlese are added to the Turkish fauna with remarks on the recorded species. Previous records of oribatid species belonging to the genera *Nothrus*, *Hermanniella*, *Autogneta* and *Phauloppia* mentioned here included *N. anauniensis* Canestrini & Fanzago (recorded as *N. biciliatus* Koch) from Erzurum, *H. punctulata* Berlese from Yozgat, *A. (Rhaphigneta) numidiana* (Grandjean) from Artvin and *P. rauschenensis* (Sellnick) (recorded as *P. saxicola* Travé) from Ankara (Ayyildiz, 1988a; Grobler et al., 2004; Toluk et al., 2006; Toluk & Ayyildiz, 2009). Prior to this study there was no record for the genera *Licnobelba*, *Jacotella* and *Adoristes (Gordeeviella)* from Turkey.

Material and Methods

The mite specimens in soil, litter and moss taken from Artvin, Antalya and Bolu provinces of Turkey between 2008 and 2014, were extracted using Berlese funnels and preserved in 75% ethyl alcohol. Measurements and descriptions are based on specimens mounted in temporary cavity slides that were studied using a compound microscope. All measurements are presented in micrometers (µm). Body length was measured in lateral view, from the tip of the rostrum to the posterior edge of the ventral plate, to avoid discrepancies caused by different degrees of notogastral distension. Notogastral width refers to the maximum width in dorsal aspect. For scanning electron microscope (SEM) investigations, the specimens were mounted on aluminum-stubs with double sided carbon tape and dried in a desiccator. Then the specimens were sputter-coated with 15 nm gold/palladium. SEM photographs were taken using a Zeiss/Leica LEO 440 scanning electron microscope at the Technology Research and Application Center of Erciyes University. The morphological terminology follows that of Norton & Behan-Pelletier (2009). The examined specimens are deposited in the Acarological Collection of the Zoological Museum, Erciyes University, Kayseri, Turkey.

Results and Discussion

A total of seven species belonging to 7 genera of oribatid mites from Artvin, Antalya and Bolu provinces of Turkey were determined. These species are given below.

Genus *Nothrus* Koch

Rostrum with median incision, bothridium present, genital and anal plate large, occupying entire ventral side posterior to epimeres, aggenital setae absent, epimeres I with 5–7 setae, legs with 1–3 claws.

Key to the species of the genus *Nothrus* of Turkey

1. Posterior notogastral setae rod like, long, not widened, barbed distally; leg tarsi with one claw, length = 710–810…….……..……..…….. *N. silvestris* Nicolet

- Posterior notogastral setae widened distally, relatively short, not much longer than the others; leg tarsi with three claws, length = 700–810 ..... *N. anauniensis* Canestrini & Fanzago

Nothrus silvestris Nicolet

Measurements: Body length: 732–773 µm, body width: 402–408 µm (n=6).

Morphological features (Fig. 1A-F): Rostrum rounded. Rostral setae (*ro*) barbed. Lamellar setae (*le*) barbed and slightly curved, set on apophyses. Interlamellar setae (*in*) phylliform, barbed. Sensilli (*ss*) long, rod-like and barbed. The middle part of notogaster, between folds, covered with distinct, large, round pits. Lateral surface with small pits. Distance between setae c_1–c_2 shorter than between c_2–c_3. Sixteen pairs of notogastral setae present, not widened, rod like. Epimeral setal formula 3-1-3-3. Genital plates with 9 pairs of setiform setae (g_{1-9}); 2 pairs of short anal setae (an_{1-2}); 3 pairs of adanal setae (ad_{1-3}). All legs monodactylous.

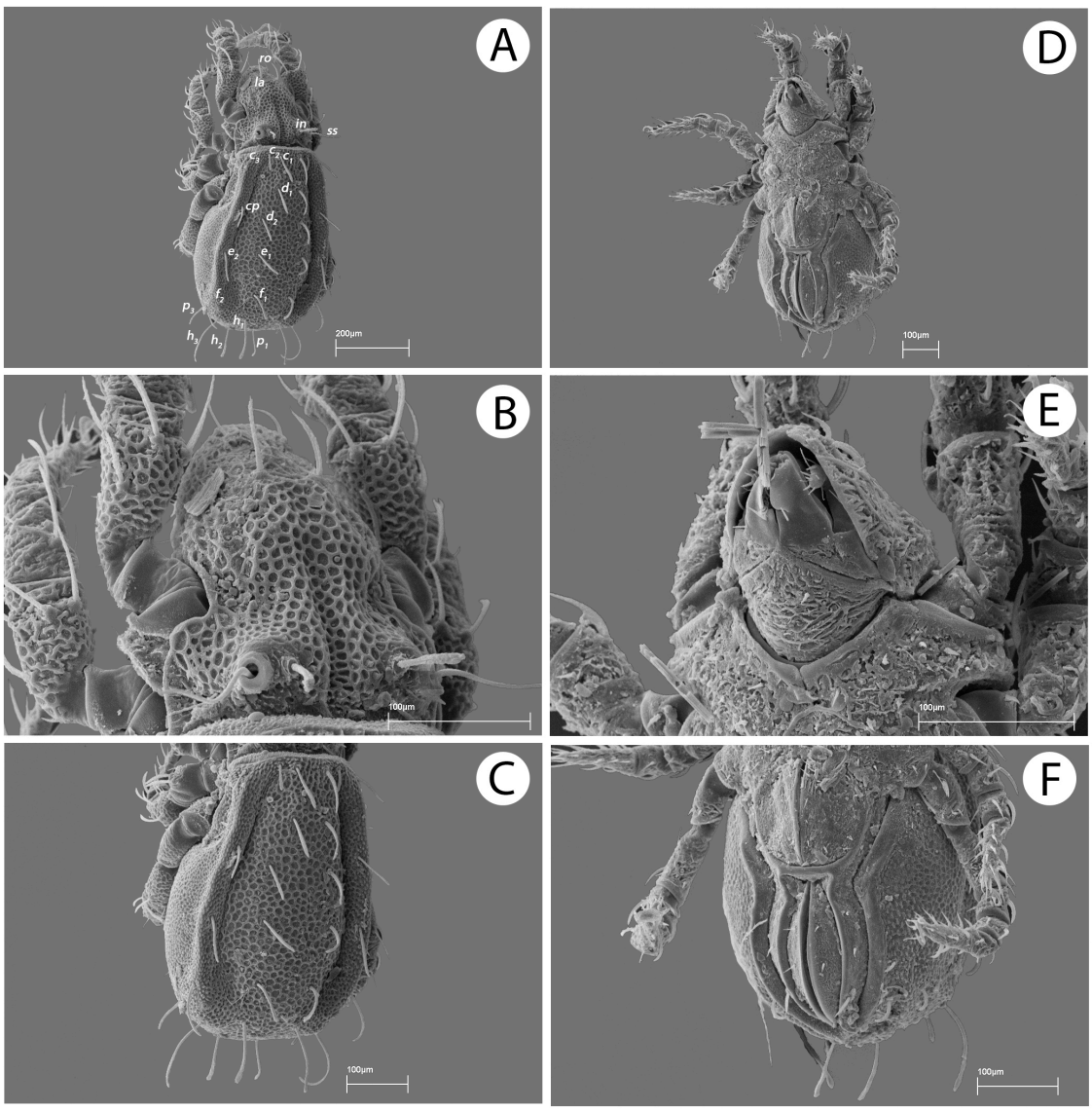

Figure 1. *Nothrus silvestris* Nicolet. A- Dorsal view; B- Prodorsum; C- Notogaster; D- Ventral view; E- Subcapitulum; F- Genito-anal region.

Material examined: Turkey, Bolu province, N: 40°56.447', E: 031°44.763', 784 m, 25.V.2014, collected in litter and soil, 6 exs. (2 of them were mounted on aluminum stubs and gold-coated for scanning electron microscopy).

Remarks: This species is new record for Turkish fauna. It is widespread in various acidophilous and mesophilic forests, also on drier peatlands (Weigmann, 2006). Turkish specimens are collected in soil and litter. The dimensions of the species are given as 710–810 µm by Weigmann (2006). The Turkish specimens (732–773 x 402–408 µm) are in the range of the known dimensions of the species. This species is well characterized by the shape of sensilli, phylliform barbed interlamellar setae, 16 pairs of notogastral setae, nine pairs of genital setae, number of claws and shape of posterior notogastral setae.

Genus *Hermanniella* Berlese

Bothridia situated near prodorsal margins, notogaster with a pair of lateral, tube-like projections, notogastral setae usually of the same type (except f_1 dilated leaf-shaped), aggenital setae absent.

Key to the species of the genus *Hermanniella* of Turkey

1. Interlamellar setae widened, strongly leaf-shaped and bent in the direction of the bothridia, much shorter than sensilli; notogastral plate without areolae, posterior notogastral setae rather widened strongly barbed, length= 516–582 µm …….…. *H. multipora* Sitnikova

- Interlamellar setae not widened, rod like and forward directed to the lamellar setae, almost the same length as sensilli; notogastral plate with areolae, posterior notogastral setae rod like, length= 510–700 µm..... *H. punctulata* Berlese

Hermanniella multipora Sitnikova

Measurements: Body length: 440–540 µm, body width: 220–321 µm (n=8).

Morphological features (Fig. 2A-F): Body surface covered with cerotegument. Rostrum widely rounded in dorsal view. Rostral and lamellar setae narrow, thickened barbed, curved inwards. Interlamellar setae strong, barbed, leaf shaped. Sensilli with long stalk (37 µm), short (13 µm) and weakly thickened head. Notogaster oval-shaped. Cerotegument forming papilliform granules. Sculpture of integument under cerotegument of dorsal part represented by a vast number of very small slitlike apertures. No areolae. Fifteen pairs of notogastral setae present, variable in length and thickness. The setae f_1 and especially h_1 and h_2 dilated, h_3 very small and barely perceptible in length. Epimeral setal formula 3:1:3:3. Seven pairs of genital setae, five pairs arising closer to inner margin than others and latter two pairs much larger than the inner pairs. One pair of aggenital setae; two pairs of anal setae; three pairs of adanal setae smooth, only adanal setae ad_1 longer and thicker than others, with bent apices. All legs monodactylous.

Material examined – Turkey, Artvin province, a forest in 7 km along Borçka-Muratlı town road, N: 41°22´39˝, E: 41°41´11˝, 210 m, 27.X.2008, collected in moss on tree, 10 exs. (2 of them were mounted on aluminum stubs and gold-coated for scanning electron microscopy).

Remarks – This species is distributed in Palaearctic region (Subías, 2004, updated 2016). It is new record for Turkish fauna. Holotype and paratypes were originally collected from litter under cypress tree and a rotten hornbeam trunk near footpath in Sochi, and litter from mixed woodland in Malesna River valley, Soviet Far East (Sitnikova, 1973). Turkish specimens are collected in moss on tree. The dimensions of the species are given as 552 µm (516–582) x 325 µm (282–382) by Sitnikova (1973). The Turkish specimens (440–540 x 220–321 µm) are in the range of the known dimensions of the species. This species is well characterized by the shape of sensilli, the vast number of very small slit like apertures on notogaster, 15 pairs of notogastral setae, dilated setae f_1, h_1 and h_2 and by the very small barely perceptible setae h_3 and the lack of areolae on the notogaster.

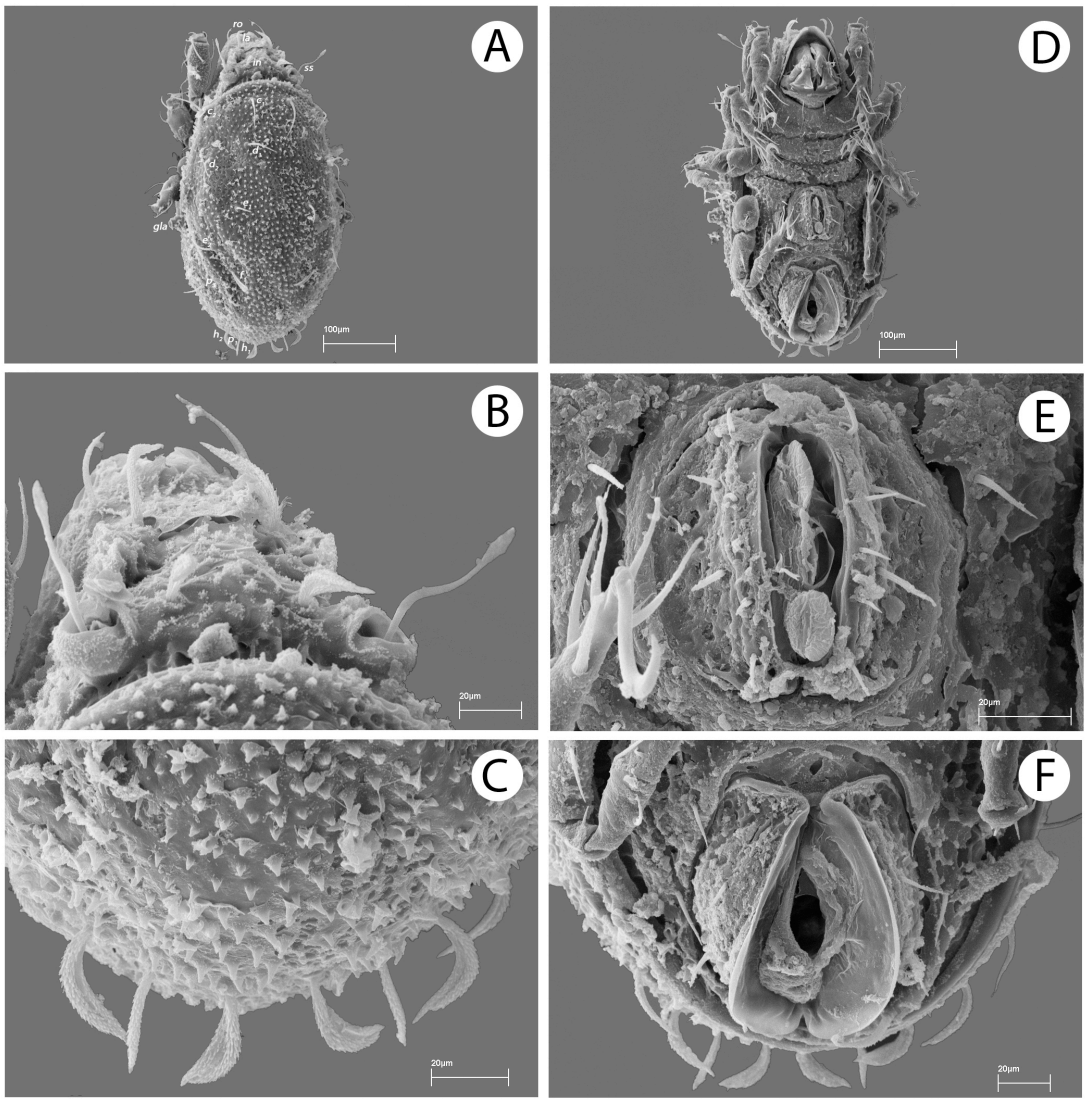

Figure 2. *Hermanniella multipora* Sitnikova. A- Dorsal view; B- Prodorsum; C-The posterior part of notogaster; D- Ventral view; E- Genital plate; F- Anal plate.

Genus *Licnobelba* Grandjean

Adults with exuviae on dorsal; thick cerotegument formed by high polygonal pieces; notogastral surface smooth, shiny and with 4–6 pairs of setae; six pairs of genital setae present.

Licnobelba caesarea (Berlese)

Measurements – Body length: 250–280 µm, body width: 120–140 µm (n = 6).

Morphological features (Fig. 3A-F): Prodorsal surface covered by a thick reticulated cerotegumental layer, forming a web-like pattern. Rostrum rounded. Rostral setae inserted from ventral side. Lamellar setae inserted on small lateral apophyses. Both of them covered by thick cerotegument with small granules. Sensilli large, leaf-shaped, with a very short stalk. Notogaster covered with unclear tubercules. Five pairs of postero-lateral notogastral setae; Lyrifissures *ia* and *im* wide and very clear. Discidium weakly developed and rounded. Epimeral setal formula 3:1:3:3. Six pairs of short and thin genital setae, one pair of aggenital setae, two pairs of anal setae and three pairs of adanal setae. All legs tridactylous and covered by a thick cerotegumental layer. Tibia I with large and well developed apophysis.

Material examined – Turkey, Antalya province, Güllük Mountain, N: 37°00´59.52˝, E: 30°00´59.52˝, 550 m, 26.VI.2010, soil and litter, 6 exs. (2 of them were mounted on aluminum stubs and gold-coated for scanning electron microscopy).

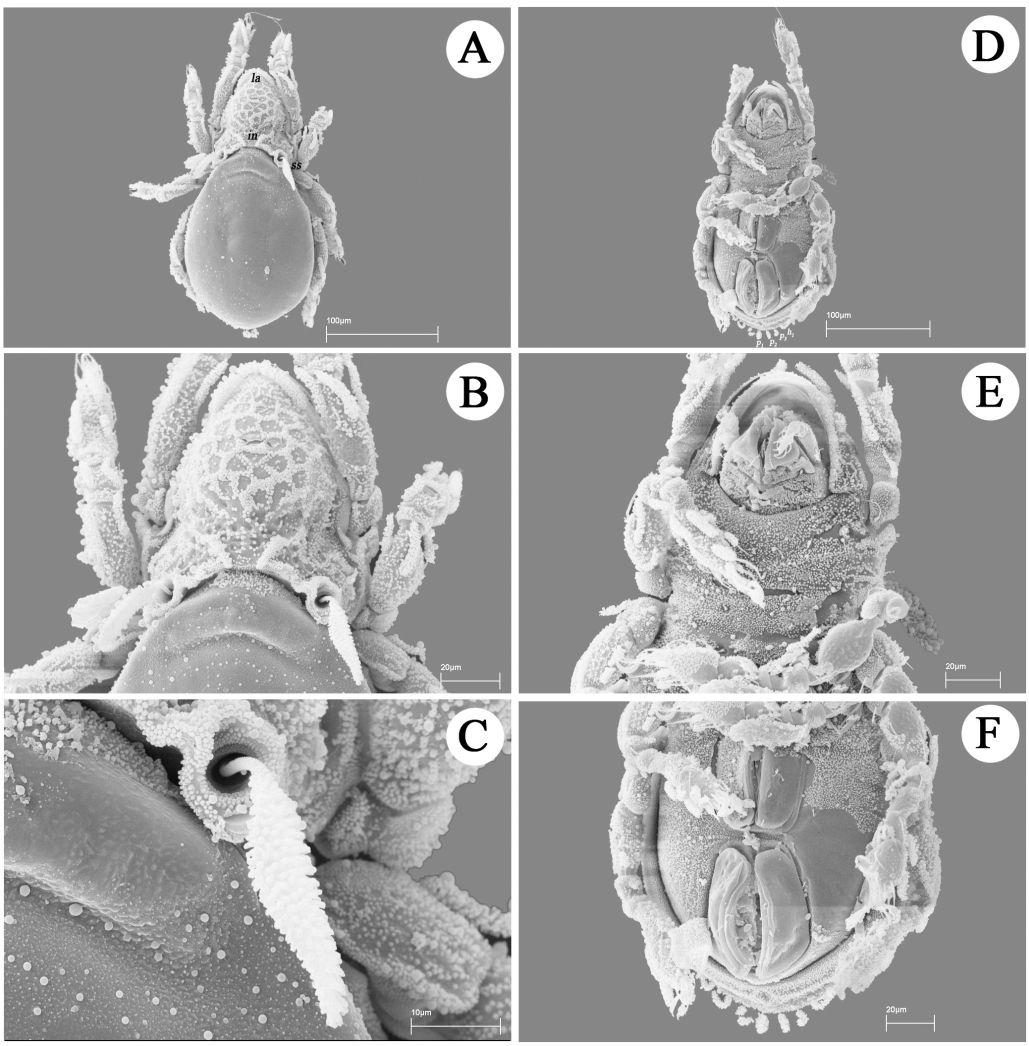

Figure 3. *Licnobelba caesarea* (Berlese). A- Dorsal view; B- Prodorsum; C- Sensillus; D- Ventral view; E- Subcapitulum and epimeral region; F- Genito-anal region.

Remarks – This species is distributed in Mediterranean (Subías, 2004, updated 2016). It is a new record for Turkish fauna. The body length is given as 270–285 µm by Grandjean (1931) and 260–302x120–160 µm by Pérez-Iñigo (1994), 275–281x125–137 µm and 270–302x125–160 µm by Kahwash et al., (1990). The Turkish specimens (250–280 x 120–140 µm) are in the range of the known dimensions of the species. This species is well characterized by the shape of sensilli; prodorsum with a polygonal sculpture; five pairs of postero-lateral notogastral setae. Pérez-Iñigo (1994) who states that notogaster is completely smooth, without any kind of network pattern. However, notogastral surface covered with unclear tubercules in the Turkish specimens. The reason for this contrast, we believe that due to the microscopic examination.

Genus *Jacotella* Banks

Adults without exuviae on dorsal; sensilli with slightly dilated head; notogastral setae h_2 absent; seven pairs of genital setae present, legs covered by a thin cerotegument layer; pedotectal tooth absent.

Jacotella frondeus (Kulijev)

Measurements: Body length: 332–392 µm, body width: 180–216 µm (n = 5).

Morphological features (Fig. 4 A-F): Body surface covered by polygonal cerotegument. Rostral setae well sclerotized. Lamellar setae inserted on short apophyses antero-dorsally on the rostrum. Interlamellar setae on small tubercle. Sensilli with broadly expanded head. Four pairs of notogastral setae (h_1, p_1, p_2 and p_3). Posterior notogastral setae h_1 and p_1 inserted on strong apophyses, h_2 absent, h_1 much longer than others. Epimeral setal formula 2:2:3:1. Seven pairs of genital setae, one pair of aggenital setae, two pairs of anal setae and three pairs of adanal setae. Genital and anal openings separated by a band of cuticle that covers the articulation of the preanal shield. Discidium absent. All legs tridactylous.

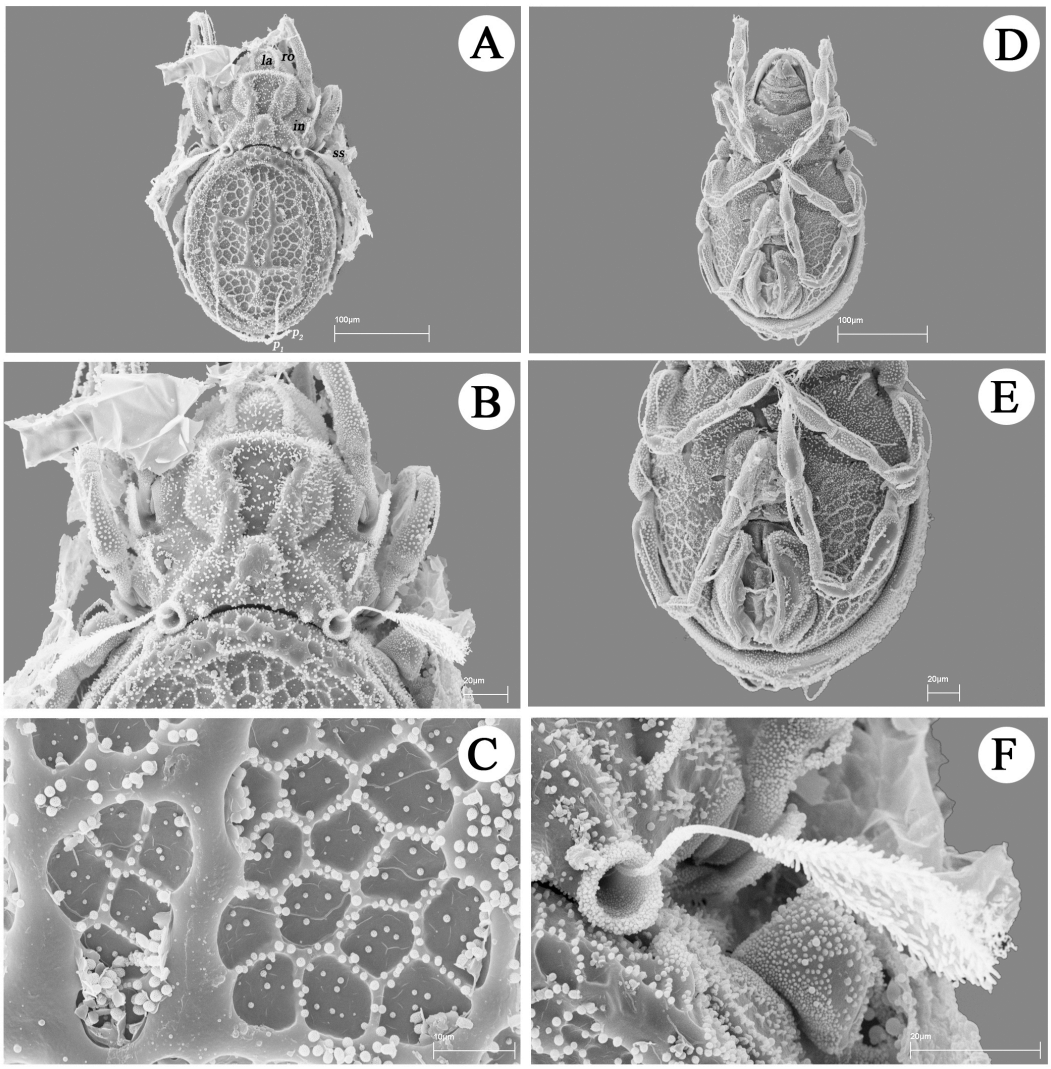

Figure 4. *Jacotella frondeus* (Kulijev). A-Dorsal view; B-Prodorsum; C- Notogastral ornamentation; D-Ventral view; E-Genito-anal region; F- Sensillus.

Material examined – Turkey, Antalya province, Güllük Mountain, N: 37°00´59.52˝, E: 30°00´59.52˝, 550 m, 26.VI.2010, soil and litter, 5 exs. (2 of them were mounted on aluminum stubs and gold-coated for scanning electron microscopy).

Remarks – This species is distributed in Eastern Mediterranean (Subías, 2004, updated 2016). It is new record for Turkish fauna. The dimensions of the type specimen are given as 422 x 266 µm by Kulijev (1979). The Turkish specimens (332–392 x 180–216 µm) are in the range of the known dimensions of the species. This species is well characterized by the shape of sensilli, notogaster with a polygonal sculpture; four pairs of notogastral setae. In this regard, the Turkish specimens closely resemble the species in all features.

Genus *Adoristes* Hull

Lamellae separated, with or without cuspis, without translamellae; sensilli clavate or sharply pointed at tip; notogaster without long humeral process and with 11 pairs of setae; five pairs of genital setae present; legs with 3 claws.

Subgenus *Adoristes (Gordeeviella)* Shtanchaeva, Subías & Arillo

Adoristes (Gordeeviella) krivolutskyi Shtanchaeva, Subías and Arillo

Measurements: Length: 480–648 µm, width: 256–344 µm (n = 6).

Morphological features (Fig. 5 A-E): Rostrum truncate swelling at middle and forward-projected. Rostral setae smooth. Lamellae long and same width over the entire length, without translamella and lamellar cuspids less obvious. Lamellar setae smooth and inserted on distal edge of lamellae. Interlamellar setae smooth and long. Bothridium hidden by anterior margin of notogaster. Sensilli spindle-form, slightly barbed, apical part longer than length of head. Anterior margin of notogaster slightly concave. Eleven pairs of very small, smooth and thin notogastral setae. Lyrifissures *im* present but difficult to see. Epimeral setae short, smooth and thin. Epimeral setal formula 3:1:3:3. Five pairs of genital setae, three anterior pairs very close to each other and longer than two posterior pairs. One pair of aggenital setae, two pairs of anal setae and three pairs of adanal setae. Adanal lyrifissures *iad* situated in paranal position. All legs tridactylous.

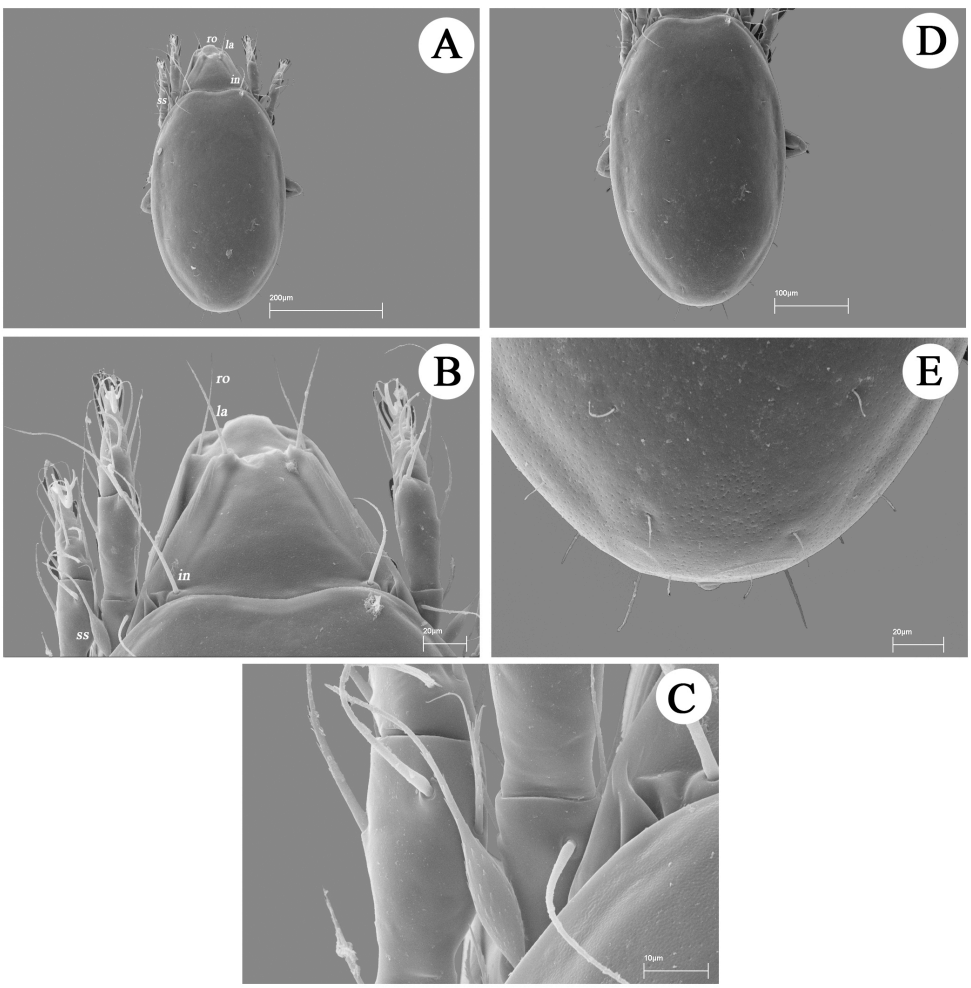

Figure 5. *Adoristes (Gordeeviella) krivolutskyi* Shtanchaeva, Subías & Arillo. A-Dorsal view; B-Prodorsum; C-Sensillus; D- Notogaster; E- The posterior part of notogaster.

Material examined – Turkey, Bolu province, Gölcük lakeside, N: 40°39´38˝, E: 31°37´598˝, 1225 m, 10.V.2008, litter, 6 exs. (2 of them were mounted on aluminum stubs and gold-coated for scanning electron microscopy).

Remarks – This species is distributed in Caucasus and Portugal (Subías, 2004, updated 2016). It is new record for Turkish fauna. It is currently known only from type locality, Batumi (Georgia) and southern Portugal. The specimens belonging to this species were extracted from soil under *Eucalyptus* and relict forest of conifers in Batumi (Georgia), and soil under *Quercus suber* in Luzianes (Portugal) (Shtanchaeva et al., 2010, 2012). The dimensions of the type specimen are given as 500–640 x 250–330 µm by Shtanchaeva et al. (2010). The Turkish specimens (480–648 x 256–344 µm) are in the range of the dimensions of the type specimen. This species is well characterized by the shape of sensilli and lamellae without translamella; eleven pairs of notogastral setae; five pairs of genital setae

Genus *Autogneta* Hull

Rostrum with a median incision; costulae long, narrow, far from each other; prodorsum laterally without granula (with granula in *Rhaphigneta*); legs with one claw.

Subgenus *Autogneta (Rhaphigneta)* Grandjean

Key to the species of the subgenus *Autogneta (Rhaphigneta)* of Turkey

1. Distal apex of notogastral setae curved like a hook, length= 456–504 µm…..... *A. (R.) flagellata* (Mahunka)

- Distal apex of notogastral setae smooth, length= 350–445 µm..... *A. (R.) numidiana* (Grandjean)

Autogneta (Rhaphigneta) flagellata (Mahunka)

Measurements: Body length: 488–504 µm, body width: 268–272 µm (n=5).

Morphological features (Fig. 6 A-F): Rostrum divided by a deep incision. Costulae long, reaching near the bothridia. Lamellar setae arising at the distal end of the costulae and reaching beyond insertion points of rostral setae. Interlamellar setae long and ciliate. Apex of sensillus weakly thickening bearing slightly dentate. Bothridia well developed. Prodorsum laterally with granula. Notogaster with a pair of prominent humeral processes. Ten pairs of notogastral setae present. Distal apex of notogastral setae curved like a hook. Epimeral setal formula 3-1-3-3. Six pairs of genital setae; one pair of aggenital; two pairs of anal; three pairs of adanal. Lyrifissures *iad* in paraanal position. The adanal setae are situated as follows: ad_1 - postanally, ad_2 - paraanally, ad_3 – preanally. All legs monodactylous.

Material examined – Turkey, Bolu province, N: 40°56´443˝, E: 031°44´816˝, 795 m, 25.V.2014, collected in soil and litter, 5 exs. (2 of them were mounted on aluminum stubs and gold-coated for scanning electron microscopy).

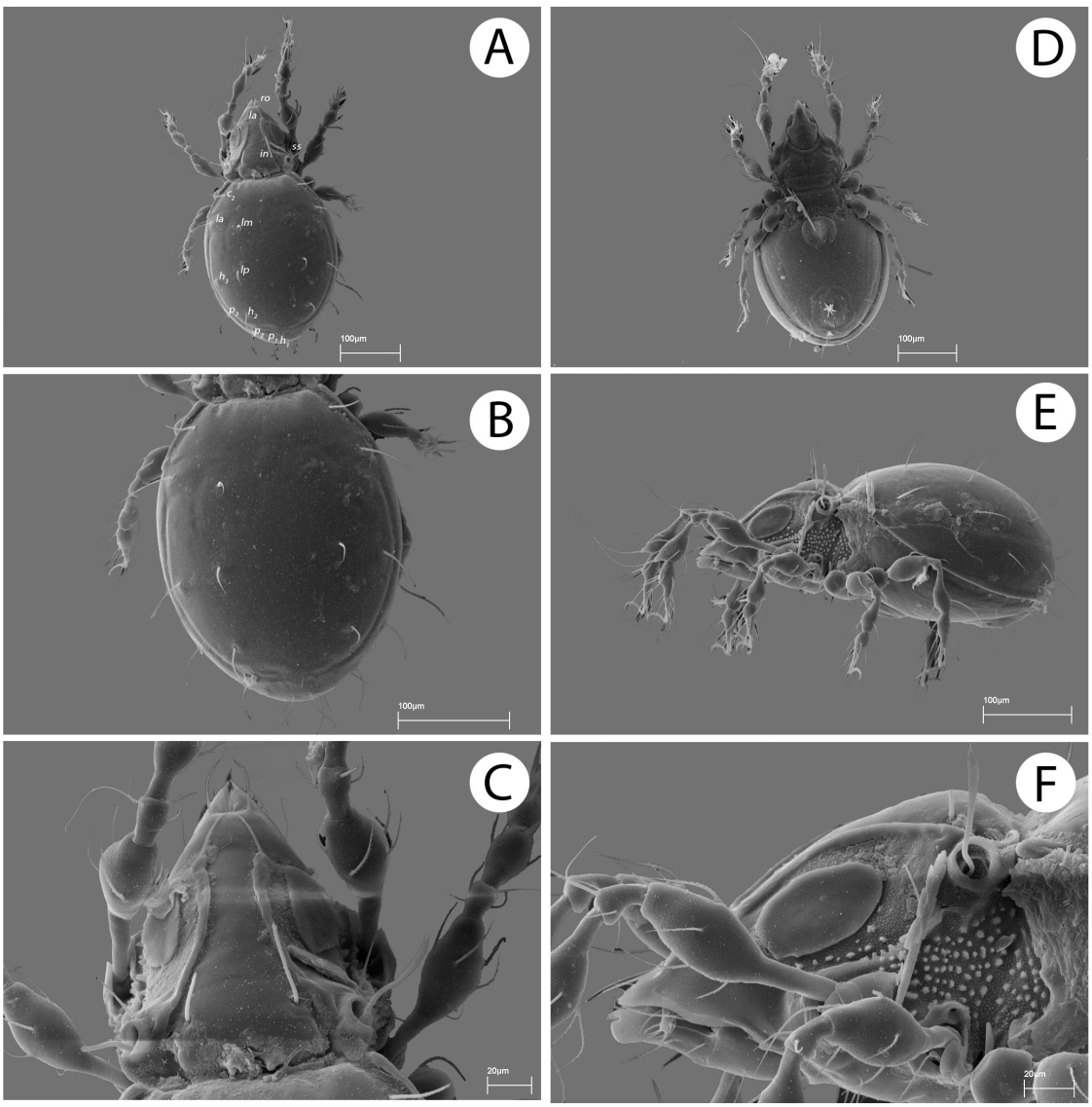

Figure 6. *Autogneta (Rhaphigneta) flagellata* (Mahunka). A-Dorsal view; B- Notogaster; C-Prodorsum; D- Ventral view; E- Lateral view of body; F- Lateral view of prodorsum. .

Remarks – This species is new record for Turkish fauna. Holotype and paratypes were originally collected from soil under *Pinus* and *Quercus coccifera* tree, ground near the summit of Mount Gournis (Mahunka, 1977). Turkish specimens are collected in litter and soil. The dimensions of the species are given as 456–483 x 249–272 µm by Mahunka (1977). The Turkish specimens (488–504x 268–272µm) are in the range of the known dimensions of the species. This species is well characterized by the shape of sensilli and costulae, 10 pairs of distal apex of notogastral setae recurving like a hook notogastral setae.

Genus *Phauloppia* Berlese

Lamellae linear; translamella absent; 14 pairs of notogastral setae present; four pairs of porose areas present; dorsosejugal suture medially interrupted or continuous; genital plates with four pairs of setae; legs with 3 claws.

Key to the species of the genus *Phauloppia* of Turkey

1. Areae porosae *Aa* significantly long, tape-shaped the others (A_1-A_3) oval; notogastral setae long (about 150 µm); dorsosejugal suture medially interrupted; length 600-900 µm …..... *P. lucorum* (C. L. Koch)

- All areae porosae (*Aa,* A_1-A_3) oval; notogastral setae short (about 25 µm); dorsosejugal suture not medially interrupted; length 310-390 µm …….. *P. rauschenensis* (Sellnick)

Phauloppia lucorum (C. L. Koch)

Measurements: Body length: 720–890 µm, body width: 440–570 µm (n= 3).

Morphological features (Fig. 7A-F): Rostrum rounded. All prodorsal setae setiform and barbed. Lamella indicated as a weak line. Sensillus with short stalk and claviform head with some small spines. Dorsosejugal suture medially interrupted. Fourteen pairs of smooth notogastral setae present, variable in length. Four pairs of porose areas present. *Aa* significantly long, tape-shaped. A_{1-3} roundish oval. Epimeral setal formula 3-1-3-3. Four pairs of genital setae, one pair of agenital setae, two pairs of anal setae, three pairs of adanal setae. All legs tridactylous.

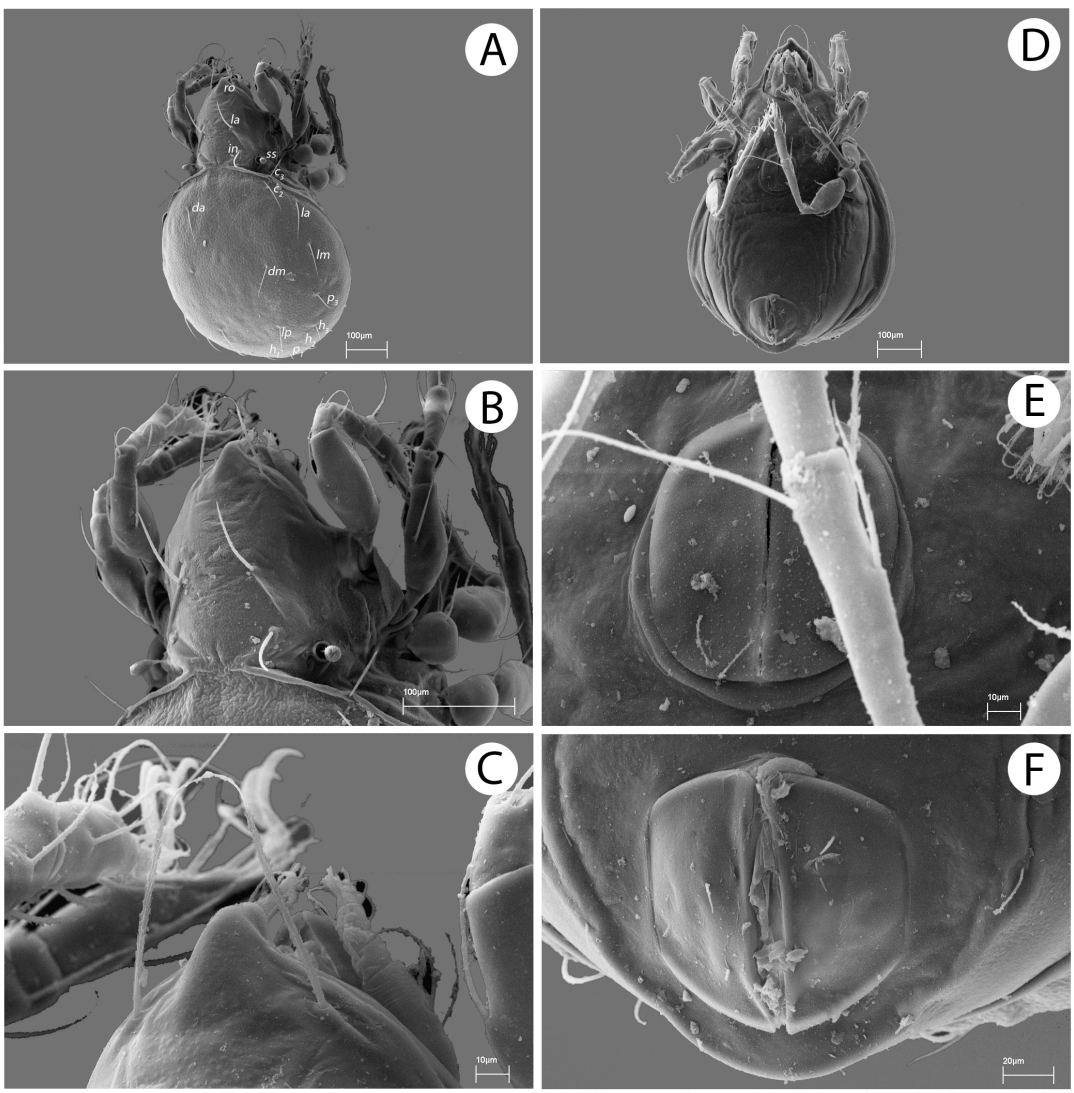

Figure 7. *Phauloppia lucorum* (Koch). A- Dorsal view; B- Prodorsum; C- Rostrum; D- Ventral view; E- Genital plate; F- Anal plate.

Material examined – Turkey, Artvin - Kafkasör road, N: 41°10´13˝, E: 41°48´42˝, 860 m, 20.IX.1992, moss on *Quercus* sp., 3 exs. (2 of them were mounted on aluminum stubs and gold-coated for scanning electron microscopy).

Remarks – This species is new record for Turkish fauna**.** It is a common in moss and lichen coatings on trees and on stones, rocks (Weigmann, 2006). Turkish specimens are collected in moss on tree. The dimensions of the species are given as 600 x 900 µm by Weigmann (2006). The Turkish specimens (720–890 x 440–570 µm) are in the range of the known dimensions of the species. This species is well characterized by the pattern shape of prodorsum and notogaster, dorsosejugal suture open in the middle, the claviform sensilli, fourteen pairs of smooth notogastral setae, four pairs of porose areas (*Aa* the longest, A_{1-3} roundish oval).

Conclusion

The present study added seven species new to the oribatid fauna of Turkey. The genera *Licnobelba* and *Jacotella,* and the subgenus *Adoristes* (*Gordeeviella*) are reported for the first time from Turkey. For *Autogneta (Rhaphigneta) flagellata* (Mahunka, 1977) and *Adoristes* (*Gordeeviella*) *krivolutskyi* Shtanchaeva, Subías & Arillo, 2010, Turkey are the first locality record outside its type locality (Greece) and the third locality record outside its type locality (Georgia) and Portugal, respectively.

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Orijinal araştırma (Original article)

Sera domatesi yetiştiriciliğinde *Eretmocerus mundus* (Hymenoptera: Aphelinidae) ve *Macrolophus melanotoma*'nın *Bemisia tabaci* (Hemiptera: Miridae, Aleyrodidae)'ye karşı etkinlikleri[1]

Efficacy of *Eretmocerus mundus* (Hymenoptera: Aphelinidae) and *Macrolophus melanotoma* aganist *Bemisia tabaci* (Hemiptera: Miridae, Aleyrodidae) in protected tomato

Kamil KARUT[2*] Cengiz KAZAK[2]
İsmail DÖKER[2] Amir Abdullahi Yousif MALİK[2,3]

Summary

In this study, the success of single and combined releases of *Eretmocerus mundus* Mercet (Hymenoptera: Aphelinidae) and *Macrolophus melanotoma* (Costa) (Hemiptera: Miridae) aganist *Bemisia tabaci* (Gennadius) (Hemiptera: (Aleyrodidae) were determined on greenhouse grown tomato plants that planted in net cages. The studies were conducted in spring growing seasons of the years 2009 and 2010. Experiments were established with four different treatments namely *B. tabaci* single (control), *M. melanotoma* single, *E. mundus* single, *M. melanotoma*+*E. mundus* combined releases with 3 replications (cage). In both years the *B. tabaci*, *E. mundus* and *M. melanotoma* were released 20, 6 and 0.5/plants, respectively. In order to determine population development of *B. tabaci* and *E. mundus*, leaf samples were taken at weekly intervals. In addition, numbers of immature and adult stages of *M. melanotoma* were counted by using visual control method on whole parts of 15 plants in each predator released cages. In the parasitoids released cages, the mean numbers of immature *B. tabaci* were not reached higher than 0.56 and 2.82 individuals/cm^2 in 2009 and 2010, respectively. These numbers were found to be lower than control and *M. melanotoma* single releases. The highest immature *B. tabaci* numbers were determined in control treatments as 51.10 and 31.12 per cm^2 in both years. Although *M. melanotoma* single release treatment reduced the whitefly numbers statistically compared to control treatment, it was not succeed as much as *E. mundus*. The results of this study showed that *B. tabaci* populations could be control without any insecticide treatments in greenhouse by using biological control programs that will be consisted with *E. mundus* and *M. melantoma* in Turkey.

Keywords: Biological control, *Bemisia tabaci*, *Eretmocerus mundus*, *Macrolophus melanotoma*

Özet

Bu çalışmada, *Eretmocerus mundus* Mercet (Hymenoptera: Aphelinidae) ve *Macrolophus melanotoma* (Costa) (Hemiptera: Miridae)'nın *Bemisia tabaci* (Gennadius) (Hemiptera: (Aleyrodidae)'ye karşı ayrı ayrı ve bir arada etkinlikleri cam sera içerisine yerleştirilmiş tül kafesler içerisinde yetiştirilen domates bitkileri üzerinde belirlenmiştir. Çalışma 2009 ve 2010 yıllarında bahar üretim sezonunda gerçekleştirilmiştir. Denemeler, tek *B. tabaci* (kontrol), tek *M. melanotoma*, tek *E. mundus* ve *M. melanotoma*+*E. mundus* bir arada olmak üzere 4 farklı uygulama ve 3 tekrarlı (kafes) olarak kurulmuştur. *B. tabaci*, *E. mundus* ve *M. melanotoma*, her iki yılda da bitki başına sırasıyla 20, 6 ve 0.5 adet olacak şekilde salınmıştır. Deneme süresince *B. tabaci* ve *E. mundus* popülasyon gelişimlerini belirlemek amacıyla haftalık aralıklarla yaprak örnekleri alınmıştır. Ayrıca, her kafeste rastgele seçilen 15 bitkinin tamamında gözle kontrol yöntemi kullanılarak *M. melanotoma*'nın nimf ve ergin dönemleri sayılmıştır. Çalışmada tek parazitiot salımı yapılan kafeslerde ergin öncesi *B. tabaci* yoğunlukları 2009 ve 2010 yıllarında sırasıyla 0.56 adet/cm^2 ve 2.82 adet/cm^2'nin üzerine çıkamamış ve kontrol ile tek avcı salımı yapılan kafeslerden düşük bulunmuştur. En yüksek *B. tabaci* ergin öncesi yoğunluğu ise aynı yıllarda 51.10 ve 31.12 adet/cm^2 ile kontrol uygulamalarından elde edilmiştir. Tek başına *M. melanotoma* uygulaması, kontrolle karşılaştırıldığında beyazsinek yoğunluğunda istatistiksel olarak önemli düşüşe sebep olsa da *E. mundus* kadar başarılı olamamıştır. Bu çalışmada elde edilen sonuçlar, Türkiye'de domates seralarında *E. mundus* ve *M. melanotoma*'nın birlikte kullanıldığı biyolojik mücadele yönteminin *B. tabaci* popülasyonunu hiç insektisit uygulamaya gerek kalmadan başarıyla baskı altına alabildiğini göstermiştir.

Anahtar sözcükler: Biyolojik mücadele, *Bemisia tabaci*, *Eretmocerus mundus*, *Macrolophus melanotoma*

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Giriş

Türkiye'de, örtüaltı sebze yetiştiriciliği, sera ve alçak plastik tünel altındaki tarımsal üretimi kapsamakta olup, toplam örtüaltı üretim alanı 2014 yılı itibarı ile yaklaşık 64.911 ha'ra ulaşmıştır (TÜİK, 2014). Türkiye 2012 üretim sezonunda 10.350.000 ton domates üretimi ile dünyada Çin, Hindistan ve A.B.D.'den sonra dördüncü sırada yer almaktadır (FAO, 2013). Örtü altında gerçekleştirilen diğer sebze üretimlerinde olduğu gibi domates üretiminde karşılaşılan sorunların başında zararlı ve hastalıklar ilk sırayı almaktadır. Bunlardan beyazsinek, *Bemisia tabaci* (Gennadius) (Hemiptera: Aleyrodidae) (i) ergin öncesi dönemlerinin bitkilerde doğrudan beslenmesi, (ii) salgıladıkları madde ile saprofit mantarların gelişmesi sonucu fumajin oluşması ve (iii) virüsleri taşıyarak ekonomik kayıplara neden olmasından dolayı önemli bir zararlı olarak karşımıza çıkmaktadır (Stansly & Naranjo, 2010). Türkiye'de bu zararlıya karşı, üreticiler tarafından öncelikli olarak kimyasal mücadele tercih edilmekte olup, bir üretim sezonunda kokteyl ilaçlardan oluşan en az 10 uygulama yapılmaktadır (Karut et al., 2012). Başarılı bir biyolojik mücadele uygulaması kimyasal mücadele uygulamalarından kaynaklanan, kalıntı ve direnç gibi olumsuzlukların önüne geçmektedir (van Lenteren, 2006; Kazak et al., 2015). Günümüzde seralarda yetiştirilen ürünlerde zararlılara karşı biyolojik mücadele tüm dünyada başarıyla uygulanmaktadır. Bu nedenle Türkiye'de de seralarda domates üretiminde *B*. *tabaci* ile mücadelede etkili bir biyolojik mücadele programının geliştirilmesi zorunludur.

Günümüzde özellikle seralarda *B*. *tabaci*'nin biyolojik mücadelesinde kullanılmak üzere birçok doğal düşman çeşitli firmalar tarafından kitle halinde üretilip satılmaktadır (Gerling et al., 2001; Alomar et al., 2006; Arno et al., 2010). Ancak ticari doğal düşman türleri ile karşılaştırıldığında yerli türlerin ortama ve zararlıya olan daha yüksek adaptasyonlarından dolayı başarılı olma şansları artmaktadır. Parazitoid *Eretmocerus mundus* Mercet (Hymenoptera: Aphelinidae) ve avcı *Macrolophus melanotoma* (Costa) (Hemiptera: Miridae) Türkiye'nin de içinde bulunduğu Akdeniz ülkelerine uyum sağlamış yerli doğal düşman türleridir (Alomar et al., 1994; Gerling et al., 1998; Ulusoy, 1999; Castañé et al., 2004; Urbaneja & Stansly, 2004; Perdikis et al., 2007; Karut et al., 2012). *E*. *mundus* Doğu Akdeniz'de seralarda saptanan en yaygın parazitoid türüdür (Karut, 2006; Karut et al., 2012). *M*. *melanotoma* ise yıl boyunca *Dittrichia viscosa* L. (W. Greuter) (Asteraceae) bitkisi üzerinde bulunmakta ve popülasyonu yaz aylarında artmaktadır (Lykouressis et al., 2012; Evangelou et al., 2013).

Beyazsineğin Türkiye'de 1928 yılından itibaren farklı kültür bitkilerinde sorun olduğu bildirilmektedir (Arık et al., 1976). Ancak zararlının seralarda biyolojik mücadelesine yönelik yapılmış çalışma sayısı oldukça sınırlıdır. Sınırlı sayıdaki bu çalışmalarda, *B*. *tabaci* ve doğal düşmanlarının sörveyleri ile durumları belirlenmiş, ayrıntılı salım ve etkinlik çalışmaları yapılmamıştır (Öncüer et al., 1994; Yaşarakıncı & Hıncal, 1996; Yoldaş et al., 1999; Bulut & Göçmen 2000; Yaşarakıncı, 2001; Karut, 2006; Karut et al., 2012). Türkiye'de yayınlanan çalışmalarla karşılaştırıldığında, yurtdışında zararlı ve doğal düşmanlarının seralardaki mevcut durumlarını bildiren çalışmaların yanında *B*. *tabaci*'nin biyolojik mücadelesine yönelik *M*. *caliginosus* (Wagner), *M*. *pygmaeus* (Rambur) (Hemiptera: Miridae) ve *E*. *mundus* gibi türlerin kullanıldığı salım çalışmaları daha fazla yürütülmüştür (Stansly et al., 2005; Alomar et al., 2006; Gabarra et al., 2006; López & Andorno, 2009). Ancak beyazsineğin diğer bir potansiyel doğal düşmanı *M*. *melanotoma* ile yapılan çalışmalar laboratuvar denemeleri ile sınırlı kalmıştır (Lykouressis et al., 2012). Ayrıca, yapılan çalışmalar incelendiğinde, domates bitkisinde *B. tabaci*'nin biyolojik mücadelesinde *M*. *melanotoma*'nın parazitoit *E*. *mundus* ile birlikte zararlıya karşı kullanım olanaklarının araştırıldığı bir çalışmaya rastlanmamıştır.

Bu bağlamda, bu çalışmada temel amaç, seralarda domates yetiştiriciliğinde *E*. *mundus* ve *M*. *melanotoma*'nın yerli popülasyonlarının, *B*. *tabaci*'nin mücadelesinde kullanım olanaklarının araştırılmasıdır. Böylece, seralarda bir üretim sezonunda 10-12 adede ulaşan ilaçlama sayısı azaltılacak, daha sağlıklı ve kalıcı olan biyolojik mücadele yönteminin seralara yerleştirilmesi için gerekli bilgi birikimine katkı sağlanacaktır.

Materyal ve Yöntem

Bemisia tabaci, *Macrolophus melanotoma* ve *Eretmocerus mundus* üretimi

Bemisia tabaci başlangıç popülasyonu, Çukurova Üniversitesi, Ziraat Fakültesi, Bitki Koruma Bölümü araştırma parselinden pamuk bitkileri üzerinden toplanmıştır. Zararlı *B*. *tabaci* üretimi, içerisinde domates, patlıcan ve pamuk bitkileri bulunan iklim odalarında gerçekleştirilmiştir. Avcı *M*. *melanotoma*, Mersin İli'nden *Dittrichia viscosa* (L.) Greuter (Asteraceae) bitkileri üzerinden toplanmıştır. Toplanan bireylerden bir kısmı ileride yapılacak moleküler tanı çalışmalarında kullanılmak üzere % 96'lık alkolde saklanmıştır. Söz konusu avcı, beyazsinek ile bulaşık bitkiler üzerinde av üretiminden farklı bir iklim odasında kültüre alınmıştır. Daha önce yapılmış çalışmalarda *M*. *caliginosus* (Wagner) olarak bilinen türün aslında *M*. *pygmaeus* (Rambur) ve *M*. *melanotoma* (Costa)'dan oluşan tür kompleksi olduğu ve bunların türe özel primer kullanılarak moleküler yöntemler ile ayrılabildiği Evangelou et al. (2013) ile Castane et al. (2013) tarafından bildirilmiştir. Ayrıca aynı araştırıcılar *M*. *pygmaeus*'un Solanaceae familyasına ait kültür bitkilerinde (domates, patlıcan vb), *M*. *melanotoma*'nın ise kültürü yapılmayan ve Akdeniz ülkelerinde yaygın olarak bulunan *D*. *viscosa* bitkisini tercih ettiğini bildirmişlerdir.

Denemelerde kullanılan ve *D*. *viscosa* bitkisinden toplanan avcı böceğin teşhisi, Castane et al. (2013)'ın bildirdiği türe özel, 154 bç ağırlığında bant veren, mitokondrial gen bölgesine ait primer çifti (Mp1F/Mp4R ve Mm1F/Mm3R) kullanılarak Çukurova Üniversitesi, Bitki Koruma Bölümü, Böcek Biyoteknolojisi Labortuvarı'nda klasik PCR yöntemi ile yapılmıştır.

Eretmocerus mundus'un başlangıç popülasyonu, Bitki Koruma Bölümü Araştırma ve Uygulama Parseli'nden beyazsinek ile bulaşık pamuk (*Gossypium hirsitum* L., Malvaceae) yapraklarından elde edilmiştir. Bunun için pamuk yaprakları laboratuvar koşullarında parazitoid elde etme kaplarına alınmış, buradan toplanan erginler ile üretim başlatılmıştır. *E*. *mundus*'un tanısı Sharaf (1982)'e göre anten, abdomenin ilk iki segmentindeki renklenme ve ovipozitör ucunun özelliklerine bakılarak yapılmıştır. *E*. *mundus* üretimi, içerisinde beyazsinek ile bulaşık bitkilerin bulunduğu iklim odasında gerçekleştirilmiştir.

Zararlı ve doğal düşman üretimlerinin tamamı 25±1 °C sıcaklık, % 70±10 orantılı nem ve 16:8 aydınlık-karanlık özelliklere sahip iklim odalarında yapılmıştır.

Denemelerin kurulması

Denemeler, 2009 ve 2010 yılları bahar sezonunda, Çukurova Üniversitesi, Ziraat Fakültesi, Bitki Koruma Bölümü Araştırma ve Uygulama Parseli'nde bulunan cam seralar içerisine kurulmuş olan 3X3X2.5 m ölçülerinde tül kafeslerde yürütülmüştür. Çalışmalarda fide üreticilerinden sağlanan, GÖKÇE çeşidi domates (*Lycopersicon esculentum* Mill., Solanaceae) fideleri kullanılmıştır. Fideler, her kafeste 30 adet bitki olacak şekilde aktarılmıştır. Denemeler tesadüf parselleri deneme desenine göre "*E*. *mundus* (1), *M*. *melanotoma* (2), *M*. *melanotoma*+*E*. *mundus* (3) ve kontrol (4) olmak üzere 4 farklı uygulama ve 3 tekrarlı olarak kurulmuştur. Birinci uygulamada içerisinde *B*. *tabaci* ile bulaşık bitkilerin bulunduğu kafeslere sadece parazitoid *E*. *mundus*, ikinci uygulamada sadece avcı *M*. *melanotoma*, üçüncü uygulamada *E*. *mundus* ve *M*. *melanotoma* birlikte salınmıştır. Dördüncü uygulamada ise hiç doğal düşman salımı yapılmayıp kontrol olarak bırakılmıştır. Her bir kafes bir tekrar olarak kabul edilmiştir. Fideler aktarıldıktan bir hafta sonra, *B*. *tabaci* üretim kolonisinden ağız aspirtatörü yardımıyla Epphendorf® tüplerine aktarılan ergin *B*. *tabaci* bireyleri bitki başına 20 adet olacak şekilde kafeslere salınmıştır. Daha sonra beyazsineğin domates bitkileri üzerinde popülasyon oluşturması ve parazitlenme için uygun dönem olan 2. nimf dönemine gelmesi için beklenmiştir (Karut, 2007). Yaklaşık 10 gün sonra, parazitoid üretim kafeslerinden üzeri bol parazitoid pupalarıyla dolu yapraklar ergin bireylerin elde

edilmesi için parazitoid çıkarma kaplarına alınmıştır. Burada pupalardan çıkan erginler ağız aspiratörü yardımıyla Epphendorf® tüplerine alınmıştır. *E. mundus* ergin dişileri 6 adet/bitki olacak şekilde salınmıştır (López & Andorno, 2009). *M. melanotoma* salımında da benzer yöntem izlenmiş, üretim kolonisinden ağız aspiratörü yardımıyla Falcon tüplerine aktarılan *M. Melanotoma*'nın çiftleşmiş ergin dişileri her iki bitkiye 1 adet olacak şekilde salınmıştır (Gabarra et al., 2006).

Örnekleme

Zararlı ve doğal düşman örneklemelerine, *E. mundus* ve *M. melanotoma*'nın yerleşip popülasyon oluşturmasını sağlamak amacıyla salımdan 2 hafta sonra başlanmıştır. *B. tabaci* ergin öncesi dönemlerinin popülasyon gelişimi ile parazitlenme oranlarının belirlenmesinde, her kafesten rastgele seçilen 15 bitkinin orta bölümünden birer yaprak (Her uygulama için toplam 45 yaprak) alınarak laboratuvara getirilmiş ve sayımlar stereobinoküler mikroskop yardımıyla yapılmıştır. Sayımlarda 2x2 cm (4 cm^2) boyutlarında, üzerinde 2 adet sayım olanağı veren şablonlar kullanılmıştır. Hazırlanan bu şablon yaprak alt yüzeyine tesadüfi olarak yerleştirilmiş ve her yaprakta toplam 8 cm^2 alanda *B. tabaci*'nin ergin öncesi dönemleri ile parazitli bireyler ayrı ayrı sayılmıştır. Parazitlenme oranı, 8 cm^2 alanda bulunan parazitli birey sayısının toplam birey sayısına bölümü ile hesaplanmıştır. *B. tabaci* erginleri sezon sonuna kadar kafes içerisinde gözle kontrol yöntemi kullanılarak sayılmış, her kafeste rastgele seçilen 15 bitkinin üst bölümünden 2 yaprak olmak üzere toplam 30 yaprak hafifçe çevrilerek yaprak alt yüzeyinde bulunan erginler kaydedilmiştir.

Macrolophus melanotoma'nın nimf ve ergin dönemleri de gözle kontrol yöntemi kullanılarak tüm sezon boyunca sayılmıştır. Bunun için her kafeste rastgele seçilen 15 bitkinin tamamı gözle kontrol edilerek avcının nimf ve erginleri sayılarak kaydedilmiştir.

İstatistiksel analizler

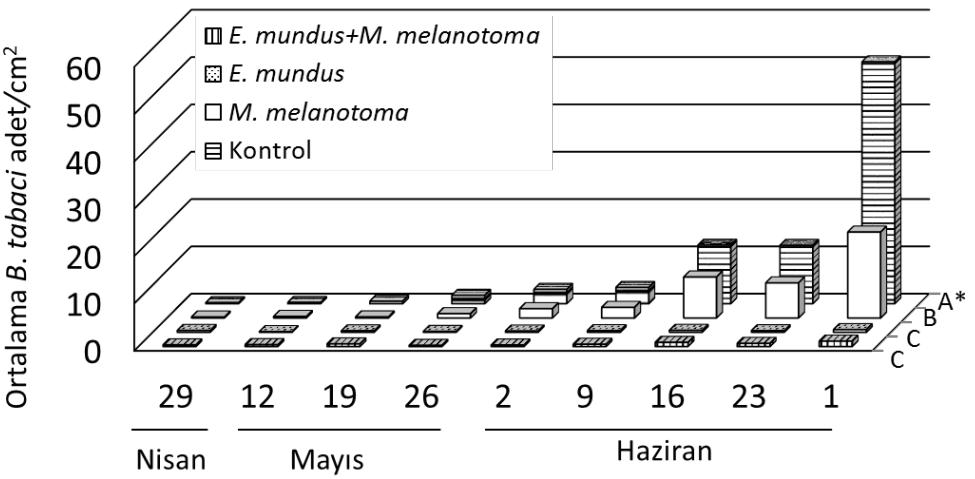
Elde edilen veriler SPSS 17.0 (Chicago IL, USA) paket programında Tekrarlı ANOVA Varyans Analizi (Repeated Measures ANOVA) kullanılarak analiz edilmiştir. Dört farklı uygulama "*between subjects-factor*" ve uygulamaların örnekleme tarihleri "*within subjects-factor*" arasındaki fark her iki yıl için ayrı ayrı Bonferroni çoklu karşılaştırma testi ile 0.05 önem seviyesinde belirlenmiştir. Analizden önce tüm verilere logaritmik transformasyon [Log10 (n+1)] uygulanmış olup, sonuçlar orijinal veriler kullanılarak sunulmuştur.

Araştırma Sonuçları

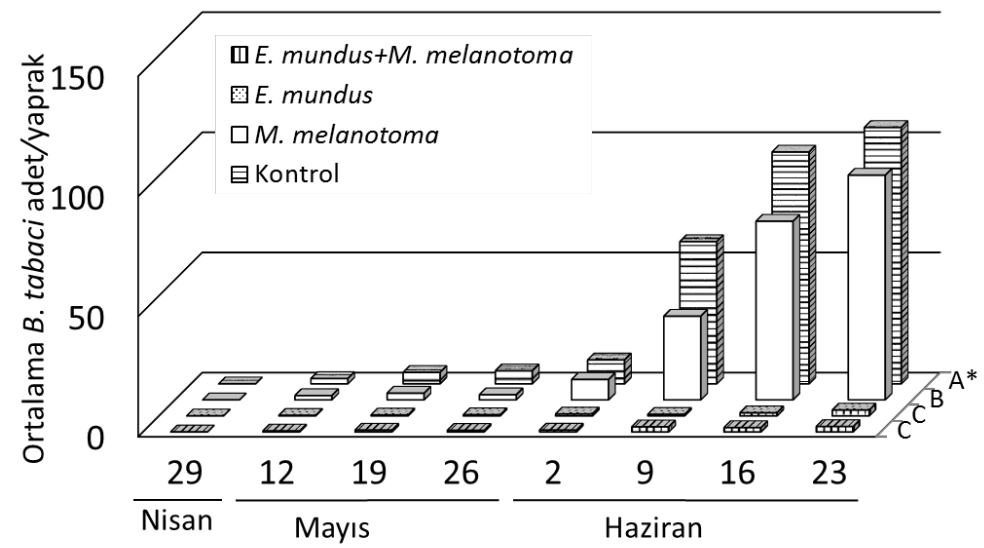
***Bemisia tabaci* popülasyon gelişimi 2009**

Ortalama *B. tabaci* ergin öncesi ve ergin yoğunlukları, dört uygulama arasında, tekrarlı ANOVA varyans analizi sonuçlarına göre önemli derecede farklı bulunmuştur (Ergin öncesi uygulama: $F_{(3,\ 177)}$ = 152.918; P < 0.001; Ergin uygulama: $F_{(3,\ 176)}$ = 892. 633; P < 0.001). Aynı uygulama içinde farklı örnekleme tarihlerinde de istatiksel fark saptanmıştır (Ergin öncesi, örnekleme tarihi: $F_{(4.7,\ 1416)}$ = 206.201; P < 0.001; Ergin örnekleme tarihi: $F_{(5.1,\ 1056)}$ = 531.938; P < 0.001). Benzer olarak örnekleme tarihleri ve uygulama X örnekleme tarihi etkileşimi de önemli derecede farklı bulunmuştur (Ergin öncesi, uygulama X örnekleme tarihi: $F_{(14.2,\ 1416)}$= 30.511; P < 0.001; Ergin, uygulama X örnekleme tarihi: $F_{(15.4,\ 1056)}$ = 101.416; P < 0.001).

Dört farklı uygulamada haftalık en düşük ergin öncesi *B. tabaci* yoğunluğu, *E. mundus*'un tek başına salındığı uygulamadan elde edilmiş olup, deneme süresince ortalama 0.56 (adet/cm^2)'yı (1 Haziran) geçmemiştir (Şekil 1). Haftalık en düşük *B. tabaci* ergin birey yoğunluğu ise parazitoit ve avcının bir arada salındığı uygulamada elde edilmiş, ortalama 2.24 (adet/yaprak)'ü (23 Haziran) geçmemiştir (Şekil 2). Beyazsineğin ergin ve ergin öncesi dönemleri için, parazitoit salınan uygulamalar istatiksel olarak birbirinden farksız, kontrol ve sadece *M. melanotoma* salınan uygulamalardan farklı bulunmuştur (P<0.001) (Şekil 1 ve 2).



Şekil 1. Domates bitkisinde 2009 yılında dört farklı uygulamada elde edilen ortalama ergin öncesi *Bemisia tabaci* yoğunlukları. *Aynı harfi taşıyan uygulamalar arasında Bonferroni testine göre fark yoktur (P<0.001).

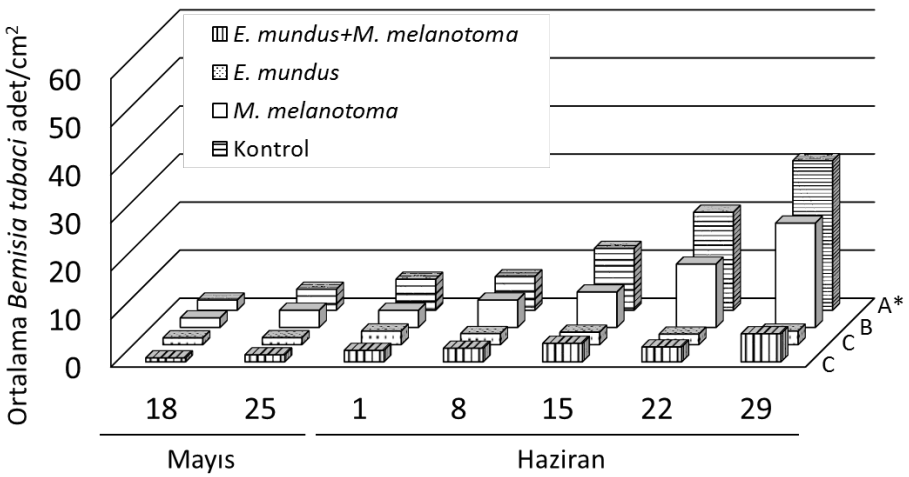


Şekil 2. Domates bitkisinde 2009 yılında dört farklı uygulamada elde edilen ortalama ergin *Bemisia tabaci* yoğunlukları. *Aynı harfi taşıyan uygulamalar arasında Bonferroni testine göre fark yoktur (P<0.001).

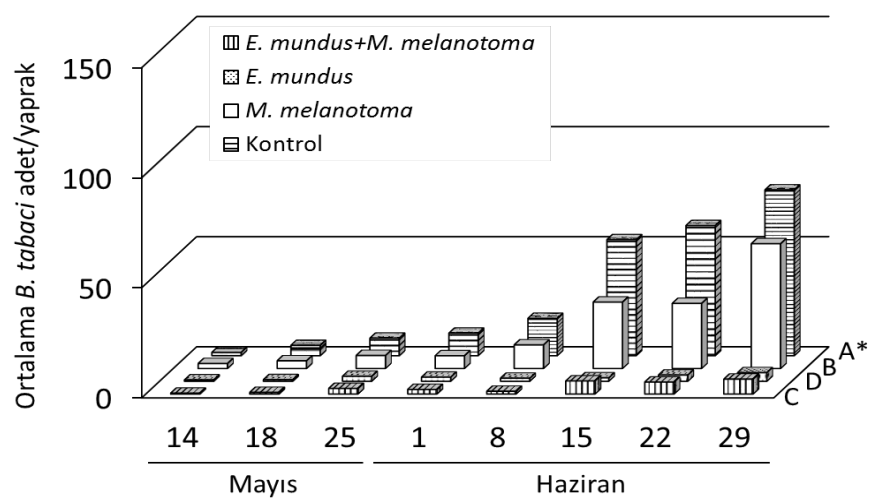
Bemisia tabaci popülasyon gelişimi 2010

Tekrarlı ANOVA varyans analizi sonuçlarına göre, ortalama *B. tabaci* ergin öncesi ve ergin yoğunlukları, 2009 yılına benzer şekilde, dört uygulamada önemli derecede farklı bulunmuştur (Ergin öncesi uygulama: $F_{(3,116)}$=60.859; P < 0.001; Ergin uygulama: $F_{(3,168)}$ = 496.470; P < 0.001). Aynı uygulama içinde farklı örnekleme tarihleri arasında da istatiksel olarak fark bulunmuştur (Ergin öncesi, örnekleme tarihi: $F_{(4,1\ 696=94.439)}$; P < 0.001; Ergin örnekleme tarihi: $F_{(6.4,\ 1176)}$=293.283; P < 0.001). Ayrıca uygulama X örnekleme tarihi etkileşimi de önemli derecede farklı bulunmuştur (Ergin öncesi, uygulama X örnekleme tarihi: $F_{(12.4,\ 696)}$=5.709; P < 0.001; Ergin, uygulama X örnekleme tarihi: $F_{(19.3,\ 1176)}$ = 28.700; P < 0.001). Haftalık en düşük ergin öncesi *B. tabaci* yoğunluğu parazitoitin tek başına salındığı uygulamadan

elde edilmiş ve 29 Haziran'da ortalama 2.82 (adet/cm^2) ile en yüksek değerine ulaşmıştır (Şekil 3). Haftalık en düşük *B*. *tabaci* ergin birey yoğunluğu, ergin öncesi dönemlere benzer şekilde, *E*. *mundus*'un tek başına salındığı uygulamadan elde edilmiş ve ortalama 3.90 (adet/yaprak)'ı (29 Haziran) geçmemiştir (Şekil 4). Beyazsinek ergin öncesi popülasyon yoğunlukları parazitoit salınan uygulamalarda (tek *E*. *mundus* ve *E*. *mundus*+*M*. *melanotoma*) istatiksel olarak farksız bulunurken, diğer uygulamalardan (Kontrol ve tek *M*. *melanotoma*) farklı bulunmuştur. Ergin yoğunluğu ise bütün uygulamalarda birbirinden farklı bulunmuştur (P<0.001) (Şekil 4).



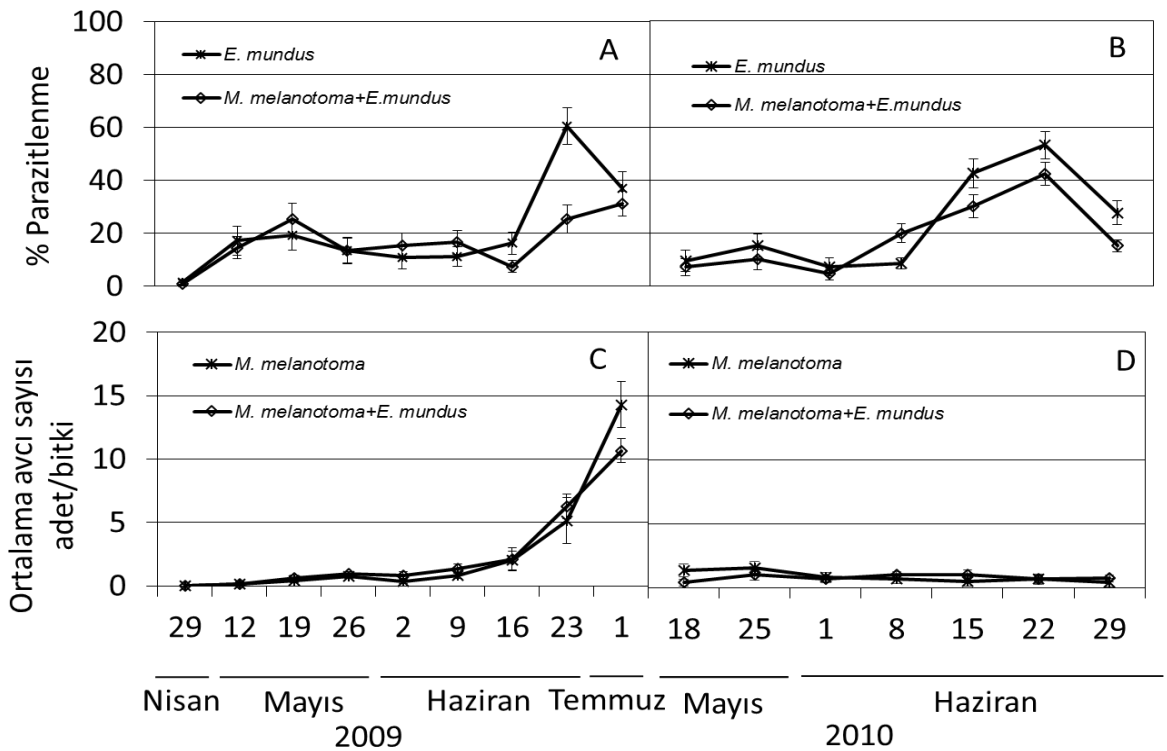
Şekil 3. Domates bitkisinde 2010 yılında dört farklı uygulamada elde edilen ortalama ergin öncesi *Bemisia tabaci* yoğunlukları.
*Aynı harfi taşıyan uygulamalar arasında Bonferroni testine göre fark yoktur (P<0.001).



Şekil 4. Domates bitkisinde 2010 yılında dört farklı uygulamada elde edilen ortalama ergin *Bemisia tabaci* yoğunlukları.
*Aynı harfi taşıyan uygulamalar arasında Bonferroni testine göre fark yoktur (P<0.001)

Parazitoit, *Eretmocerus mundus* aktivitesi

Genel olarak, her iki yılda da % parazitlenme oranı tek başına *E*. *mundus* salımı yapılan uygulamada, parazitoitin avcı ile birlikte salındığı uygulamadan daha yüksek bulunmuştur. *E*. *mundus*'un *M*. *melanotoma* ile bir arada salındığı ve tek başına salındığı uygulamalarda en yüksek parazitlenme oranı, 2009 yılında sırası ile % 31.23 ve % 60.54, 2010 yıllında ise % 42.43 ve % 53.19 olarak saptanmıştır (Şekil 5a-b).



Şekil 5. Domates bitkisinde 2009 ve 2010 yıllarında *Eretmocerus mundus* ve *Macrolophus melanotoma*'nın ayrı ayrı ve bir arada salındığı uygulamalardan elde edilen parazitlenme oranları (%) (A,B) ile ortalama avcı yoğunlukları (adet/bitki) (C, D).

Avcı, *Macrolophus melanotoma* aktivitesi

Bitki başına ortalama avcı yoğunluğu 2009 yılında 2010 yılından daha yüksek bulunmuş ve sezon sonunda (1 Temmuz) tek avcı salımı yapılan kafeslerde bitki başına ortalama 14.3 adet'e kadar çıkmıştır (Şekil 5c). Ancak 2010 yılında bitki başına ortalama avcı yoğunluğu oldukça düşük olmuş ve 0.61 adedin (25 Mayıs) üzerine çıkamamıştır (Şekil 5d). Bununla birlikte, *M*. *melanotoma* yoğunluğu düşük olmasına rağmen kontrol ile karşılaştırıldığında ergin ve ergin öncesi *B*. *tabaci* yoğunluğunda azalmaya neden olmuştur (Şekil 1, 2, 3, 4).

Tartışma

Parazitoit, *E*. *mundus* salımı yapılan kafeslerde *B*. *tabaci* popülasyonu tek başına avcı salımı yapılan ve kontrol kafeslerinden oldukça düşük bulunmuş ve *E*. *mundus*'un yerli popülasyonu zararlıyı baskı altına almada başarılı olmuştur. Stansly et al. (2005) domates ve biber bitkisinde yapmış oldukları çalışmada, *E*. *mundus*'un biber bitkisinde domates bitkisinden daha başarılı olduğunu bildirmişlerdir. Domates bitkisinde

istenilen başarının elde edilebilmesi için biberde uygulanan salım oranından daha yüksek bir salım oranı gerektiğini belirtmişlerdir. Bu çalışmada araştırıcıların domates bitkisinde uyguladıkları salım oranından (~50 adet/bitki) çok daha düşük bir salım oranında (6 adet/bitki) dahi *E*. *mundus*'un başarılı olduğu saptanmıştır. Benzer durumu Gabarra et al. (2006) da belirlemiş ve Stansly et al. (2005)'ın uyguladığı salım oranından daha düşük bir salım oranı ile başarılı sonuçlar elde etmişlerdir. Her ne kadar kültür bitkisi farklı olsa da bu çalışmada kullanılan salım oranına benzer bir oranda, Lopez & Andorno (2009) Arjantin'de biber seralarında bahar üretim sezonunda *E*. *mundus*'un % 54'lük parazitlenme oranıyla *B*. *tabaci* popülasyonunu baskı altına alabildiğini saptamışlardır.

Tek başına *M*. *melanotoma* uygulaması, kontrol ile karşılaştırıldığında *B*. *tabaci* popülasyonunda bir azalmaya neden olsa da zararlıyı baskı altına almada başarılı olamamıştır. Elde edilen bu sonuç Gabarra et al. (2006)'ın elde ettiği sonuçla benzerlik göstermiştir. Araştırıcılar bir diğer *Macrolophus* türü olan *M*. *caliginosus*'un tek başına başarılı olamamasının nedeninin uygulanan salım oranından kaynaklanmış olabileceğini bildirmişlerdir. Salım oranına ek olarak, konukçu bitki faktörünün de avcının başarısını etkileyebileceği düşünülmektedir. Lykouressis et al. (2012) üzerinde av bulunmayan Solanaceae bitkileriyle yapmış oldukları çalışmada, *M*. *melanotoma*'nın patlıcan bitkisini domates ve biber bitkilerinden daha çok tercih ettiğini saptamışlardır. Nitekim, Karut et al. (2015) patlıcan bitkisinde yapmış oldukları çalışmada *M*. *melanotoma*'nın *E*. *mundus*'un başarısına katkıda bulunduğunu belirlemişlerdir. Bu çalışmada elde edilen sonuçlar, *M*. *melanotoma*'nın domatese ek olarak farklı kültür bitkilerinde ve farklı salım oranlarında başarısının belirleneceği çalışmaların yapılmasının yararlı olacağını göstermiştir.

Parazitoit salımı yapılan kafesler kendi içerisinde karşılaştırıldığında, avcı ve parazitoidin birlikte salındığı kafesler ile parazitoidin tek başına salındığı kafeslerde ergin öncesi *B*. *tabaci* yoğunlukları her iki yılda da istatiksel olarak aynı grupta yer almasına rağmen, yoğunluk tek başına salımda görece olarak daha düşük bulunmuştur. Bu durumun doğal düşmanlar arasındaki birlik içi avcılık (intraguild predation, IGP)'tan kaynaklanmış olabileceği ve *M*. *melanotoma*'nın *E*. *mundus*'un başarısını etkilediği düşünülmektedir. Castane et al. (2000) İspanya'nın Katalan Bölgesi'nde yer alan seralarda yapmış oldukları çalışmalarda *M*. *caliginosus*'un *Encarsia formosa* Gahan (Hymenoptera: Aphelinidae) tarafından parazitlenmiş siyah pupalar ve parazitli olmayan pupalar ile beslendiklerini ancak bu durumun seralarda beyazsinek mücadelesini olumsuz etkilemediğini bildirmişlerdir. Gabarra et al. (2006) İspanya'da özellikle bahar sezonunda *E*. *mundus* ve *M*. *caliginosus*'un birlikte salındığı durumlarda *B*. *tabaci'yi* daha başarılı bir şekilde baskı altına alabildiğini bildirmişlerdir. Malo et al. (2012) ise bir başka *Macrolophus* türü olan, *M*. *pygmeous*'un, *E*. *mundus* tarafından parazitlenmiş ve parazitenmemiş *B*. *tabaci* nimfleri ile beslendiğini ancak avcının parazitlenmemiş nimfleri daha fazla tercih ettiğini ve zararlı ile mücadelede doğal düşmanların birlikte salınmasının başarıyı artırdığını saptamışlardır. Bu çalışmada her ne kadar parazitoit tek başına daha başarılı görünse de, ergin salımı yapılan *E*. *mundus*'un seraya yerleşip popülasyon oluşturana kadar geçen sürede (en az 15 gün) avcının zararlı üzerinde bir baskı oluşturacağından *B*. *tabaci* mücadelesinde her iki doğal düşmanın birlikte salınmasının daha uygun olacağı düşünülmektedir.

Bu çalışmada elde edilen sonuçlar, Türkiye'de domates seralarında *E*. *mundus* ve *M*. *melanotoma*'nın yerli popülasyonlarının birlikte kullanıldığı biyolojik mücadele yönteminin, *B*. *tabaci* popülasyonunu hiç insektisit uygulamaya gerek kalmadan başarıyla baskı altına alabildiğini göstermiştir.

Teşekkür

Bu çalışma 108O087 numaralı proje kapsamında TÜBİTAK tarafından desteklenmiştir.

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Orijinal araştırma (Original article)

Panonychus ulmi (Koch) ve *Neoseiulus californicus* (Mc Gregor)'un üreme gücü ve yaşam sürelerine bazı pestisitlerin etkisi: hormoligosis[1,2]

The effects of some pesticides on fecundity and lifespan of *Panonychus ulmi* (Koch) and *Neoseiulus californicus* (Mc Gregor): hormoligosis

Elif SARITAŞ[3] **Recep AY[3*]**

Summary

Farmers generally prefer to use chemical control methods against many of diseases, pests and weeds that cause economic losses in agricultural production. There are disadvantages of pesticides used in the control of pest, they disrupt the natural balance, causing directly or indirectly poisoning of people and animals, they have harmful effects on natural enemies which result in increase in pest populations and hormoligosis. Sublethal doses of some pesticides result in increase of the fecundity in insects and mites. This is called hormoligosis. In this study, the effect of some pesticides commonly used in apple orchards was investigated on the reproductive rate and lifespan of European red mite (*Panonychus ulmi* Koch.) (Acari: Tetranychidae) that is one of the main pests and predator mites *Neoseiulus californicus* (Mc Gregor) (Acari: Phytoseiidae). Pesticides were directly applied by sprey tower on 0-24 hours larvae of both mite that is on the leafs (plum or beans) on wet cotton in the Petri dish with spray tower. The average lifespan (larvae+nymphs+adults) of *P. ulmi* larvae exposed to distilled water (control), cypermethrin, imidacloprid, deltamethrin, spirodiclofen + abamectin and thiacloprid was found to be 25.50, 24.92, 23.97, 23.77, 21.60, 23.85 days, respectively. The average lifespan of *N. californicus* larvae exposed to control and same insecticides was detected to be 27.72, 26.94, 25.40, 27.94, 25.71, 26.88 days, respectively. The average number eggs of *P. ulmi* larvae exposed to same experimental conditions was 33.79, 35.15, 37.62, 36.17, 33.95, 36.75 eggs/female. The average oviposition for *N. californicus* larvae exposed to control and same insecticides was determined to be 36.03, 46.44, 47.46, 46.43, 35.43, 42.38 eggs/female. Except for abamectin + spirodiclofen, other pesticides caused a limited increase of eggs in individually female *P. ulmi* while they caused a greater increase of egg predatory mite, *N. californicus*.

Keywords: *Panonychus ulmi*, *Neoseiulus californicus,* hormoligosis, neonicotinoid, piretroit.

Özet

Tarımsal üretimde ekonomik kayba neden olan birçok hastalık, zararlı ve yabancı otlara karşı üreticiler kimyasal savaş yöntemini tercih etmektedirler. Kimyasal savaşımda kullanılan pestisitlerin dezavantajları arasında, doğal dengeyi bozmak, insan ve sıcakkanlılarda doğrudan veya dolaylı olarak zehirlenmelere neden olmak, doğal düşmanlara zarar vererek zararlı popülasyonlarının artmasına ve hormoligosis'e neden olması gösterilebilir. Öldürücü dozda olmayan bazı pestisitler böcek ve akarlarda yumurta verimini artırmaktadırlar. Bu duruma hormoligosis denilmektedir. Bu çalışma da yaygın kullanılan bazı pestisitlerin elma bahçelerinin ana zararlılarından biri olan Avrupa Kırmızıörümceği (*Panonychus ulmi* Koch.) (Acari: Tetranychidae) ve avcı akar *Neoseiulus californicus*'un (Mc Gregor) (Acari: Phytoseiidae) üreme gücü ve yaşam sürelerine etkileri araştırılmıştır. Pestisitler her iki akarın 0-24 saatlik larva dönemlerine ilaçlama kulesi - Petri kabı yöntemi (ıslak pamuk üzerinde bulunan erik veya fasülye yaprakları) ile doğrudan uygulanmıştır. Saf su (kontrol), cyphermethrin, imidacloprid, deltamethrin, spirodiclofen + abamectin ve thiacloprid uygulanan *P. ulmi* bireylerinin ortalama yaşam süresi (larva+nimf+ergin) sırasıyla 25.50, 24.92, 23.97, 23.77, 21.60, 23.85 gün olarak bulunmuştur. Saf su (kontrol) ve aynı insektisitlerin uygulandığı *N.californicus* bireylerinin ortalama yaşam süresi ise sırasıyla 27.72, 26.94, 25.40, 27.94, 25.71, 26.88 gün olarak saptanmıştır. Aynı deneysel koşullara maruz bırakılan *P. ulmi* dişi bireylerinin bırakmış oldukları ortalama yumurta sayısı 33.79, 35.15, 37.62, 36.17, 33.95, 36.75 yumurta/dişi olarak bulunmuştur. Saf su (kontrol) ve aynı insektisitler uygulanan *N.californicus* dişi bireylerinin bırakmış oldukları ortalama yumurta sayısı 36.03, 46.44, 47.46, 46.43, 35.43, 42.38 yumurta/dişi olarak bulunmuştur. Spirodiclofen+abamectin dışındaki ilaçlar *P. ulmi*'de dişi başına sınırlı bir yumurta artışına neden olurken, avcı akar *N. californicus*'da daha fazla bir yumurta artışına neden olmuştur.

Anahtar sözcükler: *Panonychus ulmi*, *Neoseiulus californicus,* hormoligosis, neonikotinoid, piretroit.

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Giriş

Tarımsal üretim sırasında ve depolanmasında tarım ürünlerinde ekonomik kayıplara neden olan birçok hastalık, zararlı ve yabancı ot vardır. Bunların kontrolünde hala en çok tercih edilen yöntem kimyasal savaşımdır. Kimyasal savaşımın birçok olumsuz yönünün olduğu artık bilinen bir gerçektir. Yapılan birçok çalışmada kullanılan pestisitlerin doğal düşman ve hedef dışı organizmaları etkilediği, zararlılarda direnç gelişimine, ürünlerde kalıntı ve çevre kirliğine neden olduğu bildirilmektedir (Ay et al., 2003; Karaca et al., 2005; Irigaray et al., 2007; Ay & Kara, 2011; Kaplan et al., 2012; İşçi & Ay, 2013) Bu olumsuz yönlerin azaltılması veya en aza indirilmesi için sorunların doğru olarak ortaya konması gerekir. Isparta İli'nde elma üreticileri zararlılarla savaşımda çoğunlukla kimyasal savaşımı tercih etmektedirler. Isparta İli uygun ekolojik koşulları nedeniyle elma yetiştiriciliği bakımından Türkiye'de önemli bir potansiyele sahiptir. Türkiye'de üretilen 2.889.000 ton elmanın yaklaşık 634.975 tonu Isparta'da üretilmektedir (Anonymous, 2014). Elma bahçelerinde ekonomik kayıplara neden olan birçok hastalık ve zararlı vardır. Bu zararlılar arasında elma iç kurdundan sonra en fazla savaş yapılan zararlıların arasında ilk sırada kırmızıörümcekler bulunmaktadır. Elma üretiminde zararlı olan kırmızıörümceklerin en önemlilerinin başında *Panonychus ulmi* (Koch) (Acari: Tetranychidae) gelmektedir. Bölgede yapılan birçok çalışmada elma bahçelerinden toplanan *P. ulmi* ve *Tetranychus urticae* (Koch) (Acari: Tetranychidae) popülasyonları içerisinde *Neoseiulus californicus* (Mc Gregor) (Acari: Phytoseiidae)'a rastlanılmıştır. Ancak üreticiler bölgede akarların kontrolünde çoğunlukla kimyasal savaşımı tercih etmektedirler.

İkinci dünya Savaşından önce dünyada kırmızıörümcekler genellikle tarım ürünlerinin minör zararlıları olarak bilinmekteydi. İkinci dünya Savaşı'ndan sonra DDT ve organik fosfatlı'lar gibi sentetik organik pestisitlerin aşırı kullanımı ile bu durum değişmiştir (Stern et al., 1959, Huffaker et al., 1970).1950-1960'lı yıllar arasında kırmızıörümcekler özelikle seralarda önemli zararlılara dönüşmüşlerdir (van de Vrie et al., 1972). Ayrıca pestisitlerin yoğun kullanımı seralarda bulunan bitkilerde zararlı kırmızıörümceklerin direnç geliştirmesine neden olmuştur (Hoy, 2011). Kırmızıörümceklerin ani popülasyon artışını açıklamak için 3 hipotez geliştirilmiştir (Huffaker et al., 1969, 1970). Bunlar;

1. Pestisitlerin kırmızıörümcek popülasyonlarında üremeyi uyarması

2. Pestisitlerin doğal düşmanları elimine etmesi

3. Pestisitlerin kullanımı sonucu avcıların avlardan kaçışı olarak özetlenmektedir.

Sürece bağlı olarak bu üç hipotezin belirli durumlarda doğru olduğu kanıtlanmış olup, yeni sentetik organik pestisitlerin doğal düşman popülasyonlarının kırmızıörümcek popülasyonlarından kaçmalarına neden olduğu belirlenmiştir (Huffaker et al., 1970). Sentetik organik pestisitlerin aşırı kullanımı ile üretilen bitkiler kırmızıörümcekler tarafından daha çok tercih edilmiştir. Bununla birlikte bazı pestisitlerde kırmızıörümcek popülasyonlarının artmasında uyarıcı etki yapması "hormoligosis" ile açıklanmıştır (Hoy, 2011).

Paracelsus yüzyıllar önce "Her madde zehirdir, zehir olmayan madde yoktur; zehir ile ilacı ayıran dozdur' ifadesini kullanmıştır. Başka bir deyişle yüksek dozda toksik olan bir kimyasal düşük dozda toksik olmayabilir (Duke, 2014). Toksinlere ve diğer stres faktörlerine maruz kalan organizmalar biyolojik yanıtlar vermektedirler. Bu olayda hormoligosis kavramının ortaya çıkmasına neden olmuştur. Hormoligosis kelimesi Southam ve Ehrlich tarafından 1943 yılında tanımlanmıştır. Terim Yunanca'da uyandırmak ya da hormonları teşvik etmek anlamına gelen kelimeden türetilmiştir (Guedes & Cutler, 2013). İlaçlama yapmak bazen bitkilerin yaprak yüzeyini değiştirebilir ve böylece zararlıların çoğalmasını teşvik eder. Böcek ilaçlaması bitkilerin besinsel kalitesini ya da fizyolojisini etkilediğinden, aynı şekilde zararlıların çoğalmasını tetikleyebilir. Ek olarak böcek ilaçlarının uyarıcı etkisi; kirlilikler, aktif yüzey ya da gerçek aktif bileşenler değil formülasyon içinde taşıyıcılar ile de ilgili olabilir. Pestisitler böcek bünyesine alındığında enzimler yoluyla inaktive edilip stres faktörlerine bağlı olarak böcekte yumurta verimini artırabilir. Meydana gelen bu olaya hormoligosis denmektedir (Cloyd, 2014). Başka bir deyişle hormoligosis öldürücü dozda olmayan pestisit (subletal dozda) böcek vücuduna alındıktan sonra üremeyi tetiklemesidir. Dirençli bireylerde ise bu olay farklıdır. Bireyler ilaçlara dayanıklılık kazandığı için yumurta verimi ve yaşam süreleri doğal olarak artacaktır.

Zararlı türleri baskı altına almak için kullanılan insektisit kullanımlarından sonra hedef dışı organizmalarda popülasyon artışı olabilir. Pestisit kaynaklı hormoligosis çok önemlidir. Bu konu üzerinde çalışmalar az olmakla birlikte, günümüzde bu konudaki çalışmalar önem kazanmıştır. Ayrıca yapılan bazı çalışmalarda piretroitli bileşiklerin yumurta verimini artırdığı gözlemlenmiş; piretroitli insektsitler uygulanan *Panonychus citri* (Acari: Tetranychidae) ve *T. urticae* popülasyonlarının yaşam çizelgelerin de farklılıklar oluştuğu ve yumurta verimlerinde artış meydana geldiği belirlenmiştir (Guedes & Cutler, 2013). İnsektisit uygulamaları bitkinin fizyolojisinde değişimler meydana getirerek, arthropodlarda hormoligosis'e neden olabilmektedir. İmidacloprid uygulanan *Buxus sempervirens* L. bitkisi üzerinde beslenen *Eurytetranychus buxi* Garman (Acari: Tetranychidae)'da yüksek oranda yumurta verimine neden olduğu saptanmıştır. Yumurta veriminin dışında pestisitlere maruz kalan böceklerde cinsiyet oranları (dişi: erkek) değişebilmektedir (Guedes & Cutler, 2013). *Olygonychus ilicis* (McGregor) (Acari: Tetranychidae) türüne de bu gruptan bir etken madde uygulanmış fakat hormoligosis olmadığı için türde artış meydana gelmemiştir (Guedes & Cutler, 2013).

Yapılan bu çalışmada elma bahçelerinde kullanılan bazı insektisit ve akarisitlerin elmada zararlı *P. ulmi* ve avcısı *N. californicus*'un üreme gücüne, yaşam süresine ve erkek dişi oranına etkileri yani bu pestisitlerin hormoligosis'e neden olup olmadıkları çalışılmıştır.

Materyal ve Yöntem

Denemenin ana materyalini Bayer CropScience (Almanya)'dan 2012 yılında sağlanan hassas *Panonychus ulmi* popülasyonu ve Eğirdir Meyvecilik Araştırma İstasyonu organik elma bahçesinden toplanan ve 2008 yılından bu güne kadar SDÜ. Ziraat Fakültesinde Bitki Koruma Bölümü'nde ilaçsız bir ortamda yetiştirilen avcı akar *Neoseiulus californicus*; avcı akarlara av olarak kullanılan *T. urticae*, pestisitler, konukçu bitki olarak elma ve erik fidanları, fasulye bitkisi ve denemelerde kullanılan pestisitler oluşturmuştur. Çalışmalar 2014-2015 yıllarında yapılmıştır.

Neoseiulus californicus ve *Panonychus ulmi* popülasyonlarının yetiştirilmesi

Neoseiulus californicus popülasyonları böcek yetiştirme kabinlerinde av olarak kullanılan *T. urticae* ile bulaşık fasülye bitkileri üzerinde üretilmiştir. Avcı akarlarla bulaşık olan fasülye bitkisine düzenli olarak *T. urticae* verilmiş ve avcıların beslenerek çoğalması sağlanmıştır. *Panonychus ulmi* popülasyonu elma ve erik fidanlarında yetiştirilmiştir. Her iki türde 26 ± 1°C'de ve % 60 ± 5 nispi nem, 16:8 aydınlık: karanlık koşullardaki böcek yetiştirme kabinlerinde üretilmiştir.

Biyossay çalışmaları

Çalışmada iki farklı akar türü kullanılması nedeni ile akarların üreme ve çoğalması farklı bitki yaprakları üzerinde gerçekleştirilmiştir. Avrupa kırmızı örümceği için erik (*Prunus domestica* Angeleno, Rosaceae) yaprağı, avcı akar *N. californicus* için ise fasülye yaprağı (*Phaseolus vulgaris* L., Fabaceae) kullanılmıştır. İlaç denemelerinde her ilaç için bir bahçe (elma bahçelerindeki) uygulama dozu ve kontrol grubu kullanılmıştır. İlaçların uygulama dozu saf su ile seyreltilmiş, kontrol grubuna ise saf su uygulanmıştır. Akarisit etkili ilaçların öldürücü etkisini azalmak için akarlar ilaçlı yüzeyde maksimum 30 dk tutulmuştur. Böylece akarisit etkili ilaçların subletal dozu sağlanmıştır.

Kullanılan pestisitler ve uygulama dozları

Bu çalışmada iki sentetik piretroitli, 2 neonikotinoid insektisit ile bir akarisit kullanılmıştır. Bu ilaçların isimleri Çizelge 1'de verilmiştir.

Çizelge 1. Kullanılan pestisitler ve uygulama dozları

Aktif madde	Etkili madde oranı	Kimyasal grubu	Uygulama dozu
Cypermethrin	250 g/L	Sentetik piretroit	20 µl/100 ml su
Deltamethrin	25 g/L	Sentetik piretroit	15 µl/100 ml su
Spirodiclofen+abamectin	222+18 g/L	Tetronik asit (Akarisit)	25 µl/100 ml su
Imidacloprid	350 g/L	Neonikotinoid	20 µl/100 ml su
Thiacloprid	240 g/L	Neonikotinoid	40 µl/100 ml su

***Panonychus ulmi*'nin ilaç uygulaması ve biyolojik özellikleri**

Akarların ilaç uygulaması ve uygulama sonrası etkilerinin izlenebilmesi için 9 cm çapında plastik Petri kapları kullanılmıştır. İlaç uygulamalarında bu Petri kaplarının içine pamuk yerleştirilip nemlendirilerek üzerine *P. ulmi* bireyleri için erik yaprağı alt yüzeyi üstte olacak biçimde yerleştirilmiştir. Yaprakların kenarları akarların kaçışına engel olmak için yapışkan madde (Tangle trap, Verim İnşaat, Türkiye) ile çevrilmiştir. Bu Petri kaplarındaki yaprak üzerine yaklaşık 0-24 saatlik larvalar, binoküler mikroskop altında seçilerek (60-80 adet) ince uçlu fırça ile aktarılmış ve ilaçlama kulesi ile ilaç direkt bireyler üzerine püskürtülmüştür. İlaçlama kulesi 1 bar basınçta çalıştırılarak ve her bir Petri kabına 2 ml ilaçlı sıvı püskürtülmüştür. Kontrol grubu saf su ile ilaçlanmıştır. Direkt ilaçla temas eden bireylerin bulunduğu Petriler yaklaşık 30 dk kurumaya bırakılmıştır. Daha sonra ilaçlı bireylerin biyolojik özelliklerini gözlemlemek için *P. ulmi* larvaları 3 cm çapındaki pleksiglas çerçevelerle sınırlandırılmış temiz (ilaç uygulanmamış) yapraklar üzerine bir adet akar olacak şekilde aktarılmıştır. Plexiglas çerçevelerin üzeri yine akarların kaçışını engellemek için parafilm ile kaplanmıştır.

Parafilmler üzerinden iğne yardımı ile havalandırma delikleri oluşturulmuş ve 26±1^{o} C sıcaklık, 16:8 fotoperyot ve % 60±5 oransal nem koşullarındaki iklim kabinine yerleştirilmiştir. Yapılan günlük kontrollerde her yaprak diskindeki bireyler ergin döneme geçer geçmez yanlarına iki erkek birey aktarılarak her hücrede bir dişi iki erkek birey olması sağlanmıştır. Denemeler en az 40 tekerrürlü olarak yapılmıştır. Çiftleşmeden yaklaşık 48 saat sonra erkek birey uzaklaştırılarak seçilen 3 Petri kabından dişi ergin bireyin yumurtaları günlük olarak sayılmış ve sayılan yumurtalar Petri kabından başka bir yaprak diskine aktarılmıştır. Bu Petri kaplarında yumurtaların açılma oranları ve çıkan bireylerin cinsiyet oranları belirlenmiştir.

Diğer tekerrürlerdeki dişi bireyler ve bırakılan yumurtalar günlük olarak kontrol edilmiştir. Elde edilen verilerden faydalanılarak toplam bırakılan yumurtalar, ayrıca ovipozisyon süreleri ve ortalama yaşam süreleri belirlenmiştir. Ayrıca ilaçlanan larvalardan elde edilen erkek bireylerden en az 10 tanesi ölünceye kadar gözlemlenerek ortalama yaşam süreleri belirlenmiştir.

***Neoseiulus californicus*'un ilaç uygulaması ve biyolojik özellikleri**

Akarların ilaç uygulaması ve uygulama sonrası etkilerinin izlenebilmesi için 9 cm çapında plastik Petri kapları kullanılmıştır. *N. californicus* bireyleri için bu Petri kaplarının içine pamuk yerleştirilip saf su ile nemlendirilerek üzerine fasulye yaprağı alt yüzeyi üstte olacak biçimde yerleştirilmiştir. *Neoseiulus californicus* bireylerinin kaçışlarına engel olmak için yaprak diskinin etrafı yapışkan madde (Tangle trap) ile 3 cm çevrilerek yaprak diskleri oluşturulmuştur.

Bütün çalışmalarda yaklaşık aynı yaşta bireyler kullanılmıştır. Bu amaçla öncelikle 3 cm çapında yaprak diskler oluşturulmuş ve bu Petri kaplarındaki yaprak üzerine yaklaşık 0-24 saatlik larvalar binoküler altında seçilmiş ve hazırlanan yaprak diskleri üzerine (60-80 adet) aktarılarak ilaçlama kulesi 1 bar basınçta çalıştırılarak ilaç direkt birey üzerine püskürtülmüştür. İlaçlama kulesi ile her bir Petri kabına 2 ml ilaçlı sıvı püskürtülmüştür. Kontrol grubuna ise saf su uygulanmıştır. Direkt ilaçla temas eden bireylerin bulunduğu Petri kapları yaklaşık 30 dk kurumaya bırakılmıştır. Daha sonra ilaçlı bireyler hazırlanan temiz yaprak disklerine her bir Petri kabında bir tane akar olacak şekilde ince uçlu fırça yardımıyla aktarılmış ve ayrıca avcı akar için yeterli av sağlanarak 26±1^{o}C sıcaklık, 16:8 fotoperyot ve % 60±5 oransal nem koşullarındaki iklim kabinine bırakılmıştır. Her gün kontroller yapılarak ve yine avcı akara gerektikçe *T. urticae* bireylerinin bütün dönemleri av olarak eklenmeye devam edilmiştir. Bu sırada her yaprak diskindeki bireyler ergin döneme geçer geçmez yanlarına iki erkek birey aktarılarak her hücrede bir dişi iki erkek birey olması sağlanmıştır. Denemeler en az 32 tekerrürlü olarak yapılmıştır. Çiftleşmeden yaklaşık 48 saat sonra erkek birey uzaklaştırılarak seçilen 3 Petri kabından ergin dişi bireyin yumurtaları günlük olarak sayılmış ve sayılan yumurtalar Petri kabından başka bir yaprak diske aktarılmıştır. Bu Petri kaplarından yumurtaların açılma oranları ve çıkan bireylerin cinsiyet oranları belirlenmiştir.

Diğer tekerrürlerdeki dişi bireyler ve bırakılan yumurtalar günlük olarak kontrol edilmiştir. Elde edilen verilerden faydalanılarak günlük ve toplam bırakılan yumurtalar, ayrıca ovipozisyon süreleri ve ortalama yaşam süreleri belirlenmiştir. Ayrıca ilaçlanan larvalardan elde edilen erkek bireylerden en az 10 tanesi ölünceye kadar gözlemlenerek ortalama yaşam süreleri belirlenmiştir.

Verilerin değerlendirmesi

Çalışmada beş farklı pestisit uygulanan *N. californicus* ve *P. ulmi* larvalarından elde edilen erginlerinin yumurta sayısı, ovipozisyon süresi ve ortalama yaşam süresi verilerine uygulanan Anderson-Darling normallik testi, Bartlet's varyans homojenlik testi sonuçlarında verilerin parametrik testlere uygun olmadığı saptanmıştır. Bu nedenden dolayı çalışmada uygulamaların karşılaştırılmasında parametrik olmayan testlerden Kruskal-Wallis testi uygulanmıştır. Uygulamaların Rank ortalamaları arasındaki farklılığın belirlenmesinde çoklu karşılaştırma yöntemlerinden Bonferroni-Dunn testi uygulanmıştır. Çalışmada erkek bireylerin ortalama yaşam süreleri arasındaki fark tek yönlü varyans analizi ile belirlenmiştir. Uygulamaların ortalamaları arasındaki farkların belirlenmesinde ise Tukey testi kullanılmıştır. Çalışmada dişi bireylerin bırakmış oldukları yumurtalar gözlemlenmiş ve erkek birey dişi birey oranı ikili oran karşılaştırılmasında kullanılan Z testi kullanılarak saptanmıştır.

Araştırma Bulguları

Pestisitlerin *Panonychus ulmi*'nin yumurta sayısı, ovipozisyon ve yaşam süresine etkileri

Pestisitlerin uygulandığı *P. ulmi* larvalarından elde edilen dişi bireylerin bıraktığı ortalama yumurta sayılarına yapılan Kruskal-Wallis analizi sonucunda uygulamaların Rank ortalamaları arasındaki fark istatistik olarak önemli bulunmuştur ($p<0.001$) (Çizelge 2). *P. ulmi*'nin ilaç uygulanan larvalarından elde edilen dişi bireylerinin yumurta verimi spirodiclofen + abamectin hariç diğer bütün ilaçlarda kontrol grubundan önemli derecede yüksek olmuştur. *P. ulmi*'de dişi başına ortalama en yüksek yumurta verimi imidacloprid ve thiacloprid uygulanan larvalardan elde edilen dişi bireylerde saptanmıştır ve bu değerler istatistiki olarak aynı grup içerisinde yer almıştır. Dişi başına en düşük ortalama yumurta verimi ise spirodiclofen + abamectin uygulanan larvalardan elde edilen *P. ulmi* bireylerinde görülmüş ve spirodiclofen + abamectin uygulanan dişilerin yumurta verimi kontrol ile aynı istatistiki grupta yer almıştır. *P. ulmi*'de ovipozisyon süreleri bakımından elde edilen verilere uygulanan Kruskal-Wallis analizi sonucunda uygulamaların Rank ortalamaları arasındaki fark istatistik olarak önemli olmuş ve istatistiki olarak dört farklı grup oluşmuştur ($p<0.001$). Ovipozisyon süresi kontrol grubunda diğerlerine göre daha uzun bulunmuştur. Spirodiclofen + abamectin uygulanan *P. ulmi* dişilerinin ovipozisyon süreleri kontrole ve diğer ilaçlara göre daha kısa olmuştur (Çizelge 2).

İlaç ve su (kontrol) uygulanan ve *P. ulmi* larvalarından elde edilen dişilerinin ortalama yaşam süresine ait verilere yapılan Kruskal-Wallis analizi sonucunda uygulamaların Rank ortalamaları arasındaki fark istatistik olarak önemli bulunmuştur ($p<0.001$). *P. ulmi* dişilerinin yaşam süreleri bakımından dört istatistiki grup oluşmuştur. En uzun yaşayan grubu kontrol yer alan *P. ulmi* dişileri oluşturmuştur. İkinci grubu cyphermethrin, üçüncü grubu imidacloprid, deltamethrin ve thiacloprid uygulanan larvalardan elde edilen dişi bireyler oluştururken en kısa yaşayan grubu ise spirodiclofen + abamectin uygulanan larvalardan elde edilen dişiler oluşturmuştur (Çizelge 2).

Çizelge 2. Pestisitlerin *Panonychus ulmi*'nin yumurta sayısı, ovipozisyon ve yaşam süresine etkileri*

Uygulama	N	Yumurta (adet)/ dişi ± SH	Ovipozisyon süresi (gün) /dişi±SH	Ortalama yaşam süresi (gün)/dişi ±SH**
Kontrol	200	33.79±0.23 C	21.50±0.09 A	25.50±0.09 A
Cypermethrin	40	35.15±0.47 B	20.95±0.26 B	24.92±0.25 B
Deltamethrin	40	36.17±0.63 B	20.77±0.25 B	23.77±0.25 C
Spirodiclofen + abamectin	40	33.95±0.51 C	18.60±0.30 D	21.60± 0.30 D
Imidacloprid	40	37.62±0.45 A	20.97±0.24 B	23.97±0.24 C
Thiacloprid	40	36.75±0.54 A	19.85±0.30 C	23.85± 0.30 C

*Harfler aynı sütundaki istatistiki farklılıkları göstermektedir.**Ortalama yaşam süresi larva döneminden ölünceye kadar geçen süredir.

Pestisitlerin *Neoseiulus californicus'un* yumurta sayısı, ovipozisyon ve yaşam süresine etkileri

Neosiulus californicus'ta dişi başına bırakılan ortalama yumurta sayısına ait verilere yapılan Kruskal-Wallis analizi sonucunda uygulamaların Rank ortalamaları arasındaki fark istatistik olarak önemli bulunmuştur ($p<0.001$) (Çizelge 3). Avcı akar *N. californicus'un* ilaç uygulanan larvalarında elde edilen dişi bireylerinin yumurta verimi spirodiclofen + abamectin hariç diğer bütün ilaçlarda kontrol grubundan önemli derecede yüksek olmuştur. *Neoceiulus californicus*'ta dişi başına ortalama en yüksek yumurta

verimi deltamethrin, cypermethrin ve imidaclorpid uygulanan dişi bireylerde izlenmiş ve bunlar istatistiki olarak aynı grup içerisinde yer almıştır. En az dişi başına ortalama yumurta verimi ise spirodiclofen + abamectin uygulanan dişi *N. californicus* bireylerinde olmuştur ve spirodiclofen + abamectin uygulanan dişilerin yumurta verimi kontrolle aynı istatistiki grupta yer almıştır.

Neoseiulus californicus dişi bireylerinin ovipozisyon sürelerine uygulanan Kruskal-Wallis analizi sonucunda, Rank ortalamaları arasındaki fark istatistik olarak önemli olmuştur ve iki farklı grup oluşmuştur ($p<0.001$). Ovipozisyon süresi kontrol grubu, deltamethrin ve thiacloprid uygulananlarda diğerlerine göre daha uzun olmuştur ve bunlar istatistiki olarak aynı grup içerisinde yer almıştır. Cyphermethrin, spirodiclofen + abamectin ve thiacloprid uygulanan *N. californicus* larvalarından elde edilen dişilerinin ovipozisyon süreleri kontrole göre daha kısa olmuştur ve istatistiki olarak aynı grupta yer almışlardır (Çizelge 3). İlaç ve su (kontrol) uygulanan ve *N. californicus* dişilerinin ortalama yaşam süresi verilerine uygulanan Kruskal-Wallis analizi sonucunda uygulamaların Rank ortalamaları arasındaki fark istatistik olarak önemli bulunmuştur ($p<0.001$). *N. californicus* dişilerinin yaşam süreleri bakımından üç istatistiki grup oluşmuştur. En uzun yaşayan grubu kontrol ve deltamethrin uygulanan *N. californicus* larvalarından elde edilen dişiler oluşturmuştur. İkinci grubu ise cyphermethrin ve thiacloprid uygulananlar oluşturmuştur. Üçüncü ve en kısa yaşayan grubu ise spirodiclofen + abamectin ve imidacloprid uygulanan larvalardan elde edilen dişiler oluşturmuştur (Çizelge 3).

Çizelge 3. Pestisitlerin *Neoseiulus californicus*'un yumurta sayısı, ovipozisyon ve yaşam süresine etkileri*

Uygulama	n	Yumurta (adet) /dişi±SH	Ovipozisyon süresi (gün) / dişi±SH	Ortalama yaşam süresi (gün) / dişi±SH**
Kontrol	100	36.03±0.32 C	21.72±0.14 A	27.62±0.16 A
Cypermethrin	32	46.44±0.82 A	20.94±0.34 B	26.94±0.34 B
Deltamethrin	35	46.43±0.63 A	21.89±0.19 A	27.94±0.18 A
Spirodiclofen + abamectin	35	35.43±0.74 C	20.71±0.19 B	25.71±0.19 C
Imidacloprid	35	47.46±0.57 A	20.40±0.31 B	25.40±0.31 C
Thiacloprid	34	42.38±0.57 B	21.88±0.19 A	26.88±0.19 B

*Harfler aynı sütundaki istatistiki farklılıkları göstermektedir. **Ortalama yaşam süresi larva döneminden ölünceye kadar geçen süredir.

Pestisitlerin *Panonychus ulmi*'nin ve *Neoseiulus californicus*'un erkek bireylerinin ortalama yaşam sürelerine etkileri

İlaç ve su (kontrol) uygulanan larvalardan elde edilen *P. ulmi* erkek bireylerinin ortalama yaşam süresi özelliği bakımından elde edilen verilere yapılan çoklu karşılaştırma testi Tukey testi sonucunda uygulamaların ortalamaları arasındaki fark istatistik olarak önemli bulunmuştur ($p<0.001$, df:94, F:16.85). En uzun yaşayan grubu kontrol grubu, cypermethrin erkek bireyleri oluşturmuştur. En kısa yaşayan grubu ise spirodiclofen + abamectin ve deltamethrin uygulanan erkek bireyler oluşturmuştur (Çizelge 4).

İlaç ve su (kontrol) uygulanan ve *N. californicus* erkek bireylerinin ortalama yaşam sürelerinden elde edilen verilere yapılan istatistik analiz sonucunda uygulamaların ortalamaları arasındaki fark istatistik olarak önemli bulunmuştur ($p<0.001$, df:94, F:28.08). En uzun yaşayan grubu kontrol grubu *N. californicus* erkek bireyleri oluşturmuştur. İkinci grubu deltamethrin, cyphermethrin, imidacloprid, thiacloprid uygulanan larvalardan elde edilen erkek bireyler oluşturmuştur. En kısa yaşayan grubu ise spirodiclofen + abamectin uygulanan erkek bireyler oluşturmuştur (Çizelge 4).

Çizelge 4. Pestisitlerin *Panonychus ulmi*'nin ve *Neoseiulus californicus*'un erkek bireylerinin ortalama yaşam sürelerine etkileri*

Uygulama	n	Ortalama yaşam süresi (gün) / erkek±SH (*N. californicus*)**	Ortalama yaşam süresi (gün) / erkek±SH (*P. ulmi*)**
Kontrol	50	27.24±0.267 A	25.54±0.19 A
Cypermethrin	10	23.20±0.827 BC	24.90±0.59 A
Deltamethrin	10	24.00±0.966 B	22.20±0.53 C
Spirodiclofen + abamectin	10	20.90±0.605 C	21.80±0.63 C
Imidacloprid	10	22.90±0.69 BC	22.80±0.55 BC
Thiacloprid	10	21.3±00.597 BC	24.70±0.56 AB

*Harfler aynı sütundaki istatistiki farklılıkları göstermektedir. **Ortalama yaşam süresi larva döneminden ölünceye kadar geçen süredir.

Pestisitlerin *Neoseiulus californicus* ve *Panonychus ulmi*'nin yumurta açılımına etkileri

Neoseiulus californicus ve *P. ulmi* kontrol ve pestisit uygulanmış bireylerinden elde edilen dişilerin bırakmış oldukları yumurtaların tamamı 4-5 gün içerisinde açılmıştır. Bu nedenle gruplar arasında istatistik değerlendirme yapılmamıştır.

Pestisitlerin *Panonychus ulmi*'nin cinsiyet oranına etkileri

Kontrol grubunun cinsiyet oranı 0.68 iken, cypermethrin, deltamethrin, spirodiclofen + abamectin, imidacloprid ve thiacloprid uygulanan dişi bireylerin döllerinin cinsiyet oranı sırasıyla ile 0.69, 0.66, 0.54, 0.58 ve 0.52 bulunmuştur (Çizelge 5). Dişi birey oranı Spirodiclofen + abamectin, imidacloprid ve thiacloprid uygulanan *P. ulmi* larvalarından gelişen dişi bireylerin F_1 döllerinde kontrole göre istatistiki olarak önemli derecede azalırken, sadece cypermethrin ve deltamethrin uygulanan larvalarından elde edilen dişilerin F_1 döllerinde istatistiki olarak kontrolle aynı düzeyde olmuştur.

Çizelge 5. Pestisitlerin *Panonychus ulmi*'nin cinsiyet oranına etkileri

Uygulama	N	Dişi sayısı	Erkek sayısı	D/D+E*
Kontrol	518	357	161	0.68
Cypermethrin	108	75	33	0.69
Deltamethrin	139	93	56	0.66
Spirodiclofen + abamectin	111	61	50	0.54**
Imidacloprid	114	67	47	0.58**
Thiacloprid	112	59	53	0.52**

*D: Dişi saysı, E: Erkek sayısı. **Z testine göre kontrolden farklı olan grupları göstermektedir.

Pestisitlerin *Neoseiulus californicus*'un cinsiyet oranına etkileri

Kontrol grubunun cinsiyet oranı 0.66 iken, cypermethrin, deltamethrin, spirodiclofen + abamectin, imidacloprid ve thiacloprid uygulanan dişi bireylerin F_1 döllerinin cinsiyet oranı sırasıyla 0.52, 0.62, 0.73, 0.65 ve 0.52 bulunmuştur (Çizelge 6). Dişi birey oranı cypermethrin ve thiacloprid uygulanan *N. californicus* larvalarından gelişen dişi bireylerin F_1 döllerinde kontrole göre önemli derecede azalırken, Deltamethrin spirodiclofen + abamectin ve Imidacloprid uygulanan larvalarından elde edilen dişilerin yavrularında istatistiki olarak kontrolle aynı olmuştur ($p<0.05$).

Çizelge 6. Pestisitlerin *Neoseiulus californicus*'un cinsiyet oranına etkileri

Uygulama	N	Dişi sayısı	Erkek sayısı	D/D+E*
Kontrol	576	382	194	0.66
Cypermethrin	132	69	63	0.52**
Deltamethrin	139	87	52	0.62
Spirodiclofen + abamectin	106	78	28	0.73
Imidacloprid	143	94	49	0.65
Thiacloprid	135	77	58	0.57**

*D: Dişi sayısı, E: Erkek sayısı. **Z testine göre kontrolden farklı olan grupları göstermektedir.

Tartışma ve Sonuç

Türkiye'de ve dünyada pestisit kullanımı oldukça yaygındır. Bazı araştırma sonuçlarına göre uygulanan pestisitlerin hedef organizmada sublethal dozu veya etkisinin olmadığı hedef dışı organizmalarda yumurta verimi artışına neden olduğu belirlenmiştir. Bir çok üründe değişik böceklere ruhsatlı, thiacloprid, acetamiprid ve thiamethoxam'ın *Tetranychus urticae* popülasyonlarında artış meydana getirdiği belirlenmiştir (Barati et al., 2015). Bu pestisite bağlı artışlara hormoligosis denilmektedir. Ancak bu konuda yapılmış çalışma sayısı sınırlıdır.

Çalışmada kullanılan pestisitlerden spirodiclofen + abamectin'in her iki türün dişi başına ortalama yumurta verimine bir etkisi olmazken, diğer ilaçlar her iki türde de dişi başına ortalama yumurta veriminde sınırlıda olsa bir artışa neden olmuştur. Ancak bu dişi başına ortalama yumurta verimindeki artış *P. ulmi*'de istatistiki olarak önemli olsa bile sınırlı kalırken, avcı akardaki dişi başına ortalama yumurta verimindeki artış daha çok olmuştur. Bu pestisitlerden imidacloprid'in *N. californicus ve P. ulmi* bireylerinde yumurta verimini, ovipozisyon süresini, cinsiyet oranını ve ortalama yaşam süresini etkilemiştir. Her iki türde de kontrol grubuna göre yumurta verimi artmıştır, fakat ovipozisyon ve ortalama

yaşam süresi azalmıştır. Imidacloprid uygulanmış *P. ulmi* larvasından elde edilen dişiler kontrol ile karşılaştırıldığında yaklaşık 4 yumurta fazla koyarken, *N. califronicus'*ta yaklaşık 11 yumurta fazla koymuştur. Duso et al. (2008), pyrethrin ve rotenone'nin *P. persimilis'*e toksik etki yaptığını, *Beauveria bassiana*, azadirachtin, pymetrozine, imidacloprid'inin ise toksit etki yapmadığını ve imidacloprid'inde *Phytoseiulus persimilis* (Acari: Phytoseiidae) bireylerinde yumurta verimini artırdığını saptamışlardır. Ayrıca *B. bassiana'*nın *Tetranychus cinnabarinus* (Acari: Tetranychidae) bireylerinde yumurta verimini azalttığını bildirmişlerdir. Szczepaniec et al. (2013), imidacloprid ile ilaçlanmış *Buxus sempervirens* L. bitkisi üzerinde beslenen *Eurytetranychus buxi* (Acari: Tetranychidae) bireylerinin yumurta veriminin arttığını bildirilmiştir. Qu et al. (2014), imidacloprid'in sublethal (0.005 ve 0.10 mg/L) ve letal (0.2 mg/L) dozlarının *Aphis glycines* Matsumura (Hemiptera: Aphididae)'nin farklı dönemlerine uygulamışlardır ve 0.20 mg/L imidacloprid uygulanan bireylerin, toplam yaşam süresi ve ergin ömür uzunluğunun azaldığını ve buna karşın sublethal dozdaki değerlere maruz kaldığında üremelerinde artış meydana geldiğini belirtmişlerdir. Yapılan çalışmalar bu çalışmada bulunan sonuçlar ile uyum içindedir. Sohrabi et al. (2012) ise imidacloprid'in pamuk beyazsineği parazitoidi olan *Encarsia inaron* (Walker) (Hymenoptera: Aphelinidae)'in larva ve pupa dönemlerindeki gelişimini olumsuz etkilediğini, üreme ve eşey oranlarında azalma meydana getirdiğini tespit etmişlerdir.

Çalışmada kullanılan pestisitlerden spirodiclofen + abamectin'in *N. californicus ve P. ulmi* bireylerinde yumurta verimini, ovipozisyon süresini, cinsiyet oranını ve ortalama yaşam süresini etkilemiştir. Etkili madde her iki türde de yumurta verimini, ortalama yaşam süresini ve ovipozisyon süresini kontrol grubuna göre azaltmıştır. Spirodiclofen+ abamectin akarisit olmasına rağmen doğrudan uygulama ve sınırlı sürede maruz kalma nedeniyle etkisi öldürücü dozun altında olmuştur. Sato et al. (2011), spiromesifen'in *T. urticae* ve *N. californicus*'un ovipozisyon süresine ve gelişme dönemlerine etkili olduğunu tespit etmişlerdir. Irigaray & Zalom (2006), avcı akar *Galendromus occidentalis* (Nesbitt) (Acari: Phytoseiidae)'de fenpyroximate etkili maddesinin dişi bireylerde ömür uzunluğunu azalttığını ve 24 saat geçmeden ölüm görüldüğünü, spiromesifen ve acequinocly etkili maddelerinin dişi bireylerin ömür uzunluğunun 4 gün azalttığını ve üreme ve doğurganlık oranında azalma meydana getirdiğini, etoxazole ve bifenazate etkili maddelerinin ise dişi bireylerin ömür uzunluğunu azaltmadığını, bu dişilerin döl vermediklerini tespit etmişlerdir. Çalışmamızdan elde edilen sonuçlar literatür kaynaklarındaki çalışmalar ile uyum içindedir.

Çalışmada kullanılan pestisitlerden deltamethrin'in *N. californicus ve P. ulmi* bireylerinde yumurta verimini, ovipozisyon süresini ve ortalama yaşam süresini etkilemiştir. *Neosiulus californicus* bireylerinde yumurta verimini artırmış, ortalama yaşam süresinde ve ovipozisyon süresinde etki meydana gelmemiştir. *P. ulmi* bireylerinde de ise yumurta verimini artırmış, ozipozisyon ve ortalama yaşam süresini azaltmıştır. Yapılan bazı çalışmalarda piretroit kaynaklı pestisitlerin *P. citri ve T. urticae*'de yumurta verimlerinde artışa neden olduğu belirlenmiştir (Guedes & Cutler, 2013). Deltamethrin uygulanan *Leucopetera coffeella* (Guén-Mèneville) (Lepidoptera: Lyonetiidae) ve *Sitophilus zeamais* Motsch. (Coleoptera: Curculionidae) de yumurta veriminde artışı olurken, zararlı bir akar olan *Olygonychus ilicis* (McGregor) (Acari: Tetranychidae)'de ise bu etkili madde uygulandığında hormoligosis olmadığı için artış meydana gelmemiştir (Guedes & Cutler, 2013). Hormoglosis'de esteraz enzimlerinin indüksüyonu sonucunda (vücut içine alınan ilaçların esteraz enzimi sonucunda inaktive edilip vücutta detoksifiye edilmesi ile etkinliğinin azalması sonucunda) yumurta üretimi artmaktadır (Guedes & Cutler, 2013). Bulunan sonuçlar çalışmamızda elde ettiğimiz sonuçlar ile uyum içindedir.

Çalışmada kullanılan pestisitlerden thiacloprid'in *N. californicus ve P. ulmi* bireylerinde yumurta verimini, ovipozisyon süresini, cinsiyet oranını ve ortalama yaşam süresini etkilemiştir. *N.californicus* bireylerinde yumurta verimini artırmış, ortalama yaşam süresini kısaltmıştır. Fakat ovipozisyon süresinde etki meydana gelmemiştir. *P. ulmi* bireylerinde de yumurta verimini artırmış, ovipozisyon ve ortalama yaşam süresini azaltmıştır.

Çalışmada kullanılan pestisitlerden cypermethrin'in *N. californicus ve P. ulmi* bireylerinde yumurta verimini, ovipozisyon süresini, cinsiyet oranını ve ortalama yaşam süresini etkilemiştir. *N. californicus*

bireylerinde yumurta verimini artırmış, ortalama yaşam süresini ve ovipozisyon süresini azaltmıştır. *P. ulmi* bireylerinde de yumurta verimini artırmış, ozipozisyon ve ortalama yaşam süresini azaltmıştır.

Bu çalışmayı bütün olarak değerlendirdiğimizde spirodiclofen+abamectin hariç kullanılan diğer pestisitlerin öldürücü olmayan dozlarının *N. californicus* ve *P. ulmi* dişi bireylerinde yumurta verimini artırmaktadır. Yumurta veriminde en çok artışa neden olan pestisit imidacloprid olmuştur. Çalışmada kullanılan ilaçlar, spirodiclofen+abamectin hariç genellikle akarlar dışındaki böceklere karşı kullanılmaktadır. Spirodiclofen+abamectin dışındaki ilaçlar *P. ulmi*'de dişi başına sınırlı bir yumurta artışına neden olurken, avcı akar *N. californicus*'da daha fazla bir yumurta artışına neden olmuştur. Başka bir deyişle her iki türde de hormoligosisi tetiklemişlerdir. Bu durum tarımsal üretimde avcı akar açısından istenen bir durumken, zararlı akar için istenmeyen bir durumdur. Bu nedenle avcı akarların olmadığı ve zararlı akar popülasyonlarının yoğun olduğu yerlerde alternatif savaşım yöntemlerini düşünmekte yarar vardır. Ayrıca pestisitlerin akar ve böcek türlerini nasıl etkilediğinin bilinmesi mücadele programlarının oluşturulmasında büyük önem taşımaktadır. Bu çalışmanın kimyasal savaş programında akarlara karşı savaşta kullanılan pesitisitlerin belirlenmesinde sonraki çalışmalara ışık tutabileceği ümit edilmektedir.

Teşekkür

Çalışmada istatistik analizlerini yapan Yrd. Doç. Dr. Özgür KOŞKAN (Süleyman Demirel Üniversitesi, Ziraat Fakültesi, Zootekni Bölümü) teşekkür ederiz.

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