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Original article (Orijinal araştırma)

Isolation, identification and characterization of probiotic properties of bacterium from the honey stomachs of Yigilca honeybees in Turkey

Yığılca (Türkiye) bal arılarının bal midelerinden bakteri izolasyonu, tanımlanması ve probiyotik özelliklerinin karakterizasyonu

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Summary

Honeybees are considered as a key species in nature for their vital role in the maintenance of almost all life on earth. However, the massive death of honeybee stocks worldwide, largely due to colony collapse disorder, is causing international concern. In order to avoid these losses, new approaches must be sought. In previous studies, the probiotic properties of the bacteria found in the bodies of honeybees are thought to have an active role in providing resistance against pathogens. Consequently, in this study, it is aimed to isolate probiotic lactic acid bacteria from honey stomachs of the healthy honeybee, to examine the effect of these bacteria against pathogenic bacteria and to use these bacteria to boost the immune system of bees. For this purpose, between 2015 and 2016, probiotic bacteria were screened from honey bees that provided by DAGEM (Düzce University, Beekeeping Research, Development and Application Center, Yığılca, DÜZCE). The inhibitory activity of the obtained bacteria against the bee pathogen *Melissococcus plutonius* (Trüper and de 'Clari, 1998) (Enterococcaceae) was determined by *in vitro* agar well diffusion. The bacterium with the desired characteristics were identified by biochemical, physiological and 16s rDNA analysis as *Lactobacillus kunkeei* (Edwards, 1998) (Lactobacillaceae) and its probiotic nature was investigated. With the evaluation of these findings, future preparations of the isolate are expected to support the bee immune system and, as a result, to produce resistant honeybees without resorting to treatment with antibiotics.

Keywords: Bacterium, honeybee, isolation, *Lactobacillus kunkeei*, probiotics

Özet

Balarıları, dünyadaki hemen hemen bütün canlıların idamesi için hayati değer taşıyan anahtar türler olarak nitelendirilmektedir. Ancak, tüm dünyada bal arısı stoklarının büyük oranda kitlesel ölümlerine yol açan koloni çöküşü, uluslararası endişeye sebep olmaktadır. Bu kayıpları önlemek için yeni yaklaşımların ortaya çıkarılması gerekmektedir. Yapılan çalışmalarda, arıların vücutlarında bulunan probiyotik özellik taşıyan bakterilerin, arıların patojenlere karşı direnç sağlamasında aktif bir rol üstlenecekleri düşünülmektedir. Bu bağlamda, bu çalışmada sağlıklı arıların bal midesinden probiyotik özellikli laktik asit bakterilerinin izole edilmesi, bu bakterilerin arılarda hastalık etmeni patojenlere karşı etkisinin incelenmesi ve bu bakterilerin arıların bağışıklık sistemini güçlendirmek maksadıyla kullanılması amaçlanmıştır. Bu amaç doğrultusunda, 2015-2016 yılları arasında, DAGEM (Düzce Üniversitesi, Arıcılık Araştırma, Geliştirme ve Uygulama Merkezi, Yığılca, DÜZCE)' den temin edilen arıların bal midesinden probiyotik özellikli bakteri taraması yapılmıştır. Elde edilen bakterilerin arı patojeni *Melissococcus plutonius* (Trüper and de' Clari, 1998) (Enterococcaceae)' a karşı inhibisyon aktivitesi *in vitro* agar kuyu difüzyon metodu ile belirlenmiştir. Ardından istenen özelliklere sahip bakteri biyokimyasal, fizyolojik ve 16s rDNA analizi ile moleküler olarak *Lactobacillus kunkeei* (Edwards, 1998) (Lactobacillaceae) olarak tanımlanmış ve probiyotik doğası incelenmiştir. Sonuçlar değerlendirildiğinde, elde edilen izolatın gelecekte preparatlarının hazırlanarak, arıların bağışıklık sistemlerini destekleyeceği ve sonuçta, antibiyotikler ile kimyasal tedavi yöntemlerine başvurulmadan dirençli arıların üretileceği düşünülmektedir.

Anahtar sözcükler: Bakteri, balarısı, izolasyon, *Lactobacillus kunkeei*, probiyotikler

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Introduction

Honeybees produce honey, beeswax, royal jelly, propolis and bee venom. Although they provide these exceptional products to human beings, even more importantly, bees, together with wasps and hornets, carry out pollination in cultivated crops requiring cross pollination and thus ensure the superior quantity and quality of the plants (Crane & Walker, 1984; Free, 1993; Özbek, 2002).

In this context, honeybees, as the most important pollinator insects worldwide (Corby-Harris et al., 2014), hold an exceptional position in Turkish agriculture. Bees are considered a key species in nature because of their vital role in the maintenance of almost all life on earth (Özbek, 2002). Apiculture is practiced widely throughout the world and has always had an important place in agriculture. Turkey, having a favorable climate and rich vegetation, is very suited to beekeeping.

Colony collapse disorder (CCD), however, has led to a large mass die-off of commercial honeybee stocks worldwide, causing international concern (Cox-Foster et al., 2007; Rangberg et al., 2015). Colony collapse disorder is characterized by the rapid death of adult bees. Although the reason for this mass die-off is not clearly understood, it is known to be caused by a number of pathogens (Cornman et al., 2012; Rangberg et al., 2015). Regrettably, in this regard, it must be emphasized that the density of the bee population in Turkey has been declining significantly over about 30 years (Özbek, 2002). Consequently, bee diseases have begun to receive increasing attention in recent years.

One of the biggest problems in beekeeping is attempting to treat honeybee diseases with chemical treatments. Limited success is achieved after chemical treatment, and there are problems, such as a danger to human health with chemical residues in the honey (Barganska et al., 2011). Problems are experienced in export markets for honey from treated bees, so attempts are made to sell this honey in the domestic market. As a result, the products cannot be sold easily and at their proper value.

At the same time, in order for the honeybees to be healthy, under the chemical treatment practices, antibiotics are frequently used against bacterial diseases (Mutinelli, 2003). Antibiotic use weakens the immune system of the bees and leads to antibiotic-resistant bacterial pathogens (Doğaroğlu & Samancı, 2006; Barganska et al., 2011). Unfortunately, the fight against these bacteria is self-defeating. In previous studies, positive effects have been shown by the resistance of the bacterial flora in the bodies of bees against disease (Gilliam, 1997).Thus, the idea arises that if the naturally occurring microbial flora in the bodies of the bees are supported, the bees may be more resistant to disease (Tajabadi et al., 2013) In particular, the bacteria with probiotic properties found in the honey stomachs or intestines of honeybees have been observed to provide resistance against other bee pathogens (Forsgren et al., 2010). Therefore, this study presents a new approach to combat bee diseases. Firstly, bacteria with potential probiotic properties were isolated from the honey stomach of healthy bees. Then the inhibitory activity of these bacteria against a bacterial pathogen of bees was evaluated.

A possible application of this study would be to use preparations of the probiotic bacterial isolate from the honey stomachs of healthy honeybees, identified as *Lactobacillus kunkeei* (Edwards, 1998) (Lactobacillaceae), in the beekeeping sector to support the bee immune system and accordingly, to produce resistant bees without resorting to antibiotic treatments.

Material and Methods

Sampling

The bees and all the standard bee products used in the study were provided by DAGEM (Düzce University, Beekeeping Research, Development and Application Center). The bee samples were brought to the laboratory in sterile 20-ml tubes containing 0.1% peptone-NaCl solution. After surface sterilization, the bee honey stomachs were removed in a sterile cabinet and the specimens were spread onto MRS agar and M17 agar, and incubated at 30-37ºC for 24-48 h. At the end of the incubation period, pure cultures were obtained by considering the colony morphologies and microscopic appearance. These pure cultures were stored at -20ºC in a 30%-glycerol MRS broth.

Identification of isolates

Identification of the bacterial strain was based on morphological, and biochemical characteristics, as described in Bergey's Manual of Systematic Bacteriology (Krieg & Holt, 1984). Gram staining, color and shape of the colonies were determined. For molecular identification of the strain, firstly, genomic DNA was extracted according to Sambrook et al. (1989). The PCR amplification of 16S rDNA genes using genomic DNA was performed using oligonucleotide primers (UNI16S-L: 5'-ATT CTA GAG TTT GAT CAT GGC TTC A-3' and UNI16S-R: 5'-ATG GTA CCG TGT GAC GGG CGG TGT TGT A-3') and then sequencing of the amplified DNA fragments was performed by Macrogene, Inc., Europe. Comparison of the 16S rDNA gene sequences with entries in the updated GenBank database was conducted using the BLAST program.

Phylogenetic analysis

The nucleotide sequences of the 16S rRNA genes were edited with EditSeq. The 16S rRNA gene sequences of the isolate from *Apis mellifera* L., 1761 and of six closely related strains (DAT822, Genbank Accession AB777210.1; NRIC 0778, AB559822.1; YH-15, NR_026404.1; 100-1, JQ009336.1; ANRIC 0777, B559821.1) were used in the phylogenetic analysis. The phylogenetic analysis was performed via the neighbor-joining method and carried out with MEGA 5.0 software (Tamura et al., 2004). The reliability of the phylogram was tested by the bootstrap analysis of 1000 replicates using MEGA 5.0.

Determination of resistance to low pH

Active bacterial culture (1 mL) was centrifuged at 10000 g for 5 min at 4ºC. The cells were then precipitated and the supernatant was removed. The bacterial pellet was suspended in 1 mL-solutions of PBS buffers with pH values of1.0, 2.0 and 3.0 and incubated for 3 h at 37ºC. Spectroscopic measurements were taken at 0 h and 3 h after the incubation (Maragkoudakis et al., 2006; Turhan Eryılmaz, 2011).

Determination of pepsin resistance

Active bacterial culture (1 mL) was centrifuged at 10 000 g for 5 min at 4ºC. The cells were then precipitated and the supernatant was removed. The bacterial pellet was suspended in PBS buffers containing pepsin (3 mg/mL, Merck, Kenilworth, NJ, USA) atpH values of 1.0, 2.0 and 3.0 and incubatedat 37ºC for 3 h. Spectroscopic measurements were taken at 0 h and 3 h after the incubation (Maragkoudakis et al., 2006; Turhan Eryılmaz, 2011).

Determination of pancreatin resistance

Active bacterial culture (1 mL) was centrifuged at 10 000 g for 5 min at 4ºC. The cells were then precipitated and the supernatant was removed. The bacterial pellet was suspended in PBS (pH 8.0) buffer containing pancreatin (1 mg/mL, Merck) and incubated at 37ºC for 3 h. Spectroscopic measurements were taken at 0 h and 3 h after the incubation (Maragkoudakis et al., 2006; Turhan Eryılmaz, 2011).

Determination of hemolytic activity

Bacterial cultures (18 h) were grown as stab cultures in a Columbia-agar medium containing 5% human blood. After incubation at 35ºC for 48 h, in the areas surrounding the colonies a bright-green zone was formed of α-hemolytic colonies and a clear zone of β-hemolytic, while the unformed zones were considered as γ-hemolytic (Maragkoudakis et al., 2006; Turhan Eryılmaz, 2011).

Determination of antibiotic susceptibility

An antibiotic disk diffusion method was used. The cell density of the isolate cultured at 37ºC for 18 h was adjusted to 10^6 and added to the solid medium. Antibiotic discs were then placed on the solid medium. After incubation at 30ºC for 18 h, the resistance or susceptibility was determined by measuring the diameter of the resulting inhibition zones (Wilkins et al., 1972; Turhan Eryılmaz, 2011).

Determination of antimicrobial activity

A well diffusion method was used. A 48-h bacterial culture was centrifuged at 13 000 g for 15 min. The supernatant was filtered with a 0.45-µm membrane, and stored at +4ºC for later use. Soft agar containing indicator bacteria (10^7 cells/mL) was poured into Petri dishes, allowed to solidify and wells opened. The culture supernatant (100 µL) was added to the wells. After sufficient incubation for indicator bacteria to develop, any inhibition zones were measured. The bacterial strains as indicator bacteria used in this study were obtained from the American Type Culture Collection (ATCC) and were as follows: *Enterococcus faecalis* ATCC 29212, *Salmonella typhimurium* ATCC 14028, *Klebsiella pneumonia* ATCC 13883, *Escherichia coli* ATCC 35218, *Proteus vulgaris* ATCC 13315, *Listeria monocytogenes* ATCC 7644, *Yersinia pseudotuberculosis* ATCC 911, *Pseudomonas aeruginosa* ATCC 27853, *Staphylococcus epidermidis* ATCC 12228, *Staphylococcus aureus* ATCC 25923, *Enterobacter cloacae* ATCC 13047, *Bacillus subtilis* ATCC 6633.

Determination of inhibition the bee pathogen, *Melissococcus plutonius*

The inhibitory activity of the isolate obtained in this study against *Melissococcus plutonius* (Trüper and de 'Clari, 1998) (Enterococcaceae), the cause of European foulbrood, was determined using the diffusion method of Padilla et al. (1996) in vitro. The bacterium was cultivated for 72 h in MRS broth at 30ºC. The culture was then centrifuged (10000g,15 min) and filtered with a 0.45-µm membrane. A10^6-CFU/mL suspension of *M. plutonius ATCC 35311* was spread to the surface of Columbia agar supplemented with blood. The wells were cut into the inoculated agar plates. Bacterial supernatants (100 µL) were placed in these wells. The appearance of the inhibition zone around the wells was determined after 16-18-h incubation.

Results

This study isolated bacteria with probiotic features from in the honey stomach of the Yigilca honeybee, which appears as a unique ecotype, in Düzce, Turkey (Kekeçoğlu, 2007, 2009). Priority was given to bacterium in the honey stomach that grew well on MRS agar. The isolate obtained (designated HD1) from the honey stomach formed a smooth, creamy, circular Gram-positive colony (Figure 1). Biochemical tests indicated it was indole (-), amylases (-) and catalase (-). The molecular diagnostics with PCR amplification (Figure 2) revealed it has 99% 16S rRNA sequence similarity with the six strains of *Lactobacillus kunkeei*, tested and a phylogenetic tree was constructed using these reference bacteria (Figure 3).

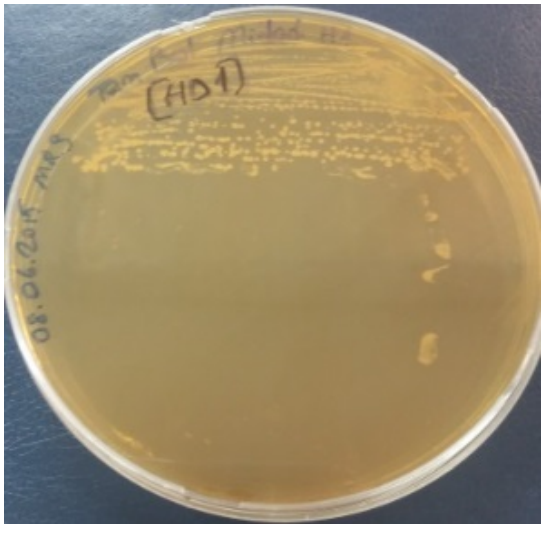

Figure 1. The isolate HD1 colony formation on MRS.

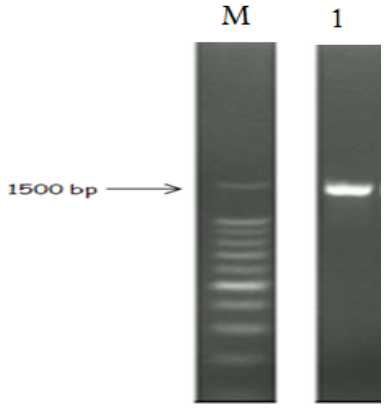

Figure 2. (1) PCR profiles of 16S rDNA fragments amplified from *Lactobacillus kunkeei* HD1 isolated from honeybee stomach, (M) marker.

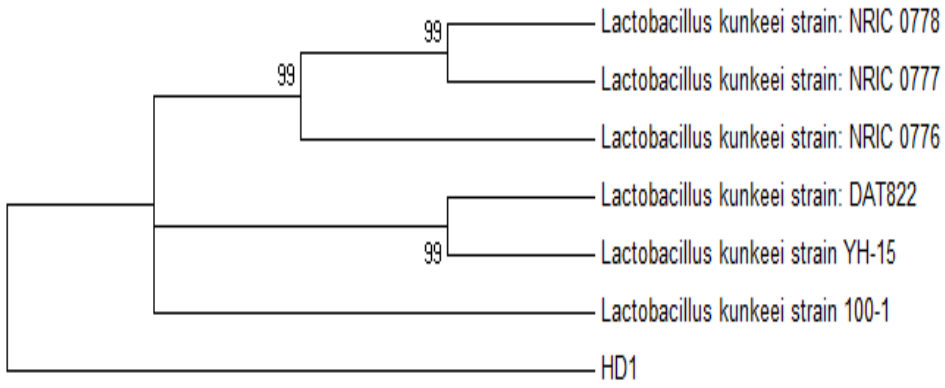

Figure 3. Neighbor-joining tree of isolate HD1.

Among lactic acid bacteria, the genus *Lactobacillus* is one of the most important and includes about 174 species (Rezvani et al., 2016). Showing a wide distribution in different habitats, *Lactobacillus* species are located in the gastrointestinal tract of bees and many other animals (Mitsuoka, 1996; Schrezenmeir & de Vrese, 2001; Tannock, 2004; Fujisawa &Mitsuoka, 1996).

The genus *Lactobacillus* is reported to be the most dominant species found in the honey stomach of bee specieslike *A. mellifera*, *Apis dorsata* Fab., 1793 and bumblebees (Olofsson & Vasquez, 2008; Vásquez et al., 2009; Tajabadi et al., 2011, 2013). In particular, *Lactobacillus* species as probiotics strengthen the immune system of bees against pathogens and have been found to help honeybees survive and to provide significant advantages for the health of honeybees (Evans & Lopez, 2004; Forsgren et al., 2010; Tajabadi et al., 2013).

In an earlier study, *Lactobacillus apinorum*, *Lactobacillus mellifer*, *Lactobacillus mellis*, *Lactobacillus melliventris*, *Lactobacillus kimbladii*, *Lactobacillus helsingborgensis* and *Lactobacillus kullabergensis* were isolated from the honey stomach of the honeybee, *A. mellifera* (Olofsson et al., 2014).

Lactobacillus kunkeei can be found in fructose-rich media like honey, bee bread, wine and flowers (Vásquez et al., 2012; Endo, 2012; Asenjo et al., 2016). In recent studies especially, *Lactobacillus kunkeei* is reported to have been isolated from the intestine of honeybees during the summer (Corby-Harris et al., 2014; McFrederick et al., 2014, Asenjo et al., 2016).

In the present study, for the first time in Turkey, *Lactobacillus kunkeei* was isolated from the honey stomach of the Yigilca honeybee. The possibility of using this isolate (HD1) in Turkey to render individual honeybees strong against diseases is promising.

HD1 was found to be substantially resistant to low pH values and highly resistant after being mixed with pepsin at pH 2 and 3. Moreover, HD1 was also found to be quite resistant against pancreatin (Figure 4). In order for bacteria with probiotic properties to reach the intestinal microbiota, they must be resistant to the acidic medium of the stomach they are required to pass through (Millette et al., 2008). HD1 was determined to be highly durable. Problematically, HD1 grows well during the first passages and tests, but slows down after 5-6 passages. CFU counts were made, but clear data were not obtained because HD1is too sensitive to growth. Observations were made only to confirm absorbance data (Figure 4). In particular, if the bacterial density is reduced, colonies do not form. If the bacterial density is increased, the bacteria survive and form colonies.

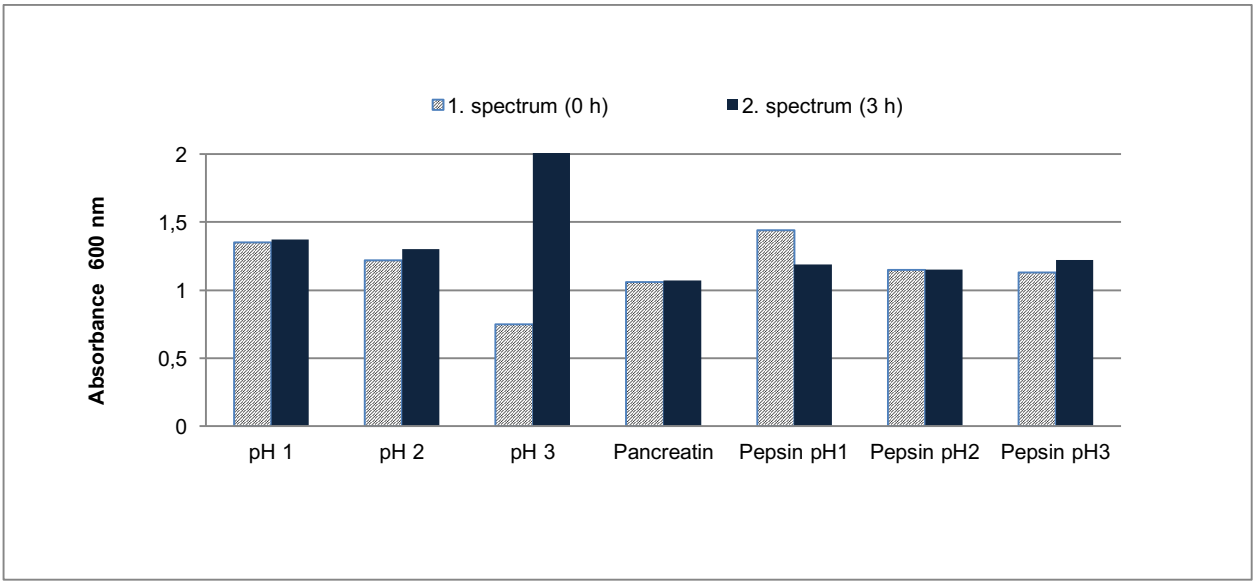

Figure 4. Tolerance to different conditions of *Lactobacillus kunkeei* HD1 isolated from honeybee stomach.

HD1 was found to exhibit γ-hemolytic activity and sensitive to multiple antibiotics (Table 1). Non-hemolytic activity and antibiotic resistance are prerequisites for the selection of probiotic strains (Hawaz, 2014). HD1 was found to be resistant to streptomycin (10 µg) and tobramycin (10 µg), and sensitive to azitromycin (15 µg), cefdinir (5 µg), ciprofloxacin (5 µg), gentamicin (10 µg), erythromycin (10 µg) and imipenem (10 µg).

Table 1. Antibiotic susceptibility of isolate HD1

Antibiotics and Inhibition zone diameter (mm)							
AZM	CD	CIP	CN	E	IPM	S	TOB
30	30	12	17	16	41	-	-

AZM: azitromycin (15 µg); CD: cefdınır (5 µg); CIP: ciprofloxacin (5 µg); CN: gentamicin (10 µg); E: erythromycin (10 µg); IPM: imipenem (10 µg); S:, streptomycin (10 µg); TOB: tobramycin (10 µg); -: no inhibition.

HD1 was determined to inhibit most of the indicator bacteria (Table 2). For two indicator strains, the inhibition was determined to be bacteriostatic because after 16-18 colonies formed in the initially large inhibition zones and the zones became smaller (Table 2).

Table 2. Inhibition activity of isolate HD1

Indicator Bacteria / Inhibition Zone (mm)											
(1)	(2)	(3)	(4)	(5)	(6)	(7)	(8)	(9)	(10)	(11)	(12)
-	-	BS	12	-	18	13	15	-	BS	-	-

1) *Enterococcus faecalis*; 2) *Salmonella typhimurium*; 3) *Klebsiella pneumonia*; 4) *Escherichia coli*; 5) *Proteus vulgaris*; 6) *Listeria monocytogenes*; 7) *Yersinia pseudotuberculosis*; 8) *Pseudomonas aeruginosa*; 9) *Staphylococcus epidermidis*; 10) *Staphylococcus aureus*; 11) *Enterobacter cloacae*; 12) *Bacillus subtilis*; BS: bacteriostatic activity; -: no inhibition.

In vitro, HD1 was found to be highly inhibitory (17 mm zone) to *Melissococcus plutonius,* the bacterial pathogen that causes European foulbrood in honeybees, a globally important honeybee brood disease (Haynes et al., 2013).

Discussion

It is suggested that preparations of the eco-friendly *Lactobacillus kunkeei* HD1*,* thanks to its probiotic properties, could be used in the beekeeping sector to support the bee immune system and produce resistant bees without resorting to treatment with antibiotics. It is hoped that future studies on the inhibitory activity of HD1 against *M. plutonius* may prove to be encouraging. Just as probiotics from fermented food products are thought to have broad application in pharmaceutical preparations (Salminen et al., 1998), this bacteria with probiotic properties might also have the potential to be used in a variety of applications.

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Original article (Orijinal araştırma)

Biological parameters and population development of *Tetranychus urticae* Koch, 1836 (Acari: Tetranychidae) on different pepper cultivars[1]

Tetranychus urticae (Koch) (Acari: Tetranychidae)'nin farklı biber çeşitlerinde biyolojik parametreleri ve popülasyon gelişimi

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Summary

Pepper cultivar preferences of *Tetranychus urticae* Koch, 1836 (Acari: Tetranychidae) were determined by comparing its development, survival, oviposition, life table parameters and population development under controlled laboratory conditions with a 16L:8D photoperiod at 27±1ºC and 65±5% RH in Bursa (Turkey) during 2015-2016. Based on assays performed on leaf discs of six pepper cvs BT-Ince Sivri, BT-Burdem, BT-Burkalem, AHCRI-Çarliston, AHCRI-Yağlık and AHCRI-Kandil Dolma, there were significant differences in egg hatch, juvenile development duration, intrinsic rate of natural increase (r_m), net reproductive rate (R_o) and mean generation time (T) of the mite. The study showed that the life table parameters, r_m (0.16-0.24), R_o (10.99-35.19) and T (14.22-16.39) for *T. urticae* were different when it was fed on different pepper cultivars. Significantly lower life table parameter values (r_m, R_o and T) for *T. urticae* were observed on cv. BT-Ince Sivri, followed by cvs BT-Burdem and BT-Burkalem. Additionally, the mite densities on pepper seedlings of these three cultivars were significantly lower compared with those on seedlings of cvs AHCRI-Çarliston, AHCRI-Yağlık and AHCRI-Kandil Dolma. Furthermore, a lower survival rate was observed during juvenile development on cv. BT-Ince Sivri. Among the pepper cultivars, cv. BT-Ince Sivri had the lowest life table parameter values. Thus, the findings suggest that cvs AHCRI-Çarliston, AHCRI-Yağlık and AHCRI-Kandil Dolma are more susceptible to *T. urticae* than cvs Bursa BT-Ince Sivri, BT-Burdem and BT-Burkalem.

Keywords: Pepper, cultivar, demographic parameters, two-spotted spider mite, population development

Özet

Bu çalışmada *Tetranychus urtic*ae Koch, 1836 (Acari: Tetranychidae)'nin farklı biber çeşitleri arasındaki konukçu tercihi Bursa (Türkiye)'da 2015-2016 yılları arasında kontrollü laboratuvar koşullarında (16:8 aydınlık: karanlık, 27±1ºC sıcaklık, %65±5 orantılı nem) elde edilen zararlının gelişim, canlılık, ovipozisyon, hayat tablosu parametreleri ve popülasyon gelişim verileri karşılaştırılarak belirlenmiştir. Altı farklı biber çeşidi (BT-Ince Sivri, BT-Burdem, BT-Burkalem, AHCRI-Çarliston, AHCRI-Yağlık ve AHCRI-Kandil Dolma) yapraklarıyla yapılan testler sonucunda akarın yumurta açılımı, ergin öncesi dönemlerinin gelişme süresi, kalıtsal üreme yeteneği (r_m), net üreme gücü (R_o) ve toplam üreme oranı (GRR) açısından istatistikî anlamda önemli farklılıklar bulunmuştur. Bu çalışmada, *T. urticae* farklı biber çeşitleri üzerinde beslendiği zaman hayat tablosu parametre değerlerinin r_m (0.16-0.24), R_o (10.99-35.19) ve T (14.22-16.39) değişiklik gösterdiği belirlenmiştir. *Tetranychus urticae*'de önemli düzeyde daha düşük biyodemografik parametre değerleri (r_m, R_o ve T) sırasıyla BT-Ince Sivri, BT-Burdem ve BT-Burkalem çeşitlerinde saptanmıştır. Buna ek olarak, bu üç çeşidin biber fideleri üzerindeki popülasyon yoğunluğu da diğer üç çeşide (AHCRI-Çarliston, AHCRI-Yağlık ve AHCRI-Kandil Dolma) göre önemli düzeyde daha düşük bulunmuştur. Ayrıca, gelişme döneminde en düşük akar canlılığı BT-Ince Sivri çeşidinde gözlemlenmiştir. Sivri biber çeşitleri arasında ise istatistiki anlamda en düşük hayat tablosu parametreleri BT-Ince Sivri çeşidinde belirlenmiştir. Sonuçta, bulgular AHCRI-Çarliston, AHCRI-Yağlık ve AHCRI-Kandil Dolma çeşitlerinin diğer üç çeşide kıyasla *T. urticae*'ye daha hassas olduğunu ortaya koymuştur.

Anahtar sözcükler: Biber, çeşit, demografik veriler, ikinoktalı kırmızıörümcek, popülasyon gelişimi

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Introduction

Tetranychus urticae Koch, 1836 (Acari: Tetranychidae) is a polyphagous plant parasitic mite that can fed on more than 1100 plant species in 140 plant families (Osborne et al., 1999; Migeon & Dorkeld, 2016). In addition, the mite is one of the most important pest species in annually cultivated plants grown in greenhouse, including pepper (Yoldaş et al., 1990; Öncüer et al., 1992; Yaşarakıncı & Hıncal, 1997; Erdoğan, 2006; Can & Çobanoğlu, 2010; Çobanoğlu & Kumral, 2016). The species is also the main pest species feeding on different pepper cultivars grown in open fields (Çobanoğlu & Kumral, 2016). As a result of the feeding of *T. urticae* on pepper, chlorophyll and pigments are broken down. These plants, which experience reduced assimilation and photosynthesis, are subject to drying and high product losses due to high water loss (Tomczyk & Kropczynska, 1985; Herrmann et al., 2012). Although biological control agents are being used around the world as well as in Turkey, synthetic acaricides or insecticide/acaricides are still commonly used (Kılınçer et al., 2010). Such chemical-based control programs can lead to pesticide resistance over a short time. In particular, this pest can quickly gain resistant to many different mechanisms of action, and pesticide residue problems could be arisen in products because of the use of high doses (van Leeuwen et al., 2010; Whalon et al., 2016). In a commercial sense, a large majority of the peppers grown in the province of Bursa are exported to foreign countries. The population of the pests reached peak populations above the economic loss threshold when the harvest began in the middle of August. Growers avoid chemicals due to residual risks in the products (Çobanoğlu & Kumral, 2016). For all these reasons, the preference for pest-resistant pepper cultivars as an alternative and/or to support chemical control may help keep the damage below the economic injury level. In addition, determining more susceptible cultivars to *T. urticae* will help producers to take precautions against this pest while growing these cultivars.

Despite the extensive list of hosts, some plant species are less preferred by the mite (Kasap, 2002). For example, sweet pepper was poorly accepted by *T. urticae* compared to soybean, red pepper and eggplant (van den Boom et al., 2003, 2004). Additionally, the preferences, development and reproduction of the mite differ not only among plant species but also on different cultivars of the same plant species (Dehghan et al., 2009; Hoy, 2011; Atalay & Kumral, 2013; Najafabadi et al., 2014; Keskin & Kumral, 2015). The most important factors that affect host acceptability and fecundity of *T. urticae* are the presence of nutrients and alkaloid contents, and physical and chemical repellents (volatile compounds, and glandular and/or non-glandular trichomes) (van den Boom et al., 2003; Erdoğan et al., 2010; Hoy, 2011). Previous studies on pepper cultivars showed that the cultivar resistance to some mite species, such as *Polyphagotarsonemus latus* (Bank, 1904) (Acari: Tarsonemidae) correlated with the contents of chlorophyll, soluble sugar, free serine, polyphenol and tannin as well as the density of pubescence on leaves (Li et al., 2011, 2015). Similarly, the white fly resistance in pepper cultivars was strongly related to protective enzyme activity and secondary metabolites, especially total phenolic content (Kong et al., 2014). However, there has been no study focusing on the mechanism of *T. urticae* resistance in different pepper cultivars.

In fact, the biology of *T. urticae* on the pepper plants has been rarely investigated, and life table parameters associated with different pepper cultivars have not been determined (Zatyko & Martinovich, 1986; van den Boom et al., 2003; Gallardo et al., 2005). The developmental duration and survival of immatures, and longevity and oviposition of adults are the most critical features in the acceptance of a host plant or cultivars (Sedaratian et al., 2011). Additionally, intrinsic rate of natural increase is the other important biological characteristic parameter in population growth of species on host plants (Birch, 1948). Given that individual tests on mites are usually carried out using leaf discs in Petri dishes, semi-field test under controlled conditions must be conducted to confirm these results (Gutierrez & Helle, 1985). For this purpose, the development, survival, oviposition and life table parameters of *T. urticae* on leaf discs in Petri dishes and its population development on potted pepper plants were determined under controlled conditions using six pepper cultivars intensively grown by producers.

Material and Methods

Pepper cultivars and growth

Six cultivated pepper cultivars (*Capsicum annuum* L.) [Bursa Tohum (BT)-Ince Sivri, BT-Burdem, BT-Burkalem, Atatürk Horticultural Central Research Institute (AHCRI)-Çarliston, AHCRI-Yağlık and AHCRI-Kandil Dolma] which are the most preferred for open field production were used. Based on Bozokalfa (2009) and Keleş (2009), the leaf and fruit features of the pepper cultivars are provided in Table 1. Pepper seeds were sown in a peat medium (Klasmann TS 1-Deilmann, Geeste, Germany) in Bursa (Turkey) during 2015-2016. Twenty to 25 d after sowing, the pepper seedlings were transplanted into 1.5-L pots (40 x 130 cm) filled with the peat and placed in a growth room with a 16L:8D photoperiod at 27±1ºC and 65±5% RH. Seedlings were irrigated every 2 d with tap water and fertilized weekly with 100 mL a water-soluble fertilizer containing 3% total nitrogen (N), 7% phosphorus (P_2O_5), 4.5% potassium (K_2O), 0.1% sulfur (SO_4-S), 0.25% iron (Fe), 0.01% copper (Cu), 0.1% zinc (Zn), 0.1% manganese (Mn), 0.01% boron (B) and 0.001% molybdenum (Mo) prepared by Uludag University, Department of Soil Science and Plant Nutrition (Bursa, Turkey). Three weeks after transplant, nine uniforms, full blooming plants with five fully developed leaves were selected for the experiments.

Table 1. The leaf and fruit features of different pepper cultivars (Keleş, 2007; Bozokalfa, 2009)

Cultivar	Leaf color	Leaf shape	Trichome density on leaf	Trichome density on stem	Leaf index (leaf length/leaf weight)	Fruit shape	Fruit bitterness
BT-Ince Sivri	DK	O	S	D	W	Tn, L	H
BT-Burdem	DK	O	S	D	W	Tn, L	H
BT-Burkalem	DK	O	S	D	W	Tn, L	Sw
AHCRI-Çarliston	DK	O	S	D	N	Tc, L	Sw
AHCRI-Kandil Dolma	G	O	S	D	N	O, Sh	Sw
AHCRI-Yağlık	G	O	S	D	W	F, L	Sw

D: dense; DK: dark green; F: Flattened; G: green; H: hot; L: long; N: narrow; O: oval; S: sparse; Sh: short; Sw: sweet; Tc: thick; Tn: thin; W: wider.

Mass rearing of *Tetranychus urticae* colony

A colony of *T. urticae*, collected from a greenhouse located Yalova city, Turkey (40.62311º N; 29.31373º E; 54 m) was reared for 8 years on potted plants, was used. The colony was reared in a growth room with a 16L:8D photoperiod at 27±1ºC and 65±5% RH. A synchronous *T. urticae* colony was provided by rearing at least two generations on each experimental pepper cultivar.

Development and survival of immature stages

The method using a leaf disc in Petri dish followed Keskin & Kumral (2015). Briefly, each leaf disc (120 mm diameter) was placed with its lower surface facing up in a Petri dish in 2% agar. The ventilation of Petri dish was ensured by holes pierced with a steel needle. For mating, one newly emerged female and two males were transferred onto each leaf disc in an insectarium (Nüve, Ankara, Turkey) with a 16L:8D photoperiod at 27±1ºC and 65±5% RH. Oviposition was monitored for 6 h, and females that had oviposited and males were removed from each Petri dish. Egg hatch and immature development were checked twice per day. The leaf discs were changed every week, and individuals were transferred to fresh leaves. Unhatched eggs and dead immatures were recorded to calculate survival rates.

Oviposition and life table parameters

When an individual was a deutonymph chrysalid female, two adult males were added onto her leaf disc for mating. After females emerged, pre-oviposition, oviposition, post oviposition periods, the daily number of eggs laid and the female longevity were recorded daily during the life of each female. For each pepper cultivar, daily age-specific survival (lx) and fecundity (mx) rates were calculated using the method described by Birch (1948), Howe (1953) and Watson (1964) using RmStat-3 software (Özgökçe & Karaca, 2010). The intrinsic rate of natural increase (r_m) was estimated based on the following equation:

$$\Sigma\ lxm\ xe^{-rmx} = 1$$

where x is female age in days, lx is the age-specific survival rate and mx is the expected number of daughters produced per female alive at age x. The net reproductive rate is given by $R_0 = \Sigma lxmx$, the mean generation time (T) in days is given by $T = \ln R_0/rm$, the finite rate of increase (λ) is given by $\lambda = erm$ and the doubling time (DT) in days is DT = ln 2/rm.

Population development on potted pepper plants

To detect the population development of the mite on potted pepper plants, full blooming plants were artificially infested with 25 deutonymph chrysalid females at a density of 5 mites/leaf. Each pepper cultivar included three replicates (with three plants). The plants were grown in a growth room with a 16L:8D photoperiod at 27±1ºC and 65±5% RH. Fourteen d after infestation, mobile and immobile (egg) stages of the mite on all surfaces of the plant leaves were counted using a template (5 cm^2) stereomicroscope (Leica, Wetzlar, Germany). For each leaf, a total area of 50 cm^2 was inspected on same locations of leaves.

Statistical analyses

The distributions of all biological data were tested by Shapiro-Wilk method (SPSS, 2015). Non-normally distributed data were $\log_{10}$ transformed, before being used in one-way analysis of variance. In addition, Levene's test for homogeneity of variance was performed. Post hoc testing ($P < 0.05$) of the multiple comparisons was performed using either the Games-Howell or Tukey test, respectively, depending on whether Levene's test was significant or not (SPSS, 2015). Hatch and survival rates were compared with the chi-square test. The bootstrap technique was used to estimate the means, variances and standard errors of the population parameters (rm, R_0 and T). To generate viable results, 10,000 replicates were used (Efron & Tibshirani, 1993).

Results

Development and survival

The total developmental time of *T. urticae* were varied from 8.15 to 11.73 d for females and 7.45 to 10.61 d for males on different pepper cultivars at long daylight, 27±1ºC and 65±5% RH (female $F_{5,204} = 50.3$, $P < 0.01$; and male $F_{5,1394} = 35.9$, $P < 0.01$; Table 2). The development times were significantly longer on cv. AHCRI-Yağlık (11.73 for females and 10.61 for males) and shorter on cv. AHCRI-Çarliston (8.15 for females and 7.45 for males). Also, there were significant variations in the development times for other stages (egg $F_{5,472} = 11.0$, $P < 0.01$; larva $F_{5,410} = 12.2$, $P < 0.01$; protonymph $F_{5,390} = 11.6$, $P < 0.01$; and deutonymph $F_{5,360} = 28.6$, $P < 0.01$).

Survival of *T. urticae* showed that the mite successfully developed on all pepper cultivars, but there were no differences between the cultivars (Table 3). The highest survival rate was found on cv. BT-Burdem (81.58%). Survival rates on cvs BT-Ince Sivri and BT-Burkalem were lowest with 53.26 and 62.50%, respectively. The most death was recorded in the larval stage on cv. BT-Ince Sivri. Similar to mite development time, the survival rates on cv. AHCRI-Çarliston were higher than those on cvs BT-Burkalem and BT-Ince Sivri.

Table 2. Developmental time (d, mean±SE) of *Tetranychus urticae* stages on different pepper cultivars

Cultivar	Egg	Larva	Protonymph	Deutonymph	Total development ♀	Total development ♂
BT-Ince Sivri	3.62±0.07ab*	1.67±0.08d	1.89±0.08a	1.59±0.10cd	8.81±0.23c	8.39±0.19c
BT-Burdem	3.25±0.05d	1.95±0.06bc	1.68±0.06ab	1.84±0.09bc	10.00±0.22b	9.14±0.08b
BT-Burkalem	3.35±0.06cd	1.98±0.06bc	1.91±0.05a	1.80±0.08bcd	9.98±0.19b	9.07±0.18bc
AHCRI-Çarliston	3.52±0.07bc	1.70±0.07d	1.31±0.06c	1.50±0.08d	8.15±0.15c	7.45±0.19d
AHCRI-Kandil Dolma	3.67±0.06ab	2.04±0.07ab	1.46±0.06bc	2.04±0.08b	10.54±0.15b	9.87±0.16a
AHCRI -Yağlık	3.90±0.09a	2.31±0.06a	1.67±0.07ab	2.70±0.07a	11.73±0.15a	10.61±0.25a

* Means followed by the same letter in a column are not significantly different (Tukey, $P < 0.05$).

Table 3. Hatch and survival rates of immature stages of *Tetranychus urticae* on different pepper cultivars

Cultivar	n	Hatch (%)	Survival of larvae (%)	Survival of protonymph (%)	Survival of deutonymph (%)	Overall survival (egg to adult) (%)
BT-Ince Sivri	93	92.47	66.28	92.98	92.45	53.26
BT-Burdem	75	98.68	95.83	95.65	86.89	81.58
BT-Burkalem	96	92.71	88.76	96.20	88.16	62.50
AHCRI-Çarliston	74	91.89	83.82	94.73	98.15	71.62
AHCRI-Kandil Dolma	93	84.95	92.41	97.26	97.18	74.19
AHCRI -Yağlık	98	82.65	95.06	92.21	91.55	66.33
X^2 (df = 5)		1.88	7.46	0.198	1.135	7.232
P		0.865	0.189	0.999	0.951	0.204

Oviposition and adult longevity

Unlike the post-oviposition period, the pre-oviposition ($F_{5,81} = 16.1$, $P < 0.01$) and oviposition ($F_{5,81} = 3.65$, $P < 0.01$) durations of *T. urticae* differed significantly among the six pepper cultivars (Table 4). The mite showed a significantly longer oviposition period of 12.60 d on cvs AHCRI-Çarliston and AHCRI-Yağlık with 11.85 d. The oviposition periods on cvs BT-Ince Sivri, BT-Burdem and BT-Burkalem were significantly lower than that on cvs AHCRI-Çarliston and AHCRI-Yağlık. There was lower fecundity on cv. BT-Ince Sivri (18.92 eggs/female) followed by cv. BT-Burdem (21.72 eggs/female) ($F_{5,81} = 7.28$, $P < 0.01$). AHCRI-Yağlık, AHCRI-Çarliston and AHCRI-Kandil Dolma showed the highest fecundities of 55.77, 52.13 and 51.33 eggs/female, respectively. Significantly differences in adult longevities on different pepper cultivars were found ($F_{5,81} = 2.53$, $P = 0.04$). The highest longevity was recorded on cv. AHCRI-Çarliston (16.33 d) and shortest female longevity was observed on cv. BT-Burkalem (10.47 d) followed by cvs BT-Burdem and BT-Ince Sivri.

Table 4. Daily egg production (mean±SE), oviposition duration and adult female longevity (mean±SE) of *Tetranychus urticae* on different pepper cultivars

Cultivar	Adult female longevity (d)	Pre-oviposition (d)	Oviposition (d)	Post-oviposition (d)	Total number of eggs/female
BT-Ince sivri	11.86±0.88b*	2.50±0.14a	7.57±0.93b	1.71±0.49a	18.92±3.16c
BT-Burdem	11.69±0.99b	1.31±0.13cd	8.39±1.17b	2.00±0.48a	21.72±6.30c
BT-Burkalem	10.47±0.64b	1.73±0.18bc	6.93±0.70b	1.80±0.47a	29.73±6.45bc
AHCRI-Çarliston	16.33±2.05a	2.00±0.19ab	12.60±1.70a	1.80±0.45a	52.13±7.65a
AHCRI-Kandil Dolma	12.50±1.49ab	1.08±0.08d	9.25±1.12ab	2.00±0.56a	51.33±7.22ab
AHCRI-Yağlık	14.54±1.35ab	1.08±0.08d	11.85±1.21a	1.69±0.26a	55.77±6.49a

* Means followed by the same letter in a column are not significantly different (Tukey, $P < 0.05$).

Life table parameters

The intrinsic rates of natural increase (r_m) of the spider mite were the lowest on cv. BT-Ince Sivri (0.1635) followed by cvs BT-Burdem (0.1688) and BT-Burkalem (0.2021) ($F_{5,53} = 962$, $P < 0.01$) (Table 5). The highest r_m value was found on cv. AHCRI-Çarliston (0.2384) followed by cvs AHCRI-Kandil Dolma (0.2325) and AHCRI–Yağlık (0.2239). Significant differences in the net reproductive rate (R_0) among different pepper cultivars were found ($F_{5,53} = 885$, $P < 0.01$). The R_0-value was significantly lowest on cv. BT-Ince Sivri (10.987) followed by cvs BT-Burdem (15.922) and BT-Burkalem (22.629) ($F_{5,53} = 885$, $P < 0.01$). The mean generation time (T) of the mite varied significantly among the pepper cultivars ($F_{5,53} = 1090$, $P < 0.01$). The T value of *T. urticae* was the lowest on cv. AHCRI-Kandil Dolma (14.220) followed by cvs AHCRI-Çarliston (14.597) and BT-Ince Sivri (14.658) ($F_{5,53} = 1090$, $P < 0.01$). The gross reproduction rate (GRR) of *T. urticae* exhibited results that were similar to those of its R_0. Similarly, the doubling time (DT) was the longest on cv. BT-Ince Sivri (3.952) following by cvs BT-Burdem (3.824) and BT-Burkalem (3.191). The other life table parameter, the finite rate of increase (λ) of the mite, was similar to T value for all cultivars.

Table 5. Life table parameters of population growth in *Tetranychus urticae* on different pepper cultivars

Cultivar	Intrinsic rate of natural increase, rm (female/female/d)	Net reproductive rate, R_0 (female/female/ generation)	Mean generation time, T (d)	Gross reproduction rate, GRR (female egg/ female/generation)	Doubling time, DT (d)	Finite rate of increase, λ (individual/ female/d)
BT-Ince Sivri	0.1635±0.001e*	10.987±0.17f	14.658±0.017d	15.022	3.952	1.192
BT-Burdem	0.1688±0.002e	15.922±0.38e	16.385±0.028a	22.058	3.824	1.199
BT-Burkalem	0.2021±0.001d	22.629±0.45d	15.424±0.021c	28.632	3.191	1.243
AHCRI-Çarliston	0.2384±0.001a	32.507±0.31b	14.597±0.025d	46.625	2.683	1.295
AHCRI-Kandil Dolma	0.2325±0.001b	27.285±0.26c	14.220±0.027e	35.643	2.749	1.287
AHCRI-Yağlık	0.2239±0.001c	35.189±0.26a	15.905±0.032b	46.104	2.886	1.271

*Means followed by the same letter in a column are not significantly different (Tukey, $P < 0.05$).

The life tables of the different pepper cultivars prepared with data obtained from emergence until death of *T. urticae* are given in Figure 1. *Tetranychus urticae* females produced the maximum number of females at 13-14 d after emergence in all cultivars. The females began to oviposit 8-10 d after emergence and terminated oviposition 27 d after emergence except on cvs AHCRI-Yağlık and AHCRI-Çarliston (31-35 d after emergence). Relatively fewer females were produced on cvs BT-Ince Sivri and BT-Burdem. The longevity of *T. urticae* was shortened depending on the pepper cultivar. For example, female longevity on cvs BT-Ince Sivri, BT-Burdem and BT-Burkalem was much lower than that on the other cultivars. The longest female longevity was found on cvs AHCRI-Yağlık and AHCRI-Çarliston.

Population development

Significant differences were found in the mean numbers of all mobile and immobile stages of the spider mite among pepper cultivars (Table 5). Similar to life table parameters, the highest number of eggs were found on cvs AHCRI-Kandil Dolma, AHCRI-Çarliston and AHCRI-Yağlık ($F_{5,53}$ = 27.8, P < 0.01). The numbers of mobile stages were the highest on cv. AHCRI-Çarliston followed by cvs AHCRI-Kandil Dolma and AHCRI-Yağlık (larva $F_{5,53}$ = 6.93, P < 0.01; nymphs $F_{5,53}$ = 45.2, P < 0.01; males $F_{5,53}$ = 6.14, P < 0.01; females $F_{5,53}$ = 76.8, P < 0.01; and all mobile stages $F_{5,53}$ = 40.4, P < 0.01). Additionally, the lowest number of eggs and mobile stages was observed on cv. BT-Burdem, followed by cvs BT-Burkalem and BT-Ince Sivri (Table 6).

Significant differences were found in the mean numbers of all mobile and immobile stages of the spider mite among the pepper cultivars (Table 5). Similar to life table parameters, the highest number of eggs were found on cvs AHCRI-Kandil Dolma, AHCRI-Çarliston and AHCRI-Yağlık ($F_{5,53}$ = 27.8, P < 0.01). The number of mobile stages was the highest on cv. AHCRI-Çarliston, followed by cvs AHCRI-Kandil Dolma and AHCRI-Yağlık (larva $F_{5,53}$ = 6.93, P < 0.01; nymphs $F_{5,53}$ = 45.2, P < 0.01; males $F_{5,53}$ = 6.14, P < 0.01; females $F_{5,53}$ = 76.8, P < 0.01; and all mobile stages $F_{5,53}$ = 40.4, P < 0.01). Additionally, the lowest number of egg and mobile stages were observed on cv. BT-Burdem followed by cvs BT-Burkalem and BT-Ince Sivri (Table 6).

Table 6. Number of mites and eggs (mean±SE) of *Tetranychus urticae* per 1 cm^2 leaf area of different pepper cultivars

Cultivar	egg	larva	nymph	male	female	all mobile stages
BT-Ince Sivri	0.44±0.17c	0.06±0.02b	0.59±0.05bc	0.20±0.02b	0.23±0.02bc	1.09±0.09cd
BT-Burdem	0.26±0.04c	0.18±0.02ab	0.25±0.02cd	0.32±0.02ab	0.02±0.01c	0.76±0.05d
BT-Burkalem	0.31±0.07c	0.17±0.03ab	0.18±0.04d	0.39±0.07ab	0.02±0.01c	0.75±0.14d
AHCRI-Çarliston	2.06±0.24b	0.32±0.07a	1.44±0.09a	0.18±0.03b	1.06±0.08a	3.00±0.22a
AHCRI-Kandil Dolma	3.53±0.45a	0.12±0.02b	0.94±0.07b	0.45±0.06a	0.39±0.04b	1.89±0.07b
AHCRI-Yağlık	1.51±0.33bc	0.08±0.02b	0.78±0.56b	0.33±0.03ab	0.31±0.06b	1.50±0.16bc

* Means followed by the same letter in a column are not significantly different (Tukey, P < 0.05).

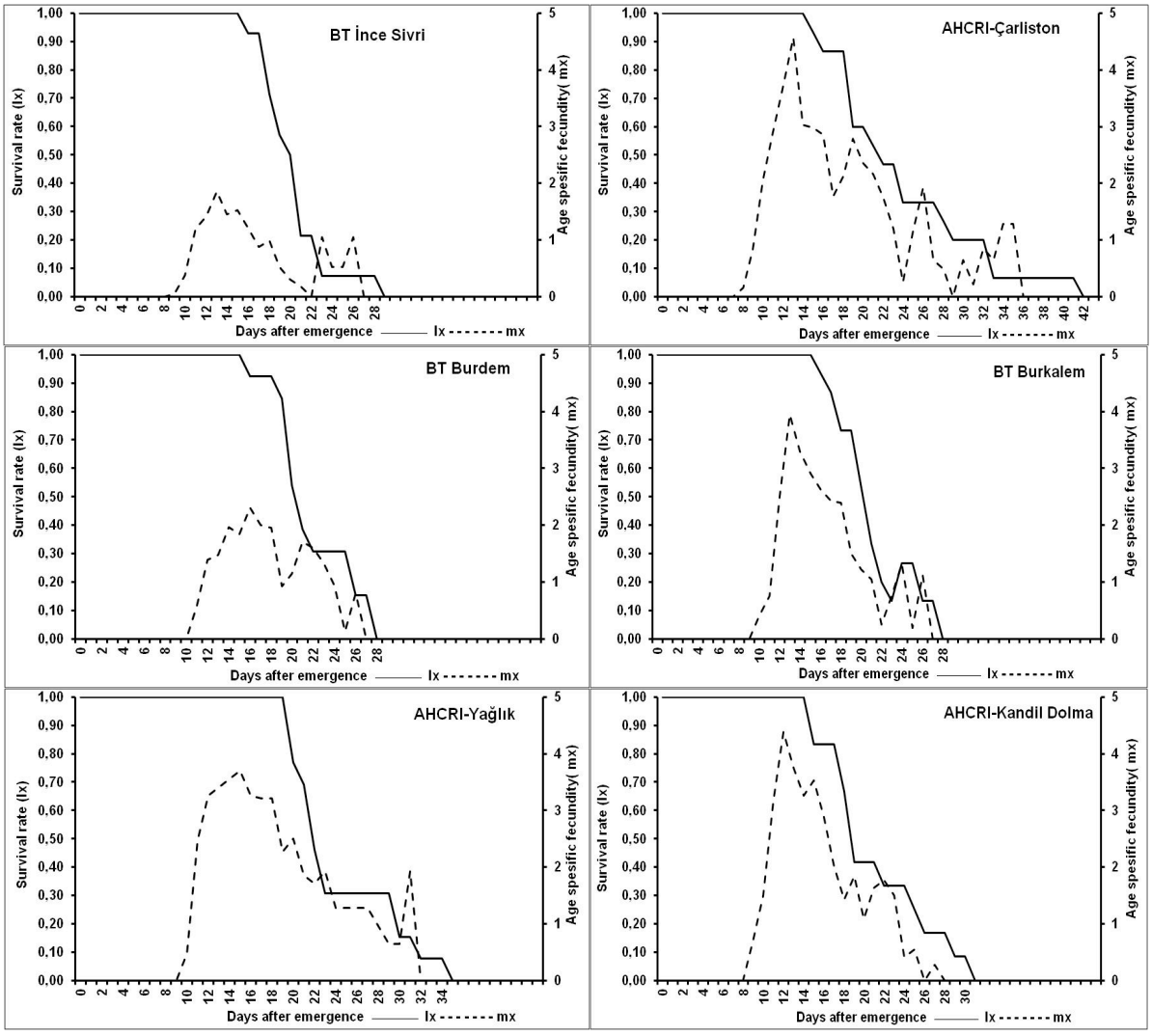

Figure 1. Age-specific survival rate (lx), age-specific fecundity rate (mx) and lxmx curves in *Tetranychus urticae* on different pepper cultivars; lx = (egg hatch) (proportion of females alive at age x), mx = (proportion of females) (age-specific oviposition).

Discussion

This study showed that the life cycle of *T. urticae* can be successfully completed on the pepper cultivars tested. However, cultivar differences significantly affected development of *T. urticae*. The total development duration of *T. urticae* was 7.5-10.6 d for males and 8.2-11.7 d for females under long day lighting, 27±1ºC and 65±5% RH. This period was very similar to those found in previous studies performed under similar conditions: sweet peppers (8.2 d), cucumber (10.4 d), beans (10.9 d) and tomato (11.4-11.6 d) (Kasap, 2002; Gallardo et al., 2005; Atalay & Kumral, 2013). The most remarkable result is the values of some pepper cultivars in current study are very similar to those for bean and sweet pepper, which are favorable hosts for *T. urticae.* As reported by Gallardo et al. (2005), the rapid development of mite on pepper cultivars was confirmed.

The other factor affecting the development of the spider mite is survival rates. Low survivability was observed some pepper cultivars in this study. These results indicate that cvs BT-Burkalem and BT-Ince Sivri are less suitable hosts because the high mortality was observed in egg and larval stages. While low survival rates were found on cvs BT-Ince Sivri and BT-Burkalem, the survival rates were quite high on cvs AHCRI-Kandil Dolma and AHCRI-Çarliston. This result is consistent with van den Boom et al. (2004), who suggested that some defensive compounds (i.e., capsaicin and dihydrocapsaicin) in pepper plants can cause mortality in some immature stages of *T. urticae* and these may be found in the tested cultivars.

Similarly, Erdogan et al. (2010) showed that the leaf extracts (12%) of hot cultivars caused high mortality in larval, nymph and adult stages of *T. urticae* and significantly reduced the reproductive capacity of females. However, further studies are needed to confirm this hypothesis.

In addition, there were significant differences in duration of oviposition, fecundity and egg production of *T. urticae.* In the current study, the oviposition period was 6.9-12.6 d and the number of eggs/female was 21.5-55.8. In some previous studies, these values were 10 d and 27.5 eggs on sweet pepper; 5-13 d and 85-276 eggs on tomato; 24 d and 231 eggs on bean and 19 d and 124 eggs on soybean and 21 d and 172 eggs on cucumber (Kasap, 2002; Dehghan et al., 2009, Atalay & Kumral, 2013). Compared with the most suitable host plants, i.e., bean and cucumber, the fecundity on all pepper cultivars of this study were lower. However, our results correspond with the findings of Gallardo et al. (2005), demonstrating that *T. urticae* feed on different sweet pepper cultivars. Thus, these results show that *T. urticae* can grow quickly on pepper cultivars, but fecundity is lower than more favorable host plants such as tomato, bean, soybean and cucumber. For example, the net reproductive rates on different pepper cultivars varied from 11.19-35.8 female/female/generation, and the intrinsic rate of natural increase was 0.18-0.26 female/female/day in this study. Similarly, these values were found to be consistent with the findings (11.5 and 0.29) from the different pepper cultivars used by Gallardo et al. (2005). In contrast, our results were low compared with the findings on some other host plants: tomato (56.9-131.2 and 0.26-0.29), beans (185.4 and 0.27), soybeans (65.7 and 0.26) and soybeans (110.7 and 0.25) (Kasap, 2002; Dehghan et al., 2009; Atalay & Kumral, 2013). Considering these results, pepper can be regarded as a less suitable host plant compared to the plants, in accordance with the findings of van den Boom et al. (2003, 2004). This research indicates that pepper has a strong direct defense mechanism against *T. urticae* and it is less preferred by the mite than tomato, eggplant and bean plants.

In this study, some cultivars were shown to be less suitable for *T. urticae* based on both the life table parameters in Petri dishes under controlled conditions and population development data on potted pepper plants under semi-field conditions. For example, the net reproductive rate and intrinsic rate of natural increase of *T. urticae* were found to be significantly lower in cvs BT-Ince Sivri, BT-Burdem and BT-Burkalem. Sabelis (1985) reported that the intrinsic rate of natural increase of *T. urticae* varies between 0.22 and 0.34 female/female/day under optimum climatic conditions depending on the condition of the host. In our study, these values were determined to be 0.163 and 0.169 female/female/day on cvs BT-Ince Sivri and BT-Burdem, respectively. Thus, these cultivars are less suitable for population development of *T. urticae*. These findings indicate that hot cultivars, cvs BT-Ince Sivri and BT-Burdem, are more resistant compared with other cultivars, probably because they have a higher capsaicin content (Table 1; Bozokalfa, 2009). In addition, the results of population development studies on potted plants at the flowering stage supported the findings obtained on leaf discs in Petri dishes. Similarly, both fewer eggs and living mites were found on cvs BT-Ince Sivri and BT-Burdem as well as cv. BT-Burkalem. In contrast, significantly more eggs and mites were found on cvs AHCRI-Çarliston, AHCRI-Kandil Dolma and AHCRI-Yağlık. Cultivars BT-Ince Sivri and BT-Burdem have strong plant habitus, dark green leaves and thin-hot fruits (Table 1). Cultivar BT-Burkalem has similar physical features but the fruits are sweet. Cultivars AHCRI-Çarliston, AHCRI-Yağlık and AHCRI-Kandil Dolma have light green leaves, thin skin and sweet fruit (Table 1). Although there are no differences among morphologically leaf features of these cultivars in terms of physical barriers such as trichome density, cvs AHCRI-Çarliston, AHCRI-Kandil Dolma and AHCRI-Yağlık are more susceptible for *T. urticae.* This difference among the cultivars is likely to be related to concentrations of phenolic compounds and alkaloids (i.e., capsaicin) in these cultivars (Keleş, 2007; Bozokalfa, 2009; Li et al., 2011, 2015) and/or possibly the lack of nutrients needed for fecundity of the mite (van den Boom et al., 2004; Antonious et al., 2006). Although the quantity of capsaicin in the hot cultivars (about 10-15 fold compared with sweet ones) is well known (Bozokalfa, 2009), the phenolic content of the pepper cultivars has not been tested.

In a conclusion, this study showed that some pepper cultivars are less suitable for population growth of *T. urticae*. Among the different pepper cultivars tested, the most suitable were cvs AHCRI-Çarliston, AHCRI-Kandil Dolma and AHCRI-Yağlık. Whereas, cvs BT-Ince Sivri, BT-Burdem and BT-Burkalem were less suitable. In the future, further pepper cultivars should be tested, and their physical and chemical properties related to population development of *T. urticae,* especially leaf phenolic content should be clarified.

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Original article (Orijinal araştırma)

Effect of botanicals and synthetic insecticides on *Pieris brassicae* (L., 1758) (Lepidoptera: Pieridae)

Bitkisel ve sentetik insektisitlerin *Pieris brassicae* (L., 1758) (Lepidoptera: Pieridae) üzerine etkileri

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Summary

Cabbage white butterfly, *Pieris brassicae* (L., 1758) (Lepidoptera: Pieridae), is one of the severe insect pests of cabbage crop which causes remarkable quantitative or qualitative crop losses. The effect of different new chemistry insecticides (thiamethoxam 25% SP, acetamiprid 20% SP and pyriproxyfen 10.8% EC) and four botanical extracts, *Aloe vera* (L.) Burm. f. leaves, grapefruit (*Citrus* × *paradisi* Macfad.) bark, spearmint (*Mentha spicata* L.) leaves and neem (*Azadirachta indica* A. Juss.) leaves, on the feeding behavior, larval growth and mortality of *P. brassicae* was studied at University of Sargodha (Pakistan). The study showed that neem extracts at 7% had a significant effect on growth parameters of *P. brassicae*. The relative growth rate was the lowest (3.05±0.27 mg/mg/d) when neem extract was applied at 7% and higher (8.59±1.38 mg/mg/d) when grapefruit extract was applied at 5% after the control treatment. In comparison to control treatment, relative feed consumption rate of *P. brassicae* and the efficiency of conversion of ingested food decreased to 66 and 58%, respectively, after the application of neem extract at 7%. Neem extracts at 7% caused up to 65% larval mortality. The neem extract (7%) in combination with pyriproxyfen also caused the significant stress on the larvae. So, neem extracts at 7% alone or in combination with insecticides can be used for control of *P. brassicae* in vegetable crops for a safer food supply.

Keywords: Botanical extracts, cabbage white caterpillar, feeding indices, insecticides, *Pieris brassicae*

Özet

Lahana beyaz kelebeği, *Pieris brassicae* (L., 1758) (Lepidoptera: Pieridae), lahanada dikkate değer nicel veya nitel ürün kayıplarına neden olan en önemli zararlılardan biridir. Bu çalışmada Sargodha Üniversitesi (Pakistan)'nde, farklı yeni kimyasal insektisitlerin (thiamethoxam 25% SP, acetamiprid 20% SP ve pyriproxyfen 10.8% EC) ve *Aloe vera* (L.) Burm. f. yaprakları, greyfurt (*Citrus* × *paradisi* Macfad.) kabuğu, nane (*Mentha spicata* L.) yaprakları ve neem (*Azadirachta indica* A. Juss.) yapraklarından elde edilen dört bitki ekstraktının, *P. brassicae*'nin beslenme davranışlarına, larva gelişimine ve öldürücü etkileri araştırılmıştır. Çalışmada *P. brassicae*'nin gelişme parametrelerinde en önemli etkiyi %7'lik neem ekstaktı göstermiştir. Göreceli büyüme oranı %7'lik neem ekstraktı uygulandığında en düşük (3.05±0.27 mg/mg/d) olurken, kontrol uygulamasından sonra %5 oranında greyfurt ekstraktı uygulandığında daha yüksek (8.59±1.38 mg/mg/d) olmuştur. Kontrol uygulamasına kıyasla, *P. brassicae*'nin göreceli yem tüketimi oranı ve yutulan yiyeceklerin dönüşüm verimliliği %7'lik neem ekstraktı uygulandıktan sonra sırasıyla %66 ve %58'e düşmüştür. Neem ekstraktının %7'lik uygulaması, %65'e kadar larva ölümüne sebep olmuştur. Ayrıca, neem ekstraktı (%7) ile pyriproxyfen kombinasyonu, larvalar üzerinde önemli strese neden olmuştur. Bu nedenle, tek başına veya insektisitlerle kombinasyon halinde %7 oranında neem ekstraktı, sebze üretiminde *P. brassicae*'nin kontrolünde daha güvenli bir gıda için kullanılabileceği söylenebilir.

Anahtar sözcükler: Bitkisel ekstraktlar, lahana beyaz kelebeği, beslenme göstergesi, insektisitler, *Pieris brassicae*

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Introduction

The reduction in cabbage production and yield is strongly related with insect herbivory (Tolman et al., 2004). Among many insect herbivores, cabbage white butterfly, *Pieris brassicae* (L., 1758) (Lepidoptera: Pieridae), is a serious insect pest which can heavily infest cabbage crops (Hasan & Ansari, 2010). This cosmopolitan pest causes severe damage to 15 plant species including cabbage, cauliflower, mustard, radish, rape and turnip (Hwang et al., 2008). This pest damages all plant stages in cabbage crops, i.e., seedlings, vegetative and flowering stages (Ullah et al., 2016b). Young caterpillars of *P. brassicae* are gregarious leaf feeders (Hasan & Ansari, 2011). The larvae of *P. brassicae* feed on all plant parts including leaves, twigs, fruits and seeds of cabbage and cauliflower (Siraj, 1999). A single larva can consume up to 74 to 80 cm^2 of leaf and causes serious damage to the host plant (Younas, et al., 2004). *Pieris brassicae* causes more than 40% yield loss in different vegetable crops in India annually (Ali & Rizvi, 2007). Extreme infestations of *P. brassicae* completely destroy the plant foliage and ultimately kill the plant (Hasan & Ansari, 2010).

Usually, farmers are dependent on synthetic insecticides to suppress *P. brassicae* infestations in cabbage crops (Ullah et al., 2016b). The frequent use of synthetic chemicals has resulted in resistance to these insecticides. Consequently, it is now difficult to control this pest using existing synthetic insecticides (Thomas, 1999). The injudicious use of insecticides has led to many biological and environmental problems, such as toxicity to non-targeted plants, insects and other organisms, environmental degradation, and human health hazards (Badshah et al., 2015; Zahid et al., 2016). Given the destructive nature of synthetic chemical insecticides, it is critical to develop safe and environment friendly resources for better and safer pest management (Rangad et al., 2014).

Plant based insecticides (botanicals) are well known for their insecticidal and insect repellent characters and lower toxicity to the environment (Zahid et al., 2016). The use of botanicals for control of *P. brassicae* can be effective, and safer than synthetic chemicals due to their lower residual effect on non-target organisms (Endersby & Morgan, 1991; Raguraman & Kannan, 2014). Crude plant extracts may alter the behavioral and physiological aspects of insects, which may result in a reduction of insect pest infestations in crops (Sharma & Gupta, 2009). Botanical insecticides are highly preferred because they are less expensive and easily available from commonly grown plants (Salim & Abed, 2015). Furthermore, botanicals usually have a slight impact on the biological activity of natural enemies and they can be safely combined with other control practices (Zahid et al., 2016). Herbivore nutritional indices are negatively affected by the application of botanicals or plant oils with a reduction in feeding and growth indices after the treatment (Huang et al., 2000; Pavela et al., 2009; Zapata et al., 2009; Taghizadeh et al., 2014). *Reynoutria* plant extracts can reduce the nutritional indices of the last instars of *Spodoptera littoralis* (Boisduval, 1833) larvae (Pavela et al., 2008). Plant extracts can lower the relative growth rate (RGR), relative consumption rate (RCR) and efficiency of conversion of ingested food (ECI), and lead to retarded larval growth and smaller pupae. These effects result into lowered fecundity and longevity of the adult insects with susceptibility to certain diseases and natural enemies (Khosravi et al., 2010).

Botanicals are comparatively safer to the parasitoids like *Cotesia glomerata* (L., 1758), which voraciously parasitizes the *P. brassicae* caterpillar (Ullah et al., 2016c), when compared to synthetic insecticides (Yi et al., 2016). Botanicals are even less toxic to the developing larvae of parasitoids in the host insects exposed to them (Tang et al., 2002). In addition, the majority of botanicals contain a diverse mix of active compounds and thus do not result in pest resistance (Pavela, 2011).

Given the above problems of chemical use in food crops, the current study aimed to evaluate the efficacy of different new chemistry insecticides and some plant extracts alone and in combination on *P. brassicae* mortality and growth performance.

Material and Methods

The experiment was conducted in Entomology Laboratory, University College of Agriculture, University of Sargodha, Pakistan in 2016. Caterpillars of *P. brassicae* were collected from the cabbage fields near the university and brought to the laboratory for rearing under controlled conditions at 25±2ºC and 65±2% RH. For the maintenance of the insect culture, fresh cabbage leaves (*Brassica oleracea* L.) were provided daily. Second instar caterpillars, from the next generation, were used for the further experiment.

Insecticides and plant extracts

Three commercially available synthetic insecticides, thiamethoxam 25% SP, acetamiprid 20% SP, pyriproxyfen 10.8% EC (Arysta Life Sciences, Karachi, Pakistan), at field recommended doses (0.024, 0.06 and 0.05 g/100 ml, respectively) and four plant extracts, *Aloe vera* (L.) Burm. f. leaves, grapefruit (*Citrus* × *paradisi* Macfad.) bark, spearmint (*Mentha spicata* L.) leaves and neem (*Azadirachta indica* A. Juss.) leaves at 5 and 7%, were used.

Preparation of plant extracts

The insecticide free plant material of the selected plant species was collected from the field and washed with distilled water. The required plant parts were sun dried. The dried plant materials were ground to powder with an electrical grinder. Five g of each plant powdered samples were placed in conical flasks (250 ml) along with 100 ml of distilled water. The flasks were placed on the heating magnetic stirrer (AM4, Velp Scientifica, Usmate, Italy) for 4 h. The solid residues were removed using muslin cloth. The liquid extracts were then filtered (Whatman No. 1) using a vacuum suction assembly (Sparmax, Taipei, Taiwan). The extracts were dried in a rotary evaporator (HB Digital, Heidolph, Schwabach, Germany) at 60ºC under vacuum. These dried plant extracts were brought to constant weight in hot air oven (60ºC). The extracts were stored at 5ºC until used. Concentrations of 5 and 7% were prepared in water for experimental evaluation.

Assay of feeding indices and mortality

Fresh cabbage leaves were cut according to fit Petri dishes. A leaf-dip bioassay was used to check the efficacy of each chemical against *P. brassicae* caterpillars. The cut cabbage leaves were dipped in 100 ml prepared solutions of the diluted botanicals and synthetic insecticides for 10 s and air dried for 30 min on filter papers before offering this leave to second instar caterpillars under ventilation. Leaves and *P. brassicae* caterpillars were weighed before transferring them into the experimental arena. The experiment was replicated four times with five larvae in each replicate. Water was used as a control treatment.

Data was recorded at 12-h intervals after the application of insecticides and botanicals. Caterpillar, fresh diet and feces weights for each replicate was recorded to estimate the feeding indices for 4 d. A high precision balance (±0.1 mg) was used to weigh all materials. The mortality of *P. brassicae* caterpillars was also recorded to check the toxicity of the treatments. The corrected percent mortality of *P. brassicae* caterpillars was obtained using Abbott's formula (Abbott, 1925). In a subsequent experiment, the most effective synthetic insecticide (pyriproxyfen) and botanical (neem 7%) were combined at ratios of 1:2 and 2:1 (insecticide: botanical) to evaluate the efficacy of these combinations. Growth indices parameters were calculated by the formulas of Ullah et al. (2016a).

Relative growth rate (RGR) = B-A/B x d

Relative consumption rate (RCR) = D/B x d

Efficiency of conversion of ingested food (ECI) = B/D x 100

Where, A is the mean weight (g) of the insects on the fourth day, B is the original mean weight of insects (g) and D is the food biomass ingested (g) per insect.

Data analysis

The mortality data was log-transformed to achieve normality before analysis. RGR, RCR, ECI and mortality data were subjected to analysis of variance and Tukey's HSD test was used to compare the means with Minitab 16.1 software to check the direct and indirect effects of the botanicals and synthetic chemicals on the larval performance. The original mean values are given below.

Results

The treatments had significant effects (P < 0.001) on RGR (F = 84.9, df = 13 and P < 0.001), RCR (F = 53.7, df = 13 and P < 0.001) and ECI (F = 64.5, df = 13 and P < 0.001) of *P. brassicae* (Table 1). Time interval was only significant for RCR and ECI. However, the interaction between treatment and time interval was highly significant for all parameters (F = 3.81, 2.32 and 2.68 for RGR, RCR and ECI, respectively, at P < 0.001 and df = 39).

Table 1. Growth indices of *Pieris brassicae* at different time intervals after the application of different synthetic insecticides and botanicals

			RGR		RCR		ECI	
Source	DF	MS	F value	P value	F value	P value	F value	P value
Treatments (A)	13	119.71	84.92	<0.001	53.67	<0.001	64.48	<0.001
Time (B)	3	0.48	0.34	>0.05	4.75	<0.05	21.84	<0.001
A x B	39	5.37	3.81	<0.001	2.32	<0.001	2.68	<0.001
Residual	168	1.41						
Total	223							

P < 0.05, significant; P < 0.001, highly significant; P > 0.05, non-significant; RGR, relative growth rate (mg/mg body weight/d); RCR, relative consumption rate (mg/mg body weight/d); ECI, efficiency of conversion of ingested food (%); DF, degree of freedom; MS, mean square.

The percent mortality data showed that the neem extract at 7% was lethal to *P. brassicae* caterpillars with the maximum mortality of 65% (Figure 1). Similarly, the combined action of pyriproxyfen with 7% neem extract also caused significant mortality of *P. brassicae* caterpillars. The insecticide and botanical combinations (1:2 and 2:1) caused 60.9% and 58.8% mortality of *P. brassicae* caterpillars, respectively. The least effective botanical was grapefruit bark extract at 5%, which caused only 26.6% mortality of *P. brassicae* caterpillars.

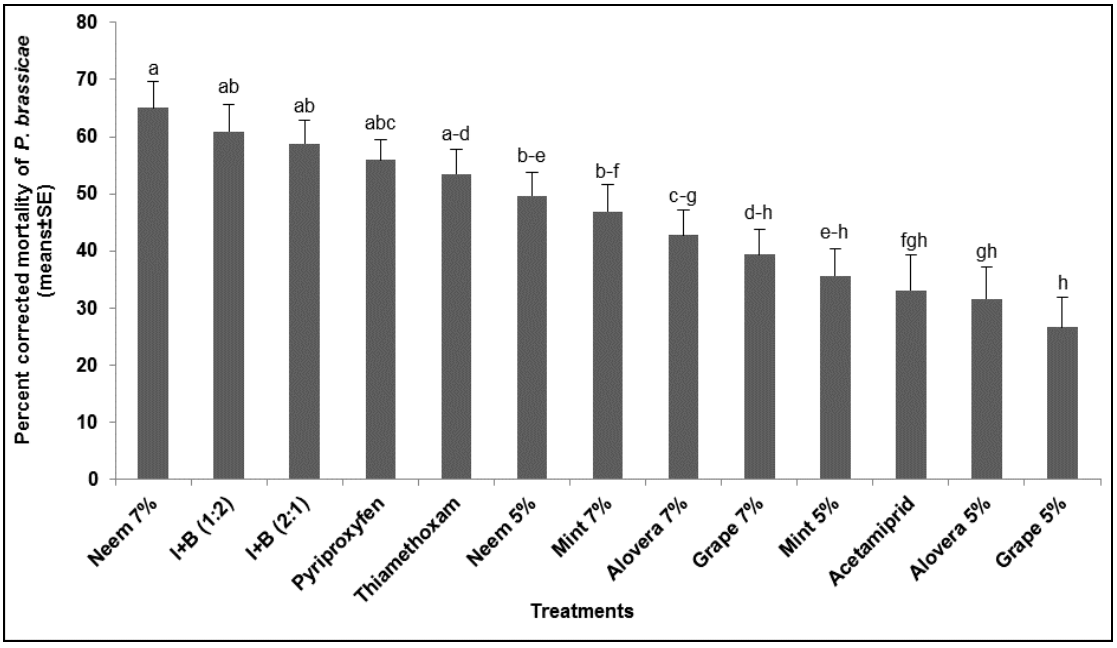

Figure 1. Percent mortality of *Pieris brassicae* after the application of different botanicals and synthetic chemicals (alone and in combination) [I+B, insecticide (pyriproxyfen) + botanical (neem 7%)], means sharing similar letters are not significantly different from each other.

The results indicated that neem leaf extract (7%) also had significant negative effects on growth parameters of *P. brassicae* (Figures 2 to 4). The RGR was the lowest (3.05±0.27 mg/mg body weight/d) with 7% neem extract application and the highest (8.59±1.37 mg/mg body weight/d) with grapefruit bark extract at 5% (Figure 2). Similar effects of 7% neem extracts were seen for ECR and ECI (Figures 3 & 4). In comparison to control treatment, the relative consumption rate of *P. brassicae* was lowered by 66% with the application of 7% neem extract (Figure 3). Similarly, the ECR decreased by 57.6% in the same treatment (Figure 4). Overall, RGR, RCR, and ECI were higher in control treatment. Both combinations of pyriproxyfen and 7% neem extract showed significant negative effects on the growth of *P. brassicae*.

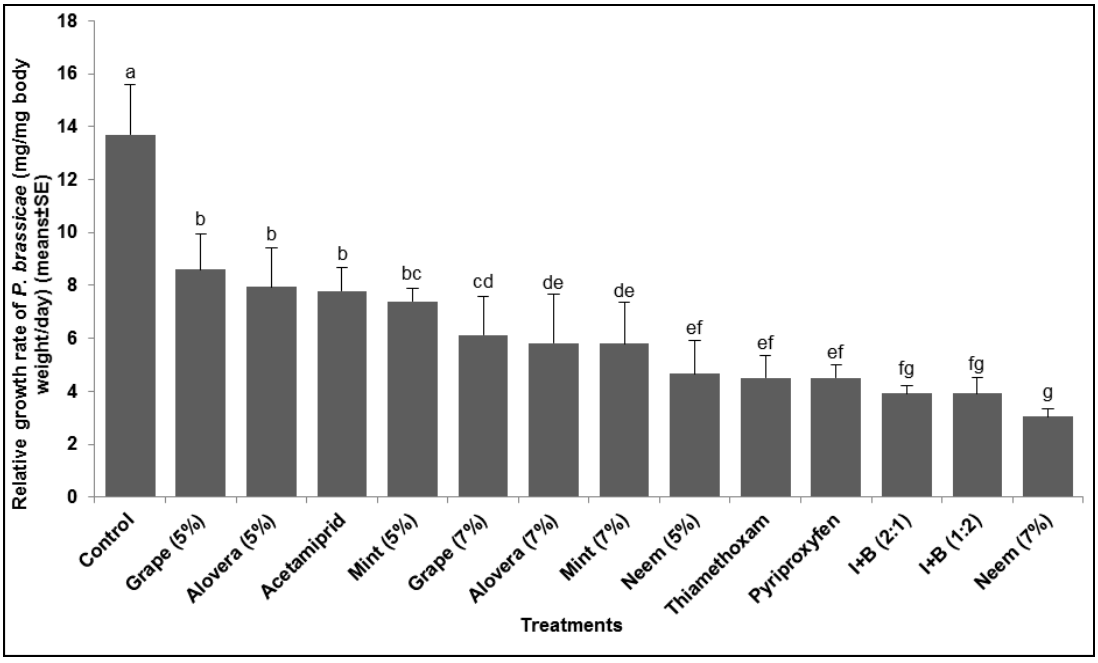

Figure 2. Relative growth rate (RGR) of *Pieris brassicae* after the application of different botanicals and synthetic chemicals (alone and in combination) [I+B, insecticide (pyriproxyfen) + botanical (neem 7%)], means sharing similar letters are not significantly different from each other.

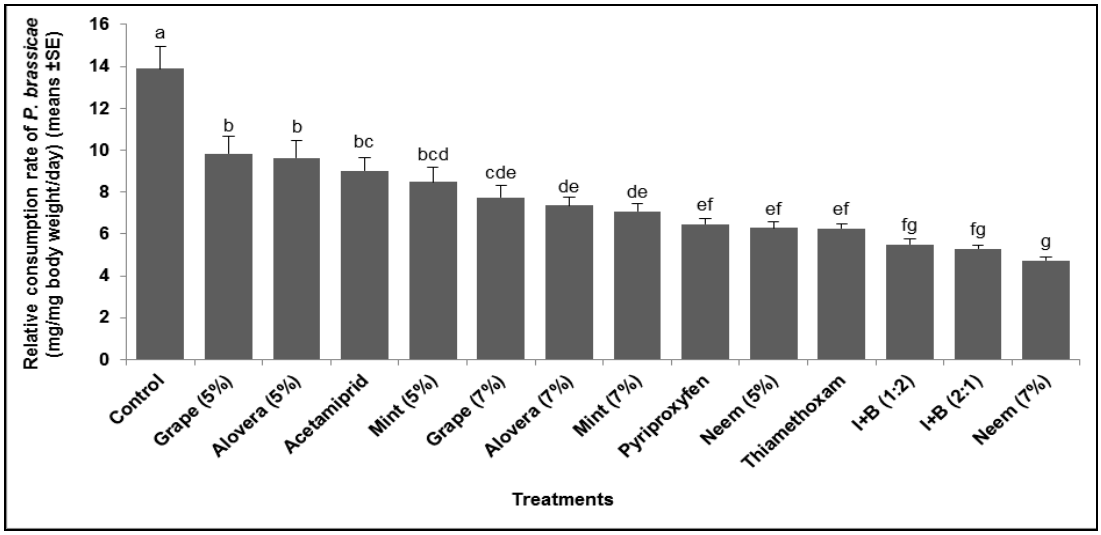

Figure 3. Relative consumption rate of *Pieris brassicae* after the application of different botanicals and synthetic chemicals (alone and in combination) [I+B, insecticide (pyriproxyfen) + botanical (neem 7%)], means sharing similar letters are not significantly different from each other.

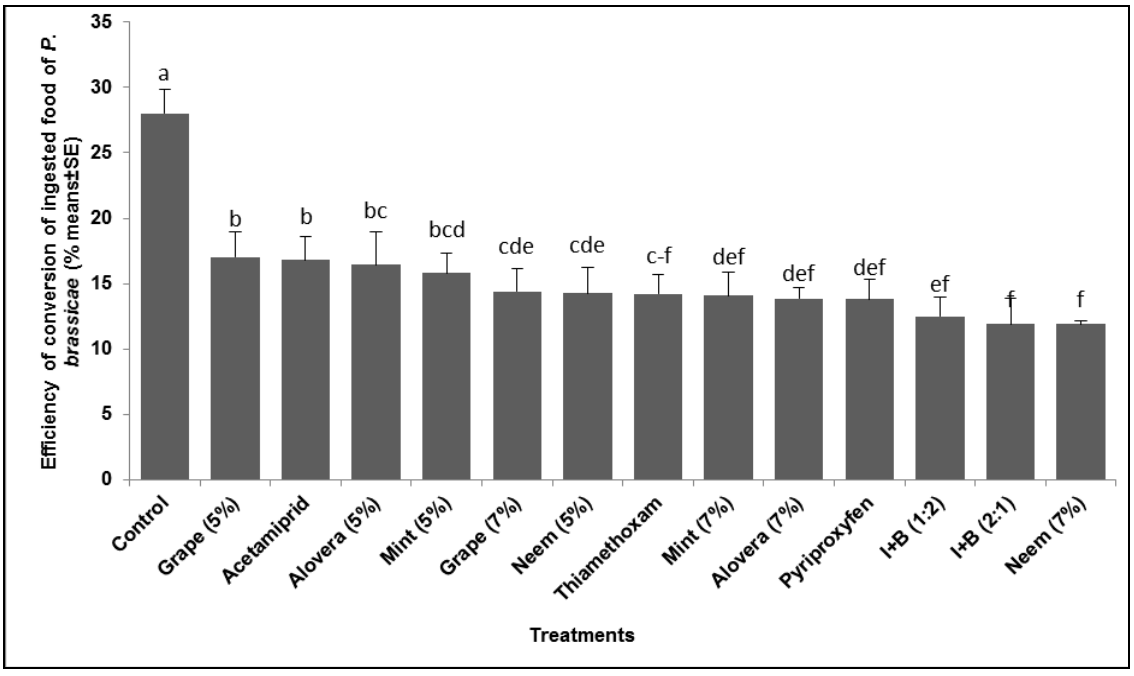

Figure 4. Efficiency of conversion of ingested food of *Pieris brassicae* after the application of different botanicals and synthetic chemicals (alone and in combination) [I+B, insecticide (pyriproxyfen) + botanical (neem 7%)], means sharing similar letters are not significantly different from each other.

Discussion

Natural plant products or botanicals can be an excellent substitute for synthetic pesticides by reducing the health and environment hazards associated with synthetics (Mohan et al., 2011). Application of natural insecticides could be a promising approach to decrease the overall use of synthetic insecticides (Pavela, 2007; Dayan et al., 2009).

In neem extracts, methyl petroselinate, hexadecamethyl cyclooctasiloxane, methyl isoheptadecanoate, oxalic acid, 2-ethylhexyl tetradecyl ester, butyl palmitate, heptacosane 7-hexyl, and eicosane are the major biologically active chemical compounds (Hossain et al., 2013). *Mentha* spp. mainly contain 1,8-cineole, limonene, carvone, linalyl acetate, linalool, menthone, menthol, piperitenone oxide and methyl acetate (Gracindo et al., 2006). The important biologically active compounds in *A. vera* are aloe-emodin, anthranol, aloetic-acid, barbaloin, emodin, isobarbaloin and esters of cinnamic acid (Moghaddasi & Verma, 2011). The grapefruit bark contains many active biocompounds including flavonoids, saponin, alkaloids, cardenolide aglycone, cardiac glycoside, tannin and terpenoids (Olabinri et al., 2014). Among these plant metabolites, many natural plant products are known for their insecticide, fumigant, repellent and antifeedant actions (Shaaya et al., 1997).

Botanicals possess insecticidal properties due to physical action and muscular poisoning. Forty-seven plant species have been listed for their toxicity to different insect species (Talukder, 2006). In our study, neem leaf extracts at 7% caused the highest larval mortality of the tested botanicals and synthetic chemicals. Neem extracts contain about 100 bioactive compounds. Among these, triterpenes (limonoids) are the most important, causing 90% of the effects on most of the insect pests. It has already been reported that neem extracts have useful insecticidal properties against many lepidopteran insect pests. Larval and nymphal mortalities in many other insect groups have also been reported (Warthen, 1989; Campos et al., 2016). Although, food consumption was lower on leaves treated with plant extracts, considerable larval mortality was recorded in all treatments, with the toxic effect of plant extracts greatest for neem extract at 7%. This shows that higher concentrations of plant extracts can cause high larval mortality even with only a small amount of treated food being consumed (Leatemia & Isman, 2004). *Capparis spinosa* L. leaf extracts caused 100% mortality of *S. littoralis* larvae through antifeeding as well

as insecticidal effects (Ladhari et al., 2013). Similarly, *Allium indica* L. (garlic) and *Melia azedarach* L. extract effectively reduced *P. brassicae* infestations in cabbage crops (Przybyszewski, 1993; Khan & Siddiqui, 1994; Grisakova et al., 2006 and Sharma & Gupta, 2009). In the current study, neem leaf extract at 7% gave significant mortality of *P. brassicae* as well as feeding disturbances to the caterpillars and reducing the growth and development of insect larvae.

Any substance reducing the food ingestion by insects is categorized as an antifeedant and generally has adverse effects on insect feeding behavior (Hummelbrunner & Isman, 2001), which was exhibited by our extracts, especially neem leaf extract at 7%, which had a significant antifeeding effect. Antifeedants can be described as allomone inhibiting insect feeding and do not kill the exposed insect pests directly but rather, they affect their developmental potential considerably by acting as a phagorepellent (Lakshmanan et al., 2012). Many botanicals offer antifeedant as well as toxic effects against *Spodoptera litura* (Fabricius, 1775) (Ulrichs et al., 2008; Arivoli & Samuel, 2012). Similarly, extracts of *Trichilia prieureana* A. Juss*., Trichilia roka* (Forssk.) Chiov. and *Trichilia connaroides* (Wight & Am.) Bentv. seeds gave high levels of feeding inhibition against *Spodoptera frugiperda* (J. E. Smith, 1797) (Mikolajczak & Reed, 1987). Leaf extracts of *Justicia vasica* L. and *C. spinosa* also caused strong feeding inhibition in *S. littoralis* (Sadek, 2003; Ladhari et al., 2013). Charleston et al. (2005) also reported aqueous extracts of *M. azedarach* and *A. indica* as antifeedants and growth inhibitors for the larvae of *Plutella xylostella* (L., 1758). From the above studies, it is inferred that the antifeeding effects of plant extracts can lead to slower growth of insect herbivores by reducing the insect growth parameters, which was also evident in our results. The botanicals alone and neem leaf extract in combination with pyriproxyfen caused significant negative effects on the food consumption and growth of the cabbage white butterfly larvae. So, certain plant part extracts can be used as insect repellents, antifeedants and growth inhibitors under certain conditions to produce toxin free food products.

However, the combination of pyriproxyfen with 7% neem extract showed lower mortality as compared to the application of 7% neem extract alone. Also, there was no significant difference in mortalities of *P. brassicae* larvae with the different ratios (1:2 and 2:1) of insecticide and botanical. The lack of synergistic effect might be due to detoxification mechanisms leading to inactivation of some metabolites. In this context, it is possible that the toxicity of pyriproxyfen and neem extract was reduced because the combination caused degradation of one or more constituents in the mixture (Yi et al., 2012).

It is also important that botanicals and pyriproxyfen have different mechanisms of action. For instance, pyriproxyfen is a juvenoid which disturbs the insect growth and causes mortality at a younger age (Ohba et al., 2013) and differential toxicities of insecticides depend on larval age, concentrations and exposure periods (Yue et al., 2003). While, neem is an antifeedant and toxicant for many insects, slowly disrupting insect growth (Morgan, 2009). The main mode of action of chemicals determines the sensitivity of exposed organisms (Sánchez-Bayo, 2012). However, botanical and insecticide synergism is important for control of insect pests. Efficacies of mixtures will depend on the insect species, strain and as well as the concentration ratio (Taillebois & Thany, 2016).

The larvae achieve faster growth when their food consumption and efficiency of conversion of ingested food is high. However, food ingestion, and its assimilation and conversion into energy, differs in accordance with the quality of the food. Feeding and growth indices are directly linked with the quality of food supplied. In this experiment, increased concentrations of plant extract greatly decreased RGR, RCR, and ECI parameters of the cabbage caterpillars. In fact, plant extract application led to reduced food quality with decreased the tendency of insects to consume food resulting in a lower growth rate. With the application of botanicals, the consumption of food can be reduced to decrease herbivore fitness. This feeding and growth relationship with food treated with botanicals can be utilized to achieve improved crop production.

Conclusions

Useful insecticidal and repellent properties of neem leaf extracts have been shown in the study, which indicates it can be used to suppress the cabbage white butterfly caterpillars in food crops. In addition, insecticide-botanical combinations can provide cost effective solutions for crop problems and can be used as an important part of integrated pest management strategies. However, achieving more acute interactions, the synergistic mechanism between insecticides and the botanicals should be the focus in future research.

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Original article (Orijinal araştırma)

A new mite species of the genus *Favognathus* Luxton, 1973 (Acari: Cryptognathidae) from Turkey

Türkiye'den *Favognathus* Luxton, 1973 (Acari: Cryptognathidae) cinsinin yeni bir akar türü

Mustafa AKYOL[1*] **Kamil KOÇ[1]**

Summary

A new species of the genus *Favognathus* Luxton, 1973, *Favognathus manisaensis* sp. nov. is described and illustrated based on the females and males collected from soil and litter under *Pinus* sp. and *Cornus* sp. in Manisa and İzmir Provinces, Turkey between 2011 and 2013. The new species is closely related to *Favognathus distortus* (Kuznetsov, 1974) and *Favognathus bafranus* Doğan, 2008. A key to all known species of *Favognathus* from Turkey is provided.

Keywords: Acari, Cryptognathidae, *Favognathus manisaensis* sp. nov., new species, Turkey

Özet

2011- 2013 tarihleri arasında Manisa ve İzmir illerinden *Pinus* sp. ve *Cornus* sp. altından alınan toprak ve döküntü örneklerinden *Favognathus* Luxton, 1973'ın yeni bir türü olan *Favognathus manisaensis* sp. nov. dişi ve erkek bireyleri üzerinden tanımlanmış ve şekilleri çizilmiştir. Yeni tür, kendisine yakın olan *Favognathus distortus* (Kuznetsov, 1974 ve *Favognathus bafranus* Doğan, 2008 türleri ile karşılaştırılmıştır. Türkiye'den bilinen *Favognathus* cinsine ait türler için teşhis anahtarı düzenlenmiştir.

Anahtar sözcükler: Acari, Cryptognathidae*, Favognathus manisaensis* sp. nov., yeni tür, Türkiye

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Introduction

Mites of the superfamily Raphignathoidea are biological control agents of spider mites, eriophyids, and scale insects of agricultural importance. Most of the species are free-living predators, but a few are phytophagous, feeding on moss, and symbionts/parasites of insects (Fan & Zhang, 2005).

The Cryptognathidae was erected by Oudemans (1902) with *Cryptognathus* Kramer as type genus. Members of this family are recognized by the presence of a protective hood anterior of the propodosoma and an extremely extendable gnathosomal base (Doğan, 2008).

The genus *Favognathus* Luxton, 1973 belongs to the family Cryptognathidae and is one of 11 families of the superfamily Raphignathoidea and includes three genera: *Cryptognathus* Kramer, 1879, *Favognathus* and *Cryptofavognathus* Doğan & Dönel, 2010. *Cryptognathus* type species, *Cryptognathus legena* Kramer, 1879, was originally described by Kramer (1879). Kramer's original description is largely insufficient for reliably distinguishing this species from all other species of *Cryptognathus*, but Luxton (1972) provided a brief redescription of the type specimen, which was accidentally destroyed in the process, and designated neotypes. Luxton (1973) established two new subgenera in the family Cryptognathidae: *Cryptognathus* (*Favognathus*) Luxton, 1973 and *Cryptognathus* (*Cryptognathus*) Luxton, 1973. Later, Luxton (1987) raised them to generic status. Doğan & Dönel (2010) proposed a new genus *Cryptofavognathus* Doğan & Dönel, 2010 based on *Cryptofavognathus afyonensis* (Koç & Akyol, 2004) as type species and a new species, *Cryptofavognathus anatolicus* Doğan & Dönel, 2010. The genus *Favognathus* is cosmopolitan. Mites of this genus are generally collected from soil, grassy soil, litter, mosses, lichens and bark. Currently, the genus *Favognathus* comprises 41 species occurring in all zoogeographical regions (Doğan, 2008; Khanjani & Ueckermann, 2008; Bagheri et al., 2015).

Ten species of *Favognathus* – *Favognathus acaciae* Doğan & Ayyıldız, 2004, *Favognathus amygdalus* Doğan & Ayyıldız, 2004, *Favognathus bafranus* Doğan, 2008, *Favognathus cucurbita* (Berlese, 1916), *Favognathus erzurumensis* Doğan & Ayyıldız, 2002, *Favognathus luxtoni* Koç & Ayyıldız, 1999, *Favognathus turcicus* Koç & Ayyıldız, 1999, *Favognathus kamili* Dönel & Doğan, 2011, *Favognathus distortus* (Kuznetsov, 1974) and *Favognathus izmirensis* Akyol, 2010 – have been reported from Turkey (Koç & Ayyıldız, 1999; Doğan & Ayyıldız, 2002; 2004; Koç & Akyol, 2004; Doğan, 2008; Akyol, 2011; Dönel & Doğan, 2011). In this paper, a new species, *Favognathus manisaensis* sp. nov. is described and illustrated based on female and male specimens. A key to the species of *Favognathus* from Turkey is also provided.

In Turkey, raphignathoid mite fauna is not known for many provinces. In order to contribute to the raphignathoid mite fauna in Turkey, we are continuing our sampling studies in provinces of the Aegean Region and this study is one of them.

Material and Methods

Collecting

The soil and litter samples were taken from *Pinus* sp. and *Cornus* sp. in Manisa and İzmir Provinces between 2011 and 2013. They were brought to the laboratory in plastic bags and extracted in Berlese funnels for 7 days. Mites were collected in vials filled with 70% ethanol.

Slide mounting

Mites were cleared in lactophenol solution and mounted in Hoyer's medium on microscopic slides. These slides were labeled with the collecting data (Akyol, 2007) and deposited in the Zoological Museum of Manisa Celal Bayar University (CBZM), Manisa, Turkey.

Illustrations and measurements

Specimens were examined and drawn using a Nikon microscope with 100 magnifications with a camera lucida. All measurements are given in micrometers (µm) with the holotype measurements followed by the minimum and maximum values of paratypes in parentheses. Chelicerae were measured from basal articulations to tips of movable digits. Palps were measured from the base of the trochanters to the tips of palp tarsi. Idiosomal lengths were measured from the anterior to the posterior margins

(including hood and anal covers). Idiosomal widths were measured across maximum width of the idiosoma between leg II and III. Setae and solenidia were measured from alveoli to tips. Legs were measured from the base of the trochanters to tips of claws.

Terminology

Terminology follows that of Luxton (1973). Dorsal setal and leg setal designations follow Kethley (1990) and Grandjean (1944), respectively.

Results and Discussion

In this paper, a brief definition of the genus species description, type materials, remarks and key to the Turkish species of *Favognathus* are given.

Taxonomy

Family Cryptognathidae Oudemans, 1902

Cryptognathidae Oudemans, 1902: 59. Type genus: *Cryptognathus* Kramer, 1879:156.

Genus *Favognathus* Luxton, 1987

Favognathus Luxton, 1987: 113. Type species: *Cryptognathus cucurbita* Berlese, 1916, was original designation by Berlese (1916).

Cryptognathus (*Favognathus*) Luxton, 1973: 62. Type species: *Cryptognathus cucurbita* Berlese, 1916, raised to genus by Luxton (1987).

Diagnosis

This genus can be defined by the wedge-shaped prosternal apron at base of gnathosoma, which is ornamented with dimples, and the presence of one or two pairs of aggenital setae and two pairs of genital setae.

Favognathus manisaensis sp. nov. (Figures 1 & 2)

Diagnosis (female and male)

The anterior margin of the hood smooth, hood with 7-8 dimples in each longitudinal row, dorsum completely reticulated and covered with punctations and faint striae, rosette patterns present, dorsum with two pairs of rosettes, prosternal apron with 12-17 faveolae, sternocoxal region non-porous and faintly striated, venter partly reticulated and with striae, genu II with solenidion *κ*, number of leg setae tarsi: 17-14-10-10 (including solenidia) and *tc* on tarsus II dissimilar.

Female (n = 8): Holotype – body length (including hood and anal covers) 301 (300-327) and width 173 (161-190).

Gnathosoma (Figure 1g): Palp 90 (76-95), chelicerae 102 (87-111), palp tarsus with four eupathidia, four setae and one solenidion, and tibia with three, genu with two and femur with three setae.

Dorsum (Figure 1a): Length of hood 72 (64-77), anterior margin of the hood smooth 7-8 dimples in each longitudinal row; dorsum completely reticulated and covered with punctations and faint striae; dorsal shield with 11 pairs of simple setae; a pair of eyes and a pair of postocular bodies laterally between setae *sci* and *sce*; two pairs of slit-like cupules (*ia* and *im*); cluster of cells associated with setae c_1 and d_1, these rosette patterns consist of three or seven cells; and surface reticulum in region of setae f_1, h_1 and h_2 not apparent. Dimensions of dorsal setae are as follows: *vi* 14 (15-21), *ve* 22 (21-28), *sci* 27 (21-28), *sce* 29 (28-31), c_1 39 (35-42), d_1 41 (35-42), e_1 41 (36-42), e_2 36 (33-42), f_1 37 (36-42), h_1 33 (30-35) and h_2 31 (19-29); and distances between setae *vi-vi* 34 (29-35), *vi-ve* 9 (10-15), *ve-ve* 34 (34-42), *ve-sci* 7 (9-12), *sci-sci* 48 (48-63), *sce-sce* 97 (97-103), *sce*-c_1 16 (16), c_1-c_1 65 (58-72), d_1-d_1 111 (114-115), d_1-e_1 42 (41-47), e_1-e_1 76 (74-86), e_1-e_2 18 (17-21), e_2-e_2 97 (97-106), e_1-f_1 53 (49-53), f_1-f_1 29 (24-34), f_1-h_1 26 (25-28), h_1-h_1 12 (13-14), h_1-h_2 28 (26-29) and h_2-h_2 70 (72-74).

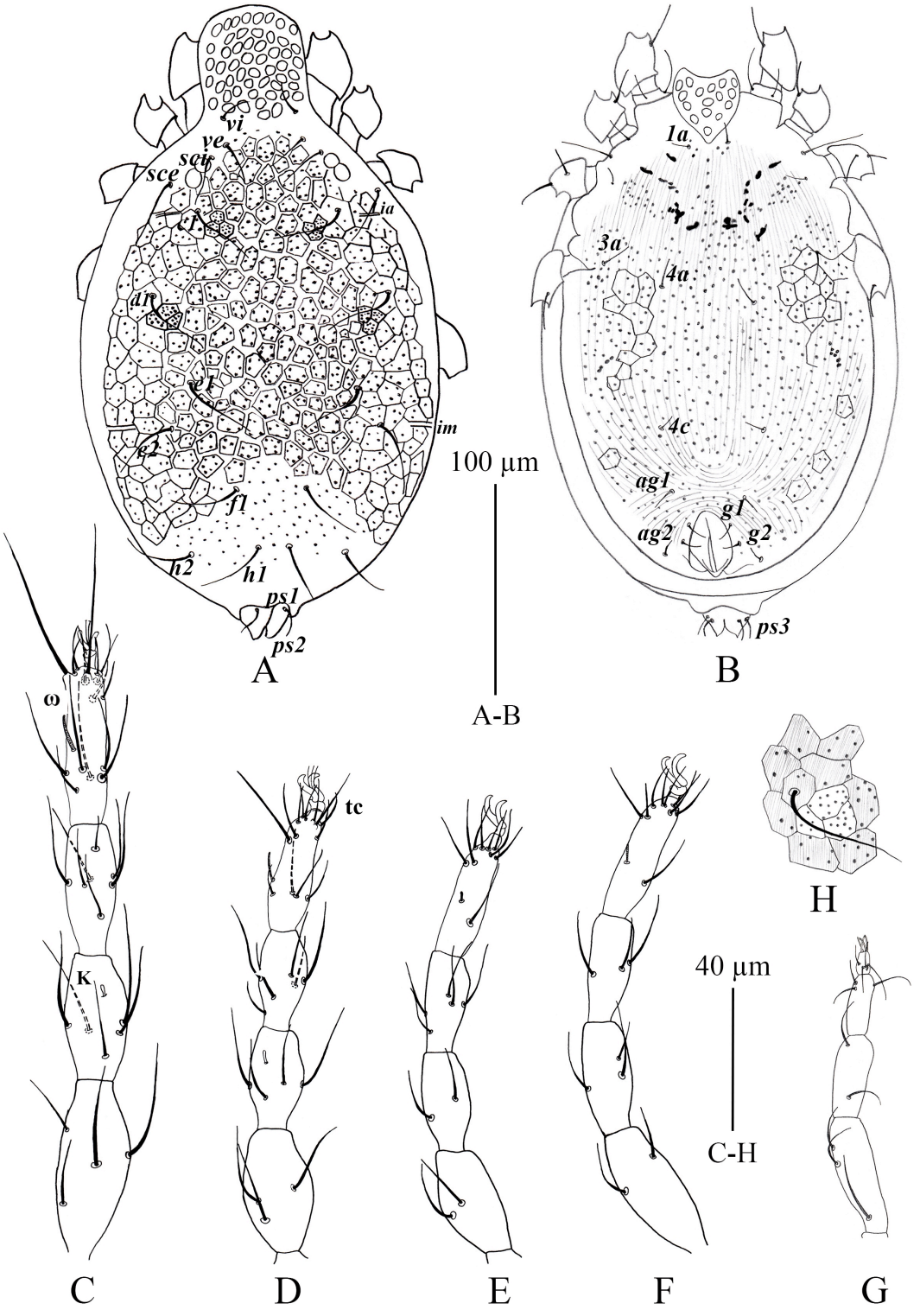

Figure 1. *Favognathus manisaensis* sp. nov. (female): A. dorsal view of idiosoma, B. ventral view of idiosoma, C. leg I, D. leg II, E. leg III, F. leg IV, G. palp, and H. setae d_1 and rosette patterns.

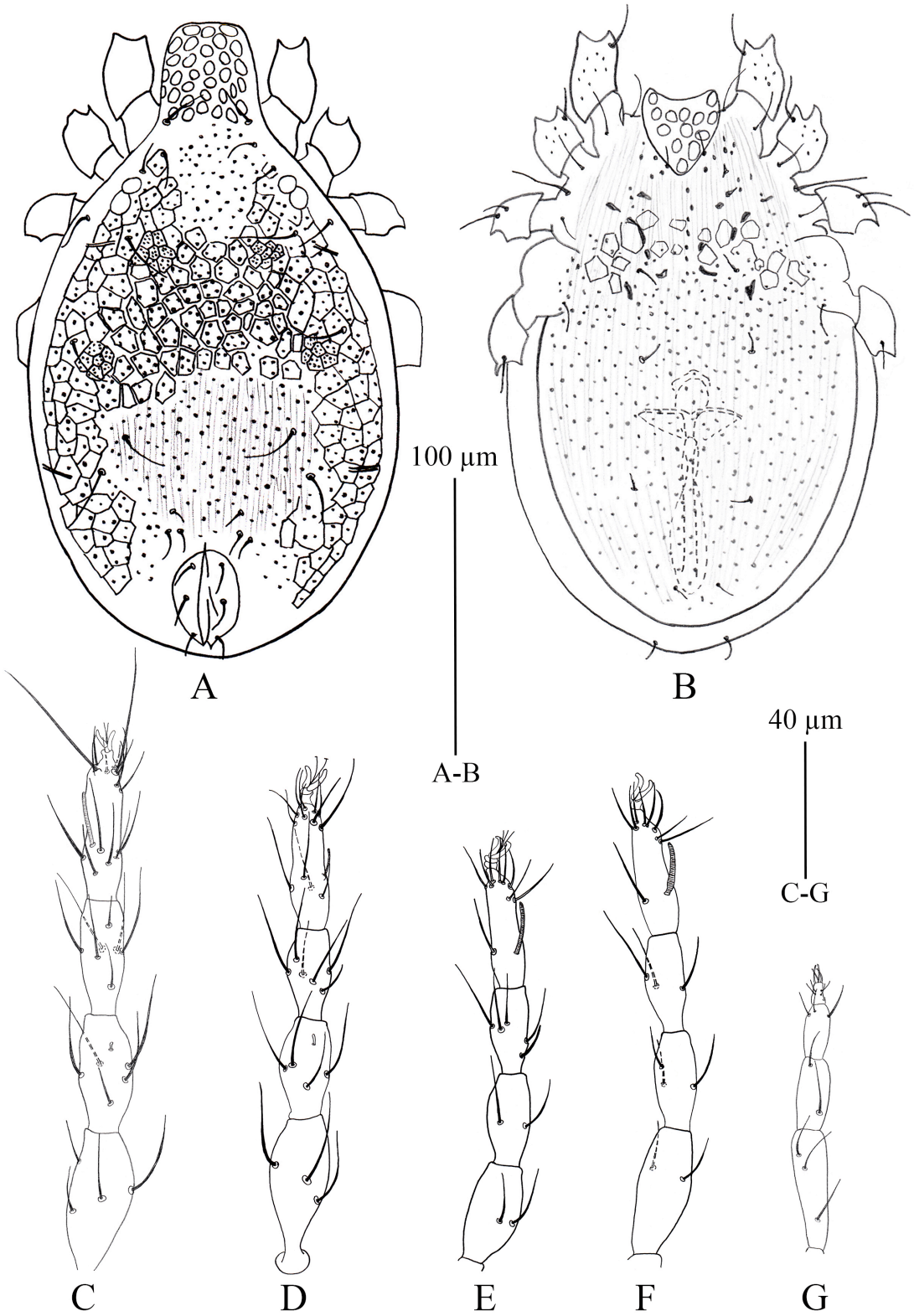

Figure 2. *Favognathus manisaensis* sp. nov. (male): A. dorsal view of idiosoma, B. ventral view of idiosoma, C. leg I, D. leg II, E. leg III, F. leg IV, and G. palp.

Venter (Figure 1b): Prosternal apron wedge-shaped, with 12-17 faveolae; venter covered with punctations; sternocoxal area nonporous and faintly striated; coxal region posterolaterally with reticulations; venter with four pairs of ventral setae *1a* 14 (14-15), *3a* 21 (15-17), *4a* 17 (14-18), *4c* 15 (13-19), genital opening with two pairs of genital setae g_1 14 (13-15), g_2 15 (13-16) and two pairs of aggenital setae ag_1 14 (12-13), ag_2 11 (8-14); distances between setae *1a-1a* 18 (17-20), *3a-3a* 34 (37-40), *4a-4a* 44 (43-54), *4c-4c* 48 (44-53), ag_1-ag_1 32 (25-34), ag_2-ag_2 43 (43-49); and anal opening terminal, with three pairs of pseudanal setae ps_1 14 (14-16), ps_2 13 (12-13) and ps_3 11 (10-12).

Legs (Figure 1c-f): Length of legs I-IV (from base of trochanter to tip of tarsal claw): 210 (196-216), 163 (144-168), 153 (148-166) and 175 (168-185), respectively. Setal formulae of legs I-IV: coxae 2-1-2-1, trochanters 1-1-2-1, femora 4-3-2-2, genua 5(+κ)-4(+κ)-2-3, tibiae 5(+φρ, +*ω*)-5(+φρ)-4(+φρ)- 3 and tarsi 15(+φρ, +ω)-12(+φρ, +ω)-9(+ω)-9(+ω). Setae *tc* on tarsi II dissimilar.

Male (n = 3) (Figure 2): Body length (including hood and anal covers) 230 (218-223) and width 138 (133-136). The male is smaller than the female.

Gnathosoma (Figure 2g): Palp 87 (75-76), chelicerae 74 (68-73), palp tarsus with four eupathidia, four setae and one solenidion, and tibia with three, genu with two, and femur with three setae.

Dorsum (Figure 2a): Length of hood 52 (55-57), anterior margin of the hood smooth 7-8 dimples in each longitudinal row; dorsum covered with punctations and faint striae, with complete reticulations; dorsal shield with 11 pairs of simple setae; a pair of eyes and a pair of postocular bodies laterally between setae *sci* and *sce*; two pairs of slit-like cupules (*ia* and *im*); and cluster of cells associated with setae c_1 and d_1; surface reticulum in region of setae e_1, e_2, f_1, h_1 and h_2 not apparent (Figure 2a). Dimensions of dorsal setae are as follows: *vi* 14 (15), *ve* 14 (16-17), *sci* 12 (14-16), *sce* 20 (17-23), c_1 28 (22-25), d_1 28 (25-26), e_1 25 (24-26), e_2 20 (18-22), f_1 7 (6-9), h_1 7 (6-8) and h_2 11 (10-11); and distances between setae *vi-vi* 29 (24-29), *vi-ve* 9 (6-7), *ve-ve* 32 (26-28), *ve-sci* 7 (7-10), *sci-sci* 45 (40-43), *sce-sce* 90 (78-84), *sce*-c_1 11 (9-14), c_1-c_1 59 (53), d_1-d_1 97 (83-91), d_1-e_1 39 (35-37), e_1-e_1 60 (52-54), e_1-e_2 16 (10-13), e_2-e_2 75 (67-75), e_1-f_1 33 (23-28), f_1-f_1 23 (21-22), f_1-h_1 6 (4-5), h_1-h_1 20 (15-17), h_1-h_2 3 (3) and h_2-h_2 29 (25-29).

Venter (Figure 2b): Prosternal apron wedge shaped, with 13-16 faveolae; venter covered with punctations except in sternocoxal area; area of coxal region posterolaterally with reticulations and faint reticulations medioventrally, and with punctations except in the sternocoxal area; venter with four pairs of ventral setae *1a* 14(11-12), *3a* 16 (14-15), *4a* 16 (14-15) and *4c* 10 (9-10), and two pairs of aggenital setae ag_1 5 (8) and ag_2 6 (5-9); distances between setae *1a-1a* 10 (14-16), *3a-3a* 34 (29-30), *4a-4a* 38 (29-36), *4c-4c* 42 (34-36), ag_1-ag_1 16 (13-14) and ag_2-ag_2 25 (22-25); and anal opening terminal, with three pairs of pseudanal setae, ps_1 8 (7), ps_2 12 (11-14) and ps_3 12 (10-12).

Legs (Figure 2c-f): Length of legs I-IV (from base of femur to tip of tarsal claw): 186 (177-182), 148 (136-144), 148 (127-145) and 169 (161-163), respectively. Setal formulae of legs I-IV: coxae 2-1-2-1, trochanters 1-1-2-1, femora 4-3-2-2, genua 5(+*k*)-4(+*k*)-2-3, tibiae 5(+φρ, +*ω*)-5(+φρ)-4(+φρ)-3 and tarsi 15(+φρ, +ω♂)-12(+φρ, +ω♂)-9(+ω♂)-9(+ω♂). Setae *tc* on tarsus II dissimilar.

Etymology: This species is named after the type locality, Manisa, where it was found.

Type materials

Holotype female, two paratype females and two males from litter and soil under *Pinus* sp., 900 m.a.s.l., Spil Mountain, Manisa Province, 23 December 2011; four females and one male from litter and soil under *Pinus* sp., 1200 m.a.s.l., Bozdağlar Mountains, Gölcük, Ödemiş District, İzmir Province, 26 November 2012; and one female from litter and soil under *Cornus* sp., 750 m.a.s.l., Gölet Region, Kula district, Manisa Province, 27 May 2013, Turkey, coll. M. Akyol.

Remarks

The new species, *F. manisaensis* sp. nov., resembles *F. distortus* and *F. bafranus* in having the anterior edge of hood smooth, dorsum completely reticulated and with two pairs of rosettes. However, it can be differentiated by the following characters: dorsal body completely punctuated and striated; venter with no reticulate pattern behind sternocoxal area medioventrally; and ratio c_1-c_1/d_1-d_1/e_1-e_1/f_1-f_1 1.8-2.8/3.4-4.8/2.4-3.1/1.0 in the new species, whereas, no punctuations in the reticulate cells on the edge of the dorsum and dorsum without striae reticulate pattern behind the sternocoxal area medioventrally; and ratio c_1-c_1/d_1-d_1/e_1-e_1/f_1-f_1 ?/2.8/2.2/1.0 in *F. distortus* (Kuznetsov & Livshitz, 1974; Fan, 1997; Dönel & Doğan, 2011). Setal formula of tarsi 17-14-10-10, hood with 6-8 dimples in each longitudinal row, prosternal apron with 12-17 foveole in the new species, whereas, setal formula of tarsi 16-12-10-10, hood with 5-6 dimples in each longitudinal row, and prosternal apron with 11 foveole in *F. bafranus* (Doğan, 2008).

The male can be distinguished from the female by the following features: anal and genital shields coalesced posterodorsally, setae *f*, h_1 and h_2 standing together as a cluster, with an aedeagus, genital setae absent, all tarsi with solenidion ω♂, and body smaller.

Key to the Turkish species of *Favognathus*

1. Genu II with solenidion *k* **3**
- Genu II without solenidion *k* **2**
2. Genu I with solenidion *k*, genu IV with 2 setae ..... ***F. luxtoni* Doğan & Ayyıldız, 1999**
- Genu I without solenidion *k*, genu IV with 3 setae ..... ***F. erzurumensis Doğan & Ayyıldız*, 2002**
3. Anterior margin of hood smooth **4**
- Anterior margin of hood denticulate ..... ***F. izmirensis* Akyol, 2011**
4. Dorsal shield partly or completely reticulated **5**
- Dorsal shield without reticulations, completely punctate ..... ***F. kamili* Dönel & Doğan, 2011**
5. Dorsal shield partly reticulated **6**
- Dorsal shield completely reticulated **9**
6. Dorsum with rosette patterns **7**
- Dorsum without rosette patterns **8**
7. Femur II with two setae ..... ***F. turcicus* Koç & Ayyıldız, 1999**
- Femur II with three setae ..... ***F. amygdalus* Doğan & Ayyıldız, 2004**
8. Prosternal apron with 14 dimples ..... ***F. acaciae* Doğan & Ayyıldız, 2004**
- Prosternal apron with 17 dimples ..... ***F. cucurbita* (Berlese, 1916)**
9. Setal formula of tarsi 16-12-10-10, hood with 5-6 dimples in each longitudinal row, prosternal apron with 11 foveole ..... ***F. bafranus* Doğan, 2008**
- Setal formula of tarsi 17-14-10-10, hood with 6-8 dimples in each longitudinal row, prosternal apron with 12- 17 foveole **10**
10. Dorsal body completely punctuated and striated ..... ***F. manisaensis* sp. nov.**
- Dorsal body partly punctuated and without striae ..... ***F. distortus* (Kuznetsov, 1974)**

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Original article (Orijinal araştırma)

Oribatid mite fauna (Acari) of Çat Forest, Sivas Province, Turkey[1]

Çat Ormanlarının (Sivas, Türkiye) Oribatid akar faunası (Acari)

Ayşe TOLUK[2*] **Ali Tuğrul AKİN[2]**

Summary

In this study, the oribatid mite fauna of Çat Forest, Sivas Province, Turkey extracting mites from soil, litter, moss, lichen and tree bark samples taken from 368 different localities in 2014 were investigated. Twenty-four species from 23 genera and 17 families of oribatid mites were found. Among them, two genera (*Bipassalozetes*, *Metabelba*) and eight species [*Licnodamaeus pulcherrimus* (Paoli, 1908), *Licnobelba latiflabellata* (Paoli, 1908), *Eupterotegaeus ornatissimus* (Berlese, 1908), *Metabelba (Metabelba) papillipes* (Nicolet, 1855), *Damaeolus bregetovae* Csiszár, 1962, *Passalozetes (Passalozetes) inlenticulatus* Mihelčič, 1959, *Camisia horrida* (Hermann, 1804) and *Bipassalozetes (Bipassalozetes) perforatus* (Berlese, 1910)] were new records for Turkey. For all new records, SEM pictures of morphological characters, as well as chorotypes for each species are provided.

Keywords: Çat Forest, fauna, new records, Oribatida, soil biodiversity

Özet

Bu çalışmada, 2014 yılında 368 farklı lokaliteden alınan toprak, döküntü, yosun, liken ve ağaç kabuğu örneklerinden oribatid akarları seçerek Sivas İli Çat Ormanlarının Oribatid akar faunası araştırılmıştır. Oribatid akarlardan 17 familyaya ait 23 cinsten 24 tür tespit edilmiştir. Bunlardan, iki cins (*Bipassalozetes*, *Metabelba*) ve sekiz tür [*Licnodamaeus pulcherrimus* (Paoli, 1908), *Licnobelba latiflabellata* (Paoli, 1908), *Eupterotegaeus ornatissimus* (Berlese, 1908), *Metabelba (Metabelba) papillipes* (Nicolet, 1855), *Damaeolus bregetovae* Csiszár, 1962, *Passalozetes (Passalozetes) inlenticulatus* Mihelčič, 1959, *Camisia horrida* (Hermann,1804) ve *Bipassalozetes (Bipassalozetes) perforatus* (Berlese, 1910)] Türkiye için yeni kayıttır. Ayrıca tüm yeni kayıtlar için morfolojik özelliklerin tarama elektron mikroskobu fotoğraflarını korotipleriyle birlikte verilmiştir.

Anahtar sözcükler: Çat Ormanları, fauna, yeni kayıtlar, Oribatida, toprak biyoçeşitliliği

[1] This study is a part of the second author's MSc thesis, and was presented as an oral presentation at the 23rd National Biology Congress (5-9 September 2016, Gaziantep, Turkey).

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Introduction

Oribatid mites (Acari) are saprophagous microarthropods inhabiting almost all types of soil ecosystems. With huge abundance, these creatures feed mainly on dead organic matter, fungi, algae and lichens, and have considerable potential as bioindicators (Ghilyarov, 1975; Weigmann, 2006; Krantz & Walter, 2009). Oribatid mites are mainly below ground animals that reach highest densities in acidic forest soils, e.g. up to 200,000 individuals per m^2 in a boreal forest (Maraun et al., 2007). They are important for litter decomposition, soil formation, nutrient cycling and the regulation of fungal and nematode populations. Mites consume various kinds of food and participate in numerous ways in the structure of the food web and found in almost every kind of habitat worldwide. Thus, they have significant interactions with their microenvironment. Given these features, Oribatid mites react to changes in environmental conditions, including chemical contamination, heavy metals and atmospheric pollutants. Therefore, they are considered useful indicators of specific soil parameters and quality (Gergócs & Hufnagel, 2009; Murvanidze et al., 2013; Vladislav et al., 2015). Oribatid mites (including Astigmata) are represented by 16 197 species and 2 399 genera within 249 families (Schatz et al., 2011) and this number is continuously increasing. More than 200 species of this suborder have been recorded from Turkey (Erman et al., 2007; Toluk & Ayyildiz, 2008a, 2009; Toluk et al., 2008). Research of the oribatid mites in Turkey goes back to the 1980s. Investigations on the oribatid diversity of Turkey are not completed for now. Taxonomic studies have been few and generally fragmentary. Despite, many studies on oribatid mites in various regions of Turkey (Özkan et al., 1994; Grobler et al., 2003, 2004), there are still regions to be investigated. One of these regions is Sivas. There is only a limited forest area (2.5%) in Sivas Province. Given the low rainfall, short vegetation season, low temperature and humidity, the forests do not grow fast and are not particularly healthy. As a result, this has a negative impact on their renewal. Within the borders of Sivas Province, the forests are generally found in the northern regions rather than western and eastern regions (Anon., 2015). There are no previously published records of oribatid mites from Çat Forest. The objective of this study was to contribute to the knowledge of the oribatid mite fauna of Turkey.

Material and Methods

The study area and sampling

Sivas Çat Forest (SÇO) is located in Gemerek District (39°28' N, 35°57' E; 1800 m a.s.l.), Sivas Province, in the north east of Central Anatolia. This area is dominated by the yellow pine tree (*Pinus sylvestris* L.). A total of 368 soil, litter, moss, lichen and tree bark samples were randomly taken from the study area on different dates in 2014 (Table 1). Mite samples were extracted using a Berlese funnel apparatus and stored in 70% ethanol for microscopic examination. Mites were sorted from the samples under a stereomicroscope and mounted on slides in modified Hoyer's medium or 35% lactic acid. Body length was measured in lateral view, from the tip of the rostrum to the posterior edge of the ventral plate. Notogastral width refers to the maximum width in dorsal aspect. The specimens examined are deposited in the Acarological Collection of the Zoological Museum, Erciyes University, Kayseri, Turkey.

Table 1. Details of collection sites under *Pinus sylvestris* in the Sivas Çat Forest (SÇO), Turkey

Site code	Habitat	Elevation (m)	Sampling date
SÇO-1 to 46	Litter	1534-1550	04.II.2014
SÇO-47 to 92	Soil and litter	1557-1664	01.III.2014
SÇO-93 to 138	Soil, tree bark, moss, lichen and litter	1415-1638	05.V.2014
SÇO-139 to 184	Soil, tree bark, moss, lichen and litter	1602-1639	04.VI.2014
SÇO-185 to 230	Soil, tree bark, moss, lichen and litter	1602-1639	04.VI.2014
SÇO-231 to 276	Soil, tree bark, moss, lichen and litter	1640-1686	05.VIII.2014
SÇO-277 to 322	Soil, tree bark, lichen and litter	1614-1685	09.IX.2014
SÇO-323 to 368	Soil, tree bark, moss, lichen and litter	1604-1627	06.X.2014

Scanning electron microscopy

The specimens for SEM were cleaned by soaking in Terg-a-zyme solution for 6-12 h, followed by brief (1-2 s) immersion in an ultrasonic bath. They were dried using critical point method, mounted on Al-stubs, and gold-coated in a Polaron sputter coater apparatus. Photographs were taken with an LEO 440 computer controlled digital SEM.

Terminology

The taxa were identified with reference to Woas (1986), Subías & Balogh (1989), Pérez-Íñigo, (1997) and Weigmann (2006). Terminology followed Norton & Behan-Pelletier (2009).

Results and Discussion

Twenty-four species belonging to 17 families of oribatid mites from Çat Forest, Sivas Province, Turkey were determined. The diagnostic features of new records are given below.

1. *Camisia (Camisia) horrida* (Hermann, 1804)

Measurements: Body length, 836-952 µm and body width, 336-544 µm (n = 10).

Diagnostic characters (Figure 1): Lamellar setae barbed, situated on small apophyses. Interlamellar setae minute. Sensilli short, with globose club. Lateral notogastral setae short. Lateral notogastral margin with transverse ridges. Setae h_1 barbed, on apophyses. Setae h_2 on short apophysis near posterolateral corner of notogaster. Transverse ridge present between setae e_1. Dorsocentral ridges extending from bases of setae d_1, bifurcating at medial level of setae f_2, forming W-shaped posterior ridge. Epimeral setal formula, 3-1-2-3. Nine pairs of genital setae. All legs tridactylous.

Material examined: SÇO-190, 2 exs.; SÇO-193, 7 exs.; SÇO-207, 4 exs.; SÇO-213, 2 exs.; SÇO-311, 4 exs.

Distribution: Holarctic region and the northern part of the Neotropical Region (Subías et al., 2012, updated 2017).

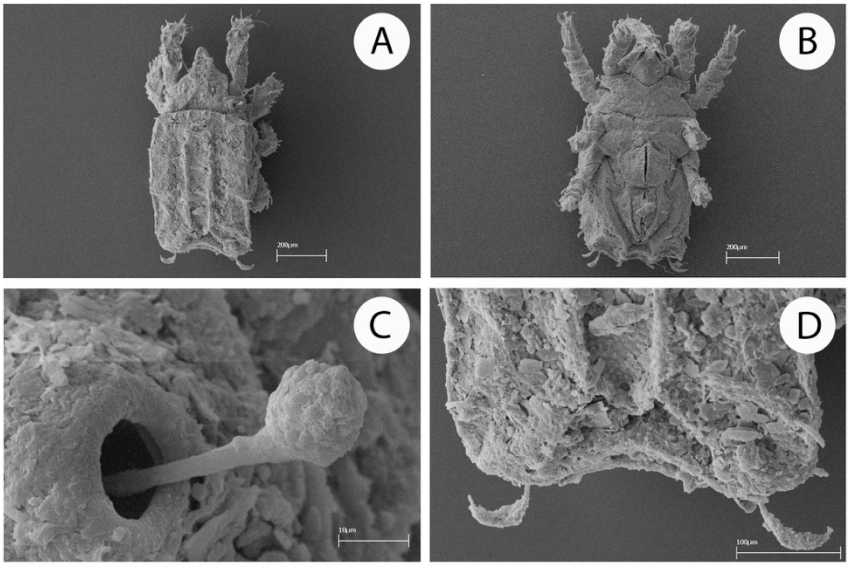

Figure 1. *Camisia (Camisia) horrida* (Hermann, 1804): A) dorsal view; B) ventral view; C) sensillum; D) posterior of notogaster.

Remarks: This species is a new record for Turkish fauna. Specimens belonging to this species have been extracted from the pine forest litter in Poland; bark and old wood from a tree stump, coniferous forest of cedar and hemlock in USA; mixed vegetation among rocks with *Silene, Antennaria, Saxifraga* and *Potentilla* in Canada; lichen on stone in Norway; moss and lichen in Austria and lichen in India; canopy samples in Georgia (Colloff, 1993; Murvanidze & Mumladze, 2014; Murvanidze & Arabuli, 2015; Murvanidze & Mumladze, 2016). Turkish specimens were collected in litter and soil. The body length of the species was given as 825 x 960 µm by Weigmann (2006). The dimensions for the Norway and Indian specimens were 796-998 x 431-522 and 819-866 x 437-451 µm, respectively (Colloff, 1993). The Turkish specimens (836-952 x 336-544 µm) examined were in the range of the known dimensions of the species. This species is well characterized by minute interlamellar setae, short lateral notogastral setae, seta h_2 on short apophysis near posterior edge of the notogaster and lateral notogastral margin with transverse ridges transverse carina between bases of setae e_1.

2. *Licnodamaeus pulcherrimus* (Paoli, 1908)

Measurements: Body length, 240-270 µm and body width, 122-136 µm (n = 10).

Diagnostic characters (Figure 2): Prodorsum, notogaster and ventral plate with reticulated cerotegument. Rostral and lamellar seta inserted on dorsal side of rostrum and covered by cerotegument. Sensilli with short stalk and expanded head. Four pairs of notogastral setae present, all of them covered by cerotegument. Notogastral lyrifissures *ia, im* and *ip* present. Five pairs of genital setae.

Material examined: SÇO-69, 17 exs.; SÇO-70, 2 exs.; SÇO-88, 1 ex.; SÇO-89, 7 exs.

Distribution: Palearctic region (Subías et al., 2004, updated 2017).

Remarks: This species is a new record for Turkish fauna. According to Weigmann (2013), and is found in dry bush and meadows soil. Bayartogtokh & Smelyansky, (2004) found this species in steppe soils dominated by *Stipa* sp. and *Festuca valesiaca* Schleich. ex Gaudin. Turkish specimens were collected in litter and soil. The dimensions of the species were given as 271 µm (269-275) x 128 µm (122-134) by Bayartogtokh & Smelyansky (2004). In this regard, the Turkish specimens (240-270 x 122-136 µm) examined were in the range of the known dimensions of the species. This species is well characterized by body surface with reticulated cerotegument, the shape of sensilli and four pairs of notogastral setae covered with cerotegument.

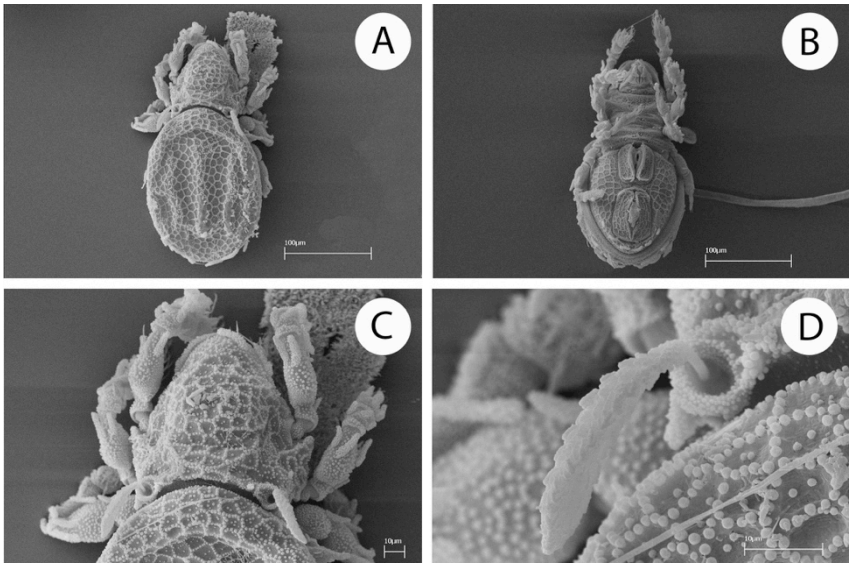

Figure 2. *Licnodamaeus pulcherrimus* (Paoli, 1908): A) dorsal view; B) ventral view; C) prodorsum; D) sensillum.

3. *Licnobelba latiflabellata* (Paoli, 1908)

Measurements: Body length, 280-320 µm and body width, 148-164 µm (n = 10).

Diagnostic characters (Figure 3): Adult with exuvial scalps of juveniles. Body covered by granules of cerotegument. Rostral and lamellar seta inserted on dorsal side of rostrum and covered by cerotegument. Sensilli spatulate, long and its head barbed. Anterior part of notogaster with transverse ridge. Four pairs of posterior notogastral setae present (h_1, p_{1-3}). Epimeral setal formula, 3-1-2-2. Six pairs of genital setae. All legs tridactylous.

Material examined: SÇO-86, 1 ex.; SÇO-97, 1 ex.; SÇO-114, 8 exs.

Distribution: Palearctic region (Subías et al., 2004, updated 2017).

Remarks: This species is new record for Turkish fauna. According to Pérez-Íñigo (1993), this species lives in the soil under dry litter, especially of pine, juniper and box trees, but also mosses on soil. Grandjean (1934) found it to be very common in the forest litter. We found this species in soil and litter. The dimensions of this species have been reported in the range of 276-400 x 145-280 µm (Pérez-Íñigo, 1997). The Turkish specimens (280-320 x 148-164 µm) examined were in the range of the known dimensions of the species. This species is well characterized by long sensilli with a spatulate head, four pairs of posterior notogastral setae (h_1, p_{1-3}) and six pairs of genital setae.

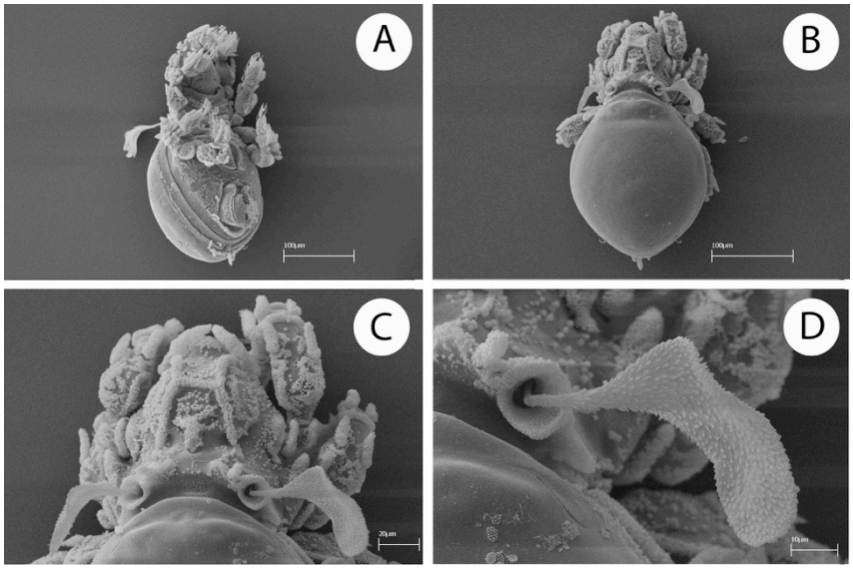

Figure 3. *Licnobelba latiflabellata* (Paoli, 1908): A) lateral view; B) dorsal view; C) prodorsum; D) sensillum.

4. *Damaeolus bregetovae* Csiszár, 1962

Measurements: Body length, 264-300 µm and body width, 140-156 µm (n = 7).

Diagnostic characters (Figure 4): Lamellar setae near rostral setae. Sensilli fusiform with an apical flagellum. Eleven pairs of notogastral setae present, all of them flagelliform. Dorsosejugal suture straight. Epimeral setal formula, 3-1-3-3. Six pairs of genital setae, three pairs of aggenital setae, two pairs of anal setae and three pairs of adanal setae present.

Material examined: SÇO-94, 3 exs.; SÇO-179, 4 exs.

Distribution: Mediterranean (Subías, 2004, updated 2017).

Remarks: This species is a new record for Turkish fauna. It is found in the soil and on grass (Pérez-Íñigo, 1997). We found this species abundant in soil and litter. The dimensions of this species were given as 347 x 162 µm by Csiszár & Jeleva (1962). The Turkish specimens (264-300 x 140-156 µm) examined

were smaller than the known body length of the species. These differences in dimensions are considered within the variation limits. This species is well characterized by fusiform sensilli, eleven pairs of flagelliform notogastral setae, straight dorsosejugal suture and six pairs of genital setae.

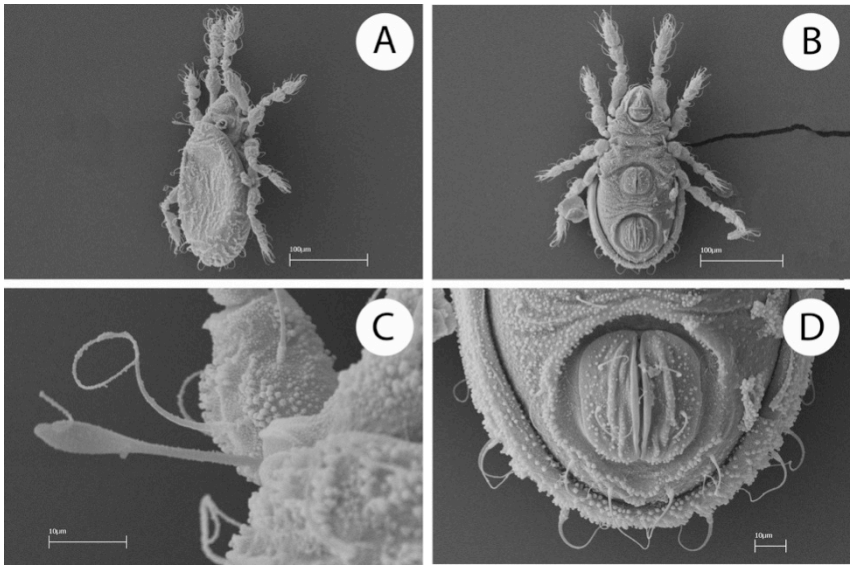

Figure 4. *Damaeolus bregetovae* Csiszár, 1962: A) laterodorsal view; B) ventral view; C) sensillum; D) anal plate.

5. *Bipassalozetes (Bipassalozetes) perforatus* (Berlese, 1910)

Measurements: Body length, 325-364 µm and body width, 200-205 µm (n = 2).

Diagnostic characters (Figure 5): Prodorsum structure with narrow, branched ridges. Notogaster with round knots. Sensilli setiform, apically pointed. Dorsosejugal suture not evident medially. Lenticulus circular. Four pairs of small porose areas. Ten pairs of small, smooth notogastral setae. Epimeral setal formula, 3-1-2-2. Four pairs of genital setae.

Material examined: SÇO-28, 2 exs.

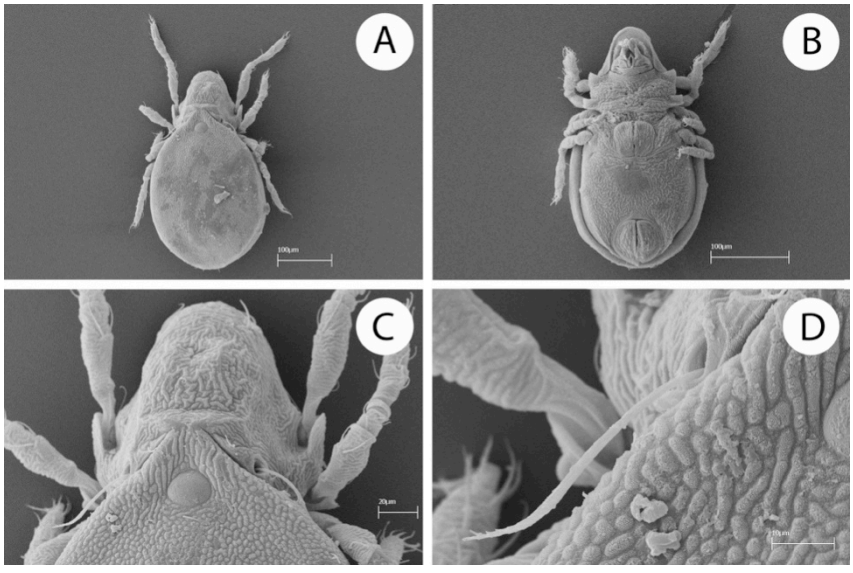

Figure 5. *Bipassalozetes (Bipassalozetes) perforatus* (Berlese, 1910): A) dorsal view; B) ventral view; C) prodorsum and lenticulus; D) sensillum.

Distribution: Palearctic region (Subías et al., 2004, updated 2017).

Remarks: This species is a new record for Turkish fauna. It is a xerothermophilous species (Lazarus & Krisper, 2014). The specimens belonging to this species are extracted from samples taken in salt marshes and dunes, in montane and subalpine meadows (Weigmann, 2006). It is also recorded from dry meadow in Tbilisi, Georgia (Murvanidze & Mumladze, 2016). Turkish specimens were collected in litter. The body length was given as 350 x 210 µm by Woas (1998) and 365 x 390 µm by Weigmann (2006). The Turkish specimens (325-364 x 200-205 µm) examined were in the range of the known dimensions of the species. This species is well characterized by the shape of sensilli, narrow and branched ridges on the prodorsum and round knots on notogaster.

6. *Passalozetes (Passalozetes) inlenticulatus* Mihelčič, 1959

Measurements: Body length, 280-304 µm and body width, 140-148 µm (n = 10).

Diagnostic characters (Figure 6): The surface of prodorsum and notogaster with three or four branched linear ridges. Sensilli filiform, flat in section, with small barb on their posterior edge. Notogaster with humeral projection. Ten pairs of notogastral setae present. Lenticulus present anteriorly with indistinct borders. All legs tridactylous

Material examined: SÇO-9, 32 exs.; SÇO-114, 12 exs.

Distribution: Mediterranean (Subías et al., 2004, updated 2017).

Remarks: This species is a new record for Turkish fauna. The body length was given as 210-280 x 140-160 by Mihelčič (1959) and 234-243 x 112-125 µm by Gil & Subías (1990). The Turkish specimens (280-304 x 140-148 µm) examined were in the range of the known dimensions of the species. This species is well characterized by the filiform sensilli, notogaster with small humeral projection, narrow, branched ridges on the prodorsum, round knots on the notogaster and lenticulus with indistinct borders.

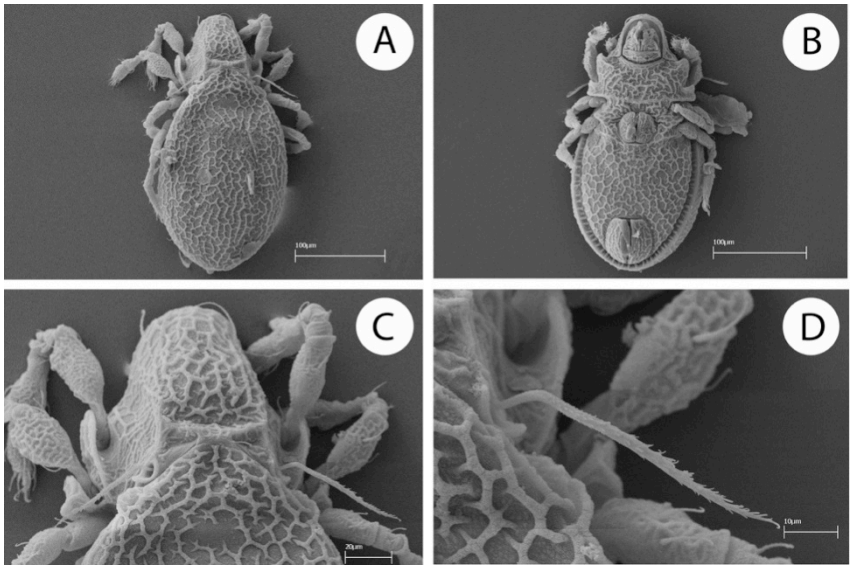

Figure 6. *Passalozetes (Passalozetes) inlenticulatus* Mihelčič, 1959: A) dorsal view; B) ventral view; C) prodorsum; D) sensillum.

7. *Metabelba (Metabelba) papillipes* (Nicolet, 1855)

Measurements: Body length, 448-452 µm and body width, 280-304 µm (n = 10).

Diagnostic characters: Anterior tubercle (Da) and posterior centrodorsal tubercle (Dp) present on prodorsum. Sensilli setiform, with flagelliform tips. Notogaster setae smooth and arranged radially. Six pairs of genital setae, one pair of aggenital setae, two pairs of anal setae and three pairs of adanal setae present.

Material examined: SÇO-49, 19 exs.; SÇO-69, 1 ex.; SÇO-109, 2 exs.

Distribution: Holarctic region (Subías et al., 2004, updated 2017).

Remarks: This species is a new record for Turkish fauna. It is known to inhabit lower layers of the forest litter (Weigmann, 2006). We found this species in the litter under *P. sylvestris*. The dimensions of this species were given as 410-520 µm by Weigmann (2006). The Turkish specimens (448-452 x 280-304 µm) examined were in the range of the known dimensions of the species. This species is well characterized by anterior tubercle (Da) and posterior centrodorsal tubercle (Dp) on the prodorsum, setiform sensilli, smooth notogastral setae arranged radially and six pairs of genital setae.

8. *Eupterotegaeus ornatissimus* (Berlese, 1908)

Measurements: Body length, 608-728 µm and body width, 360-480 µm (n = 10).

Diagnostic characters (Figure 7): Surface of body with foveolate cerotegument**.** Lamellar cusps projecting well beyond rostrum. Sensilli expanded distally. Humeral processes projected forwards to the level of bothridia. Eight pairs short, smooth notogastral setae present. Anterior margin of notogaster with a triangular projection. Epimeral setal formula, 3-1-3-3. Adanal setae ad_1 and ad_2 in postanal, ad_3 in adanal positions.

Material examined: SÇO-4, 8 exs.; SÇO-196, 5 exs.

Distribution: Holarctic region (Subías et al., 2004, updated 2017).

Remarks: This species is a new record for Turkish fauna. According to Trave (1982), this is a forest and mountain species and an inhabitant of litter, moss and decomposed wood. It is found at altitudes above 500 m, with an optimum between 500 and 1200 m. This species is also found in oak and chestnut, beech and pine (Pérez-Íñigo, 1997). We found this species in the litter under *P. sylvestris*. The dimensions of this species were given as 750 x 450 µm by Berlese (1908), 616-704 x 360-408 by Pérez-Íñigo (1990) and 629-713 x 365-425 by Kunst (1958). The Turkish specimens (608-728 x 360-480 µm) examined were in the range of the known dimensions of the species. This species is well characterized surface of body by with foveolate cerotegument, the shape of sensilli, anterior margin of the notogaster with a triangular projection and eight pairs of notogastral setae.

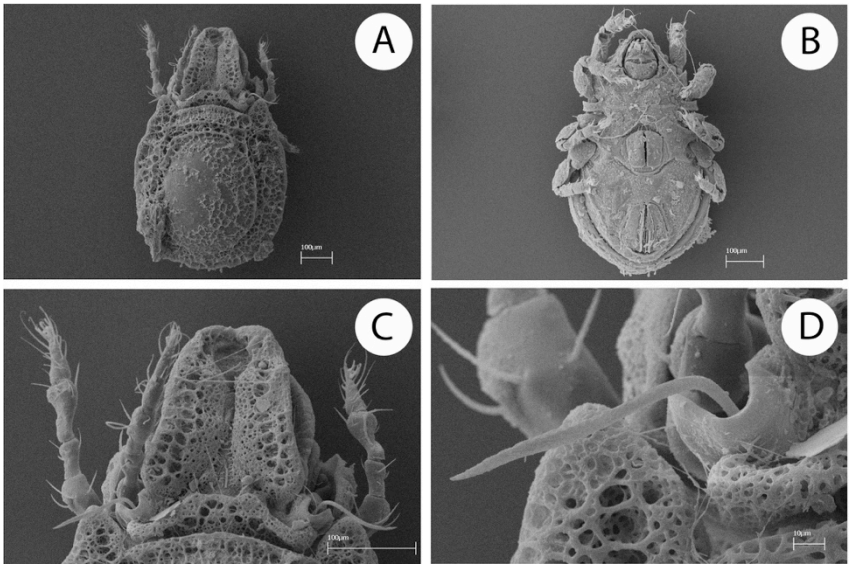

Figure 7. *Eupterotegaeus ornatissimus* (Berlese, 1908)*:* A) dorsal view; B) ventral view; C) prodorsum; D) sensillum.

9. *Neoliodes ionicus* Sellnick, 1931

Material examined: SÇO-59, 6 exs.; SÇO-76, 2 exs.; SÇO-119, 1 ex.; SÇO-192, 6 exs.; SÇO-238, 15 exs.

Distribution: Mediterranean (Subías et al., 2004, updated 2017). This species was previously recorded from Turkey (Sevimli & Baran, 2016).

10. *Gymnodamaeus barbarossa* Weigmann, 2006

Material examined: SÇO-239, 2 exs.

Distribution: Central Europe (Subías et al., 2004, updated 2017). This species was previously recorded from Turkey (Toluk & Ayyildiz, 2014).

11. *Aleurodamaeus setosus* (Berlese, 1883)

Material examined: SÇO-49,8 exs.

Distribution: Palearctic region (Subías et al., 2004, updated 2017). This species was previously recorded from Turkey (Seniczak et al., 2012).

12. *Eueremaeus oblongus oblongus* (Koch, 1835)

Material examined: SÇO-59, 6 exs.; SÇO-94, 2 exs.; SÇO-165, 3 exs.; SÇO-344, 3 exs.; SÇO-355, 6 exs.

Distribution: Holarctic region (Subías et al., 2004, updated 2017). This subspecies was previously recorded from Turkey (Per & Ayyildiz, 2005).

13. *Ramusella (Insculptoppia) insculpta* (Paoli, 1908)

Material examined: SÇO-6, 8 exs.; SÇO-12, 3 exs.; SÇO-54, 15 exs.; SÇO-60, 29 exs.; SÇO-62, 86 exs.; SÇO-68, 21 exs.; SÇO-70, 8 exs.; SÇO-83, 14 exs.; SÇO-85, 11 exs.; SÇO-86, 63 exs.; SÇO-88, 24 exs.; SÇO-89,14 exs.; SÇO-90, 21 exs.; SÇO-91, 67 exs.; SÇO-97, 26 exs.; SÇO-114, 31 exs.; SÇO-141, 53 exs.; SÇO-142, 37 exs.; SÇO-143, 123 exs.; SÇO-144, 214 exs.; SÇO-146, 92 exs.; SÇO-147, 96 exs,; SÇO-148, 69 exs.; SÇO-149, 66 exs; SÇO-150, 76 exs.; SÇO-152, 54 exs.; SÇO-153, 12 exs.; SÇO-154, 121 exs.; SÇO-155, 33 exs.; SÇO-157, 24 exs.; SÇO-158, 24 exs.; SÇO-159, 207 exs.; SÇO-161, 93 exs.; SÇO-166, 72 exs.; SÇO-167,13 exs.; SÇO-170, 30 exs.; SÇO-172, 56 exs.; SÇO-173, 91 exs.; SÇO-176, 137 exs.; SÇO-177, 41 exs.; SÇO-178, 24 exs.; SÇO-180, 84 exs.; SÇO-182, 37 exs.; SÇO-183, 72 exs.; SÇO-186, 72 exs.; SÇO-189, 45 exs.; SÇO-190, 32 exs.; SÇO-196, 11 exs.; SÇO-197, 9 exs.; SÇO-199, 64 exs.; SÇO-200, 61 exs.; SÇO-201, 60 exs.; SÇO-205, 121 exs.; SÇO-211, 82 exs.; SÇO-212, 48 exs.; SÇO-213, 38 exs.; SÇO-215, 13 exs.; SÇO-216, 9 exs.; SÇO-218, 62 exs.; SÇO-220, 66 exs.; SÇO-221, 62 exs.; SÇO-223, 34 exs.; SÇO-225, 27 exs.; SÇO-226, 23 exs.; SÇO-227, 69 exs.; SÇO-228, 13 exs.; SÇO-229, 63 exs.; SÇO-231, 2 exs.; SÇO-240, 152 exs.; SÇO-242, 13 exs.; SÇO-243, 13 exs.; SÇO-245, 8 exs.; SÇO-248, 21 exs.; SÇO-264, 16 exs.; SÇO-265, 61 exs.; SÇO-272, 12 exs.; SÇO-273, 8 exs.; SÇO-279, 28 exs.; SÇO-284, 10 exs.; SÇO-289, 36 exs.; SÇO-291, 21 exs.; SÇO 296, 26 exs.; SÇO-299. 11 exs.; SÇO-301, 61 exs.; SÇO-304, 11 exs.; SÇO-305, 9 exs.; SÇO-309, 56 exs.; SÇO-310, 17 exs.; SÇO-312, 46 exs.; SÇO-313,11 exs.; SÇO-314,120 exs.; SÇO-315, 10 exs.; SÇO-316, 12 exs.; SÇO-318, 11 exs.; SÇO-322, 11 exs.; SÇO-333, 102 exs.; SÇO-335, 21 exs.; SÇO-336, 31 exs.; SÇO-353, 38 exs.; SÇO-363, 86 exs.

Distribution: Palearctic region (Subías et al., 2004, updated 2017). This species was previously recorded from Turkey (Toluk & Ayyildiz, 2008b).

14. *Rhinoppia (Rhinoppia) obsoleta obsoleta* (Paoli, 1908)

Material examined: SÇO-4, 3 exs.; SÇO-13, 9 exs.; SÇO-21, 1 ex.; SÇO-22. 8 exs.; SÇO-94, 24 exs.; SÇO-114, 9 exs.; SÇO-155, 7 exs.; SÇO-221, 2 exs.; SÇO-322, 3 exs.; SÇO-335, 8 exs.; SÇO-336, 6 exs.

Distribution: Palearctic region (Subías et al., 2004, updated 2017). This subspecies was previously recorded from Turkey (Toluk & Ayyildiz, 2008b).

15. *Rhinoppia (Bipectinoppia) tasdemiri* Toluk & Ayyildiz, 2008

Material examined: SÇO-16, 5 exs.; SÇO-20, 2 exs.; SÇO-49, 4 exs.; SÇO-55, 1 ex.; SÇO-57, 6 exs.; SÇO-70, 2 exs.; SÇO-74, 3 exs.; SÇO-91, 3 exs.; SÇO-94, 24 exs.; SÇO-106, 2 exs.; SÇO-107, 2 exs.; SÇO-114, 9 exs.; SÇO-115, 6 exs.; SÇO-116, 4 exs.; SÇO-118, 41 exs.; SÇO-129,1 ex.; SÇO-131, 2 exs.; SÇO-152, 4 exs.; SÇO-155, 7 exs.; SÇO-163, 2 exs.; SÇO-172, 2 exs.; SÇO-185, 9 exs.; SÇO-190, 4 exs.; SÇO-197, 2 exs.; SÇO-199, 6 exs.; SÇO-200, 4 exs.; SÇO-201, 2 exs.; SÇO-221, 2 exs.; SÇO-223, 7 exs.; SÇO-227, 4 exs.; SÇO-240, 3 exs.; SÇO-273, 3 exs.; SÇO-322, 3 exs.; SÇO-335, 8 exs.; SÇO-336, 6 exs.

Distribution: Turkey and Iran (Subías et al., 2004, updated 2017). This species was previously recorded from Turkey (Toluk & Ayyildiz, 2008d).

16. *Berniniella (Berniniella) serratirostris hauseri* (Mahunka, 1974)

Material examined: SÇO-16, 8 exs.; SÇO-28, 9 exs.; SÇO-54, 41 exs.; SÇO-56, 5 exs.; SÇO-57, 14 exs.; SÇO-58, 120 exs.; SÇO-59, 17 exs.; SÇO-60, 72 exs.; SÇO-62, 46 exs.; SÇO-68, 34 exs.; SÇO-76, 15 exs.; SÇO-77, 9 exs.; SÇO-79, 16 exs.; SÇO-80, 45 exs.; SÇO-81, 8 exs.; SÇO-86, 33 exs.; SÇO-89, 12 exs.; SÇO-90, 27 exs.; SÇO-91, 92 exs.; SÇO-94, 162 exs.; SÇO-98, 14 exs.; SÇO-99, 8 exs.; SÇO-107, 65 exs.; SÇO-108, 19 exs.; SÇO-109, 76 exs.; SÇO-111, 32 exs.; SÇO-116, 1 ex.; SÇO-118, 24 exs.; SÇO-123, 7 exs.; SÇO-131, 60 exs.; SÇO-136, 72 exs.; SÇO-139, 7 exs.; SÇO-148, 196 exs.; SÇO-158, 18 exs.; SÇO-166, 44 exs.; SÇO-193, 6 exs.; SÇO-196, 6 exs.; SÇO-198, 6 exs.; SÇO-199, 17 exs.; SÇO-201, 82 exs.; SÇO-203, 6 exs.; SÇO-206, 48 exs.; SÇO-209, 11 exs.; SÇO-211, 7 exs.; SÇO-221, 41 exs.; SÇO-223, 5 exs.; SÇO-227, 13 exs.; SÇO-265, 6 exs.; SÇO-282, 8 exs.; SÇO-283, 48 exs.; SÇO-284, 63 exs.; SÇO-285, 54 exs.; SÇO-287, 9 exs.; SÇO-288, 7 exs., SÇO-289, 47 exs.; SÇO-290, 27 exs.; SÇO-291, 28 exs.; SÇO-292, 57 exs.; SÇO-294, 30 exs.; SÇO-296, 34 exs.; SÇO-297, 18 exs.; SÇO-299, 91 exs.; SÇO-300, 27 exs.; SÇO-301, 26 exs.; SÇO-303, 9 exs.; SÇO-304, 32 exs.; SÇO-307, 8 exs.; SÇO-309, 21 exs.; SÇO-310, 13 exs.; SÇO-311, 13 exs.; SÇO-312, 16 exs.; SÇO-314, 45 exs.; SÇO-315, 14 exs.; SÇO-316, 18 exs.; SÇO-320, 330 exs.; SÇO-333, 27 exs.; SÇO-336, 21 exs.

Distribution: Holarctic region and U.S.A (Subías et al., 2004, updated 2017). This subspecies was previously recorded from Turkey (Toluk & Ayyildiz, 2008c).

17. *Lauroppia fallax* (Paoli, 1908)

Material examined: SÇO-48, 1 ex.; SÇO-49, 16 exs.; SÇO-93, 9 exs.; SÇO-95. 19 exs.; SÇO-96, 16 exs.; SÇO-97, 11 exs.; SÇO-98, 5 exs.; SÇO-114, 11 exs.; SÇO-118, 34 exs.; SÇO-121, 4 exs.; SÇO-123, 5 exs.; SÇO-127, 2 exs.; SÇO-139, 3 exs.; SÇO-154, 9 exs.; SÇO-212, 26 exs.; SÇO-263, 2 exs.

Distribution: Semicosmopolitan (Subías et al., 2004, updated 2017). This species was previously recorded from Turkey (Baran, 2003).

18. *Moritzoppia escotata escotata* (Subías & Rodríguez, 1986)

Material examined: SÇO-50, 2 exs.; SÇO-53, 1 ex.; SÇO-54, 5 exs.; SÇO-55, 1 ex.; SÇO-56, 1 ex.; SÇO-59, 42 exs.; SÇO-60, 57 exs.; SÇO-66, 1 ex.; SÇO-68, 18 exs.; SÇO-69, 5 exs.; SÇO-74, 1 ex.; SÇO-75, 7 exs.; SÇO-77, 2 exs.; SÇO-80, 30 exs.; SÇO-81, 108 exs.; SÇO-84, 1 ex.; SÇO-86, 94 exs.; SÇO-97, 3 exs.; SÇO-100, 6 exs.; SÇO-116, 1 ex.; SÇO-119, 7 exs.; SÇO-136, 52 exs.; SÇO-173, 4 exs.; SÇO-189, 2 exs.; SÇO-193, 42 exs.; SÇO-195, 1 ex.; SÇO-203, 4 exs.; SÇO-209, 4 exs.; SÇO-218, 14 exs.; SÇO-226, 18 exs.; SÇO-256, 1 ex. SÇO-260, 2 exs.; SÇO-286, 2 exs.; SÇO-298, 11 exs.

Distribution: Mediterranean (Subías et al., 2004, updated 2017). This subspecies was previously recorded from Turkey (Toluk & Ayyildiz, 2008c).

19. *Oppiella (Oppiella) nova nova* (Oudemans, 1902)

Material examined: SÇO-20, 4 exs.; SÇO-49, 45 exs.; SÇO-57, 4 exs.; SÇO-59, 42 exs.; SÇO-70,16 exs.; SÇO-71, 2 exs.; SÇO-76, 4 exs.; SÇO-88, 10 exs.; SÇO-93, 51 exs.; SÇO-94, 44 exs.; SÇO-95, 121 exs.; SÇO-96, 17 exs.; SÇO-97, 5 exs.; SÇO-98, 34 exs.; SÇO-99, 5 exs.; SÇO-102, 18 exs.; SÇO-108, 7 exs.; SÇO-109, 44 exs.; SÇO-111, 21 exs.; SÇO-113, 1 ex.; SÇO-118, 13 exs.; SÇO-119,18 exs.; SÇO-123, 13 exs.; SÇO-125, 17 exs.; SÇO-127, 21 exs.; SÇO-128, 61 exs.; SÇO-129, 34 exs.; SÇO-132, 37 exs.; SÇO-133, 1 ex.; SÇO-134, 1 ex.; SÇO-136, 540 exs.; SÇO-137, 46 exs.; SÇO-141, 32 exs.; SÇO-142, 18 exs.; SÇO-143, 138 exs.; SÇO-144, 193 exs.; SÇO-147, 62 exs.; SÇO-148, 122 exs.; SÇO-149, 38 exs.; SÇO-150, 52 exs.; SÇO-152, 35 exs.; SÇO-153, 9 exs.; SÇO-154, 80 exs.; SÇO-155, 25 exs.; SÇO-157, 21 exs.; SÇO-161, 27 exs.; SÇO-166, 15 exs.; SÇO-167, 7 exs.; SÇO-170,15 exs.; SÇO-172, 16 exs.; SÇO-176, 48 exs.; SÇO-177, 36 exs.; SÇO-178, 7 exs.; SÇO-180, 142 exs.; SÇO-182, 12 exs.; SÇO-183, 43 exs.; SÇO-185, 28 exs.; SÇO-186, 29 exs.; SÇO-193, 8 exs.; SÇO-219, 26 exs.; SÇO-220, 19 exs.; SÇO-223, 11 exs.; SÇO-225, 6 exs.; SÇO-227, 52 exs.; SÇO-228, 19 exs.; SÇO-229, 96 exs.; SÇO-240, 154 exs.; SÇO-243, 14 exs.; SÇO-245, 14 exs.; SÇO-246, 19 exs.; SÇO-264, 12 exs.; SÇO-282, 28 exs.; SÇO-283, 26 exs.; SÇO-284, 14 exs.; SÇO-288, 18 exs.; SÇO-291, 56 exs.; SÇO-293,8 exs.; SÇO-294, 16 exs.; SÇO-296, 18 exs.; SÇO-301, 12 exs.; SÇO-309, 50 exs.; SÇO-314, 72 exs.; SÇO-318, 13 exs.; SÇO-320, 220 exs.; SÇO-321, 22 exs.; SÇO-322, 14 exs.; SÇO-336, 34 exs.; SÇO-363, 51 exs.

Distribution: Cosmopolitan (Subías et al., 2004, updated 2017). This subspecies was previously recorded from Turkey (Toluk & Ayyildiz, 2008b).

20. *Coronoquadroppia nasalis* Gordeeva, 1983

Material examined: SÇO-94, 12 exs.; SÇO-97, 8 exs.; SÇO-114, 13 exs.; SÇO-141, 6 exs.; SÇO-144, 56 exs.; SÇO-146, 6 exs.; SÇO-147, 26 exs.; SÇO-148, 7 exs.; SÇO-150, 11 exs.; SÇO-161,19 exs.; SÇO-166, 23 exs.; SÇO-172, 6 exs.; SÇO-176, 14 exs.; SÇO-180, 32 exs.; SÇO-185, 13 exs.; SÇO-206, 26 exs.; SÇO-240, 40 exs.; SÇO-248, 6 exs.; SÇO-309. 9 exs.; SÇO-310, 6 exs.; SÇO-311, 7 exs.; SÇO-312, 6 exs.; SÇO-313, 6 exs.; SÇO-314, 19 exs.; SÇO-315, 30 exs.; SÇO-316, 8 exs.; SÇO-318, 10 exs.; SÇO-321, 9 exs.; SÇO-333, 7 exs.; SÇO-363, 32 exs.

Distribution: Palearctic region (Subías et al., 2004, updated 2017). This species was previously recorded from Turkey (Toluk & Ayyildiz, 2008b).

21. *Tectocepheus alatus* Berlese, 1913

Material examined: SÇO-69,17 exs.; SÇO-86, 11 exs.

Distribution: Palearctic region (Subías et al., 2004, updated 2017). This species was previously recorded from Turkey (Per et al., 2015).

22. *Scutovertex sculptus* Michael, 1879

Material examined: SÇO-10, 2 exs.; SÇO-24,1 ex.

Distribution: Palearctic region and New Zealand (Subías et al., 2004, updated 2017). This species was previously recorded from Turkey (Başẗürk & Toluk, 2016).

23. *Eupelops acromios* (Hermann, 1804)

Material examined: SÇO-19, 1 ex.; SÇO-213, 2 exs.; SÇO-266, 2 exs.

Distribution: Semicosmopolitan (Subías et al., 2004, updated 2017). This species was previously recorded from Turkey (Taşdemir et al., 2010).

24. *Oribatula (Zygoribatula) cognata* (Oudemans, 1902)

Material examined: SÇO-78,7 exs.

Distribution: Palearctic region (Subías et al., 2004, updated 2017). This species was previously recorded from Turkey (Taşdemir et al., 2010).

Conclusion

In total, 13151 individual oribatid mite were extracted from the samples collected. They represented by 24 species belonging to 23 genera and 17 families (Table 2). Among them, two genera, (*Bipassalozetes* and *Metabelba*) and eight species (*L. pulcherrimus*, *L. latiflabellata*, *E. ornatissimus*, *M.* (*M.*) *papillipes*, *D. bregetovae*, *P.* (*P.*) *inlenticulatus*, *C. horrida* and *B.* (*B.*) *perforatus*) were new records for the Turkish fauna.

Of the whole fauna picnonotic oribatids represented 79% and poronotic oribatids 21%. The faunistic analysis shows that picnonotic oribatids are the dominant major group. Oribatid mites can be found in almost every kind of habitat worldwide, most importantly in the layers of soil containing organic materials, but they also occur in several other kinds of microhabitat (e.g. lichen, moss and tree bark) (Gergócs & Hufnagel, 2009). In our findings, the 24 species were recorded in litter samples and 14 species from soil samples. In the other three habitats, species numbers were seven in lichen, seven in moss and six in tree bark samples. Oppiidae species were found in all microhabitats. They were abundant in soil and litter microhabitats with a higher number of individuals (12428) and species (7). According to Maraun & Scheu (2000), Oppiidae species are abundant because they have a wider food spectrum, and reproduce and develop rapidly. Also, they are generally abundant in forest soils, whereas their density is usually low in heavily disturbed habitats. Four oppiid species, *R.* (*I.*) *insculpta, B.* (*B.*) *serratirostris hauseri*, *O.* (*O.*) *nova nova* and *Q.* (*C.*) *nasalis,* were found in all microhabitats. The subspecies *O.* (*O.*) *nova nova* is cosmopolitan and parthenogenetic (thelytokous), known from many habitats (Von Saltzwedel et al., 2014). Ten species, *D. bregetovae*, *B.* (*B.*) *perforatus, P.* (*P.*) *inlenticulatus*, *M.* (*M.*) *papillipes*, *E. ornatissimus*, *G. barbarossa*, *A. setosus*, *E. oblongus oblongus*, *S. sculptus*, *O.* (*Z.*) *cognata,* were found only in litter samples. Passalozetidae species are commonly found in arid environments (desert valleys and hills) of Holartic, Ethiopian and Neotropical regions (Martínez & Herrero, 2006)**.** The species *G. barbarossa* inhabits warm and dry litter layer (Weigmann & Mourek, 2008). According to Steiner (1990), the species *E. oblongus oblongus* and *S. sculptus* are indicators of air pollution. The presence of these species in the study area gives us some information about the environmental conditions of these habitats. According to Murvanidze & Mumladze (2014) finding of the species *E. ornatissimus* in litter samples indicates an organic matter decay process is operating. Similarly, we found this species only in litter samples.

Zoogeographical analysis of the oribatid fauna of Çat Forest shows that Palearctic species are the most numerous, representing 50%, followed by the Holarctic species at 20.8%, cosmopolitan and semicosmopolitan species at 12.5% and Mediterranean species at 16.6%.

Table 2. The distribution of the microhabitats of the oribatid species determined from the study area

Family	Taxa	Microhabitats				
		Soil	Litter	Moss	Lichen	Tree bark
Crotoniidae	*Camisia (Camisa) horrida* (Hermann, 1804)	2	17			
Licnodamaeidae	*Licnodamaeus pulcherrimus* (Paoli, 1908)	8	17			
Licnobelbidae	*Licnobelba latiflabellata* (Paoli, 1908)	9	1			
Damaeolidae	*Damaeolus bregetovae* Csiszár, 1962		7			
Passalozetidae	*Bipassalozetes (Bipassalozetes) perforatus* (Berlese, 1910)		2			
	Passalozetes (Passalozetes) inlenticulatus Mihelčič, 1959		44			
Damaeidae	*Metabelba (Metabelba) papillipes* (Nicolet, 1855)		22			
Compactozetidae	*Eupterotegaeus ornatissimus* (Berlese, 1908)		13			
Neoliodidae	*Neoliodes ionicus* Sellnick, 1931	2	21	7		
Gymnodamaeidae	*Gymnodamaeus barbarossa* Weigmann, 2006		2			
Aleurodamaeidae	*Aleurodamaeus setosus* (Berlese, 1883)		11			
Eremaeidae	*Eueremaeus oblongus oblongus* (Koch, 1835)		14			6
Oppiidae	*Ramusella (Insculptoppia) insculpta* (Paoli, 1908)	918	2959	82	381	258
	Rhinoppia (Rhinoppia) obsoleta obsoleta (Paoli, 1908)	7	59		14	
	Rhinoppia (Bipectinoppia) tasdemiri Toluk & Avvildiz, 2008	75	155		25	10
	Berniniella (Berniniella) serratirostris hauseri (Mahunka, 1974)	830	1647	39	182	98
	Lauroppia fallax (Paoli, 1908)	54	93	26		
	Moritzoppia escotata escotata (Subías & Rodríguez, 1986)	143	1388	7	11	
	Oppiella (Oppiella) nova nova (Oudemans, 1902)	596	2875	39	46	18
Quadroppiidae	*Coronoquadroppia nasalis* Gordeeva, 1983	63	357	6	7	40
Tectocepheidae	*Tectocepheus salatus* Berlese, 1913	11	17			
Scutoverticidae	*Scutovertex sculptus* Michael, 1879		3			
Phenopelopidae	*Eupelops acromios* (Hermann, 1804)	4	1			
Oribatulidae	*Oribatula (Zygoribatula) cognata* (Oudemans, 1902)		7			

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Original article (Orijinal araştırma)

Toxicological and behavioral effects of some plant extract on Colorado potato beetle, *Leptinotarsa decemlineata* Say, 1824 (Coleoptera: Chrysomelidae)[1]

Bazı bitki ekstraktlarının Patates böceği [*Leptinotarsa decemlineata* Say,1824 (Coleoptera: Chrysomelidae)]'ne karşı toksikolojik ve davranışsal etkileri

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Summary

Repellent, ovicidal and oviposition deterrent effects of six plant extracts [*Heracleum platytaenium* Boiss (Apiaceae), *Humulus lupulus* L. (Cannabaceae), *Achillea millefoliu*m L. (Asteraceae), *Acanthus dioscoridis* L. (Acanthaceae), *Phlomoides tuberosa* (L.) Moench (Lamiaceae), *Bifora radians* Bieb. (Apiaceae)] were tested on *Leptinotarsa decemlineata* Say, 1824 (Coleoptera: Chrysomelidae) under laboratory conditions. Methanol extracts prepared from plant vegetative components were tested on *L. decemlineata*. *Heracleum platytaenium* extract was significantly more toxic against the egg stage than all other extracts, except for *A. millefolium* 5 d after treatment. It was followed by *A. millefolium* extract reducing the egg hatch rate to 15%. Significant mortality was not observed in the case of other plant extracts. In the second series of experiments, different dose-response bioassays with *H. platytaenium* against *L. decemlineata* eggs were conducted. The lowest egg hatch rate of 1% was observed at 7.5% [w/v (plant extract/acetone]. The greatest oviposition deterrent effect was seen with the *H. platytaenium* extract treatment, which resulted in no egg laying. Plant extracts showed a high level of repellent activity to *L. decemlineata* and their activity increased with extended incubation time. The greatest repellency was observed with the *A. millefolium* extract treatment, which gave 0.01% repellency in the first 15 min. These results show that *H. platytaenium* extract could be a useful toll in the control of *L. decemlineata*.

Keywords: Biopesticide, Colorado potato beetle, ovicidal effect, plant extract, repellency

Özet

Altı bitki ekstraktının [*Heracleum platytaenium* Boiss (Apiaceae), *Humulus lupulus* L. (Cannabaceae), *Achillea millefoliu*m L. (Asteraceae), *Acanthus dioscoridis* L. (Acanthaceae), *Phlomoides tuberosa* (L.) Moench (Lamiaceae), *Bifora radians* Bieb. (Apiaceae)], *Leptinotarsa decemlineata* Say 1824 (Coleoptera: Chrysomelidae)'ye karşı repellent, ovisidal ve yumurta bırakmayı engelleyici etkileri test edilmiştir. Bitkilerin vejetatif aksamlarından hazırlanan methanol ekstraktları *L. decemlineata* üzerinde test edilmiştir. En yüksek ovisidal etki beşinci gün itibari ile *H. platytaenium* uygulamasında gözlemlenmiş ve bunu *A. millefolium* ekstraktının etkisi izlemiştir. *Heracleum platytaenium* ekstraktının etkinliğini %15 yumurta açılım oranı ile *A. millefolium* ekstraktının etkinliği takip etmiştir. Diğer bitki ekstraktlarında önemli ölüm gözlenmemiştir. İkinci seri denemelerde Patates böceğinin yumurtalarına karşı *H. platytaenium*'un doz-etki çalışmaları yürütülmüştür. Önemli derecede düşük yumurta açılım oranı sadece %7.5 [w/v (bitki ekstraktı/aseton)] konsantrasyonda %1 etki ile gözlemlenmiştir. En yüksek yumurta bırakmayı engelleyici etki *H. platytaenium* ekstraktında görülmüş ve bu muamelede hiç yumurta bırakılmamıştır. Bitki ekstraktları yüksek oranda repellent aktivite göstermiş ve bu aktivite inkübasyon süresine bağlı olarak artmıştır. Test edilen bitki ekstraktları arasında en yüksek derecede uzaklaştırıcı etki %0.01'lik oransal değer ile ilk 15 dakikada *A. millefolium* ekstraktında saptanmıştır. Bu sonuçlar *H. platytaenium* ekstraktının *L. decemlineata* kontrolünde önemli bir potansiyele sahip olduğunu ortaya koymuştur.

Anahtar sözcükler: Biyopestisit, Patates böceği, yumurta bırakmayı engelleyici etki, bitki ekstraktı, repellent

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Introduction

Leptinotarsa decemlineata Say, 1826 (Coleoptera: Chrysomelidae), Colarado potato beetle is a polyphagous pest, and occurs over an area of 12 million km^2 in North America, Asia and Europe (Alyokhin, 2009). It causes serious damage to various crops from the Solanaceae family including potato, tomato and eggplant (Hsiao, 1978; Hare, 1990) and in the absence of pest control losses may reach 100% (Christie et al., 1991). Additionally, it is also a vector of certain plant viruses (Borror & De Long, 1966; Kısmalı, 1973; Jolivet et al., 1988; Booth et al., 1990).

Control of *L. decemlineata* almost totally relies on insecticides. Heavy insecticide usage to control *L. decemlineata* has led to many environmental problems (Ioannidis et al., 1991; Stewart et al., 1997; Mota-Sanchez et al., 2000). Insecticide resistance is the most serious problem in managing *L. decemlineata,* since it has become resistant to 54 compounds (Whalon et al., 2013). Alternative control methods for *L. decemlineata* are urgently needed for insect resistance management programs. Use of biopesticides in the control of pests has become common since 1990 following outbreaks of resistance of many insect species to synthetic insecticides. Biopesticides including microorganisms, including fungi, nematodes, bacteria and plant secondary metabolites, are important tools for management of resistant pest species. Plant secondary metabolites, e.g., terpenes, nitrogen containing compounds and phenolic compounds, are mainly produced when plants are stressed, e.g., by drought or pest attacks. These metabolites have been tested against many insect pest species and promising results for control of *L. decemlineata* have been reported (Hough-Goldstein, 1990; Scott et al., 2003, 2004; Gökçe et al., 2005, 2006, 2011; Alkan et al., 2015; Tampe et al., 2015).

Solvent systems used for extraction of plant secondary metabolites have effects on both yield and composition. Especially plant waxes, that cause low viscosity in the plant extracts suspension, are removed from the plant residue using non-polar solvent, e.g., hexane is used to obtain a more uniform plant residue (Hassan & Gökçe, 2014). Additionally, removing of plant waxes from the extracts may also increase bioactive compound concentrations in the plant extract suspension. In the present study, secondary metabolites from plants having various activities to different insect species (Gökçe et al., 2005, 2011), were extracted with different solvent systems and tested against the important potato to pest, *L. decemlineata*. The objectives of the study were to evaluate the selected plant extracts behavioral effects, including repellence, oviposition deterrence and ovicidal effects and to determine effective dose for specific activity. To achieve these objectives, the plant extracts were tested against *L. decemlineata* adults and dose-response bioassays were also conducted with promising extracts.

Material and Methods

Collection of plant materials

Details of the plant material used are presented in Table 1. The plants were identified to species at Istanbul University with reference to herbarium samples. The plants were collected in the spring and summer of 2009 as described by Gökçe et al. (2006). Plant materials were passed through preliminary purification process in that the plants parts to be used were separated from other parts. The plants parts, cone, leaf and stem, were placed on blotting papers in a dark room and were left to dry at room temperature (25°C) for 2 weeks. The dry plant materials were ground into small pieces by using a mill (M20 IKA Universal Mill, IKA Group, Wilmington, NC, USA) and transferred to 5-L glass jars and placed in a dark room at 15±5°C until used.

Table 1. Plants, analyzed parts and place of collection in Turkey

Botanical Name	Family	Analyzed part	Sample location
Humulus lupulus L.	Cannabaceae	Cone	Tokat
Heracleum platytaenium Boiss	Apiaceae	Leaf, Stem	Trabzon
Achillea millefolium L.	Asteraceae	Leaf, Stem, Flower	Tokat
Acanthus dioscoridis L.	Acanthaceae	Leaf, Stem, Flower	Erzincan
Phlomoides tuberosa (L.) Moench	Lamiaceae	Leaf, Stem, Flower	Erzincan
Bifora radians Bieb.	Apiaceae	Leaf, Stem	Tokat

Preparation of plant extracts

Plant extracts were obtained by maceration method as described by Alkan & Gökçe (2012). *Acanthus dioscoridis* L., *Heracleum platytaenium* Boiss and *Phlomoides tuberosa* (L.) Moench extracts were processed using three different solvents (hexane, ethyl acetate and methanol) to remove the plant waxes that prevent homogenous plant extract suspension being obtained. Two hundred g of dried plant material for each species were put into separate glass jars and then treated with solvents (hexane, ethyl acetate and methanol) according to their polarity range. The plant materials were first treated with hexane for 48 h, then the plant suspension was separated from plant materials using Whatman No. 4 filter paper. After that, the separated plant materials were treated with ethyl acetate further 48 h at room temperature. The plant suspensions were again filtered through the filter paper to separate the plant parts. Finally, methanol was added on the plant materials and then incubated 48 h under the same conditions. Similarly, the methanol soluble plant extracts were separated from the plant material using the filter paper. Excess hexane, ethyl acetate and methanol were evaporated from the plant suspension using a rotary evaporator (RV 05 Basic 1-B, IKA-Werke GmbH & Co. KG, Staufen, Germany). *Humulus lupulus* L., *Bifora radians* Bieb. and *Achillea millefolium* L. were only extracted with methanol because these plant species contain limited amount of the plant waxes. The total yield of plant extracts was about 10% of their dry weight. The plant residues were transferred into glass tubes, and stored at 4ºC. The methanol extracts were used in all the experiments described below.

Rearing of *Leptinotarsa decemlineata*

Leptinotarsa decemlineata larvae were reared at Gaziosmanpasa University, Faculty of Agriculture, Department of Plant Protection as described by Gökçe et al. (2006). *Leptinotarsa decemlineata* colony was continuously reared on potato plants (*Solanum tuberosum* L. cv. Granola) which were planted at Gaziosmanpasa University Research Station (Taşliçiftlik, Tokat, Turkey) in a 0.2-ha field used for organic potato production with no pesticide application in the previous 3 years. The potato tubers were planted in the first week of April each year. When the potato plants reached to three- to five-leaf stage*, L. decemlineata* adults from a lab colony were released into the field and all stages used in for this study collected when needed.

Single dose ovicidal effects

The ovicidal effects of the six plant extracts were tested on *L. decemlineata* egg masses 1 to 3 d old. Plant extracts were diluted with 70% acetone to give the concentration of 10% (w/v) plant extract/acetone suspension. Twenty µl of each plant extract suspension were applied to each egg mass (n ≈ 20 eggs) using a hand spray. In the control group, each mass was treated with 20 µl of 70% acetone. The egg masses on potato leaflets were then transferred into Petri dishes and the petiole of each leaflet was covered with a distilled water soaked cotton wool to prevent the leaflets withering. Egg hatch was recorded up to 7 d. Bioassays were set up in the randomized block design. The experiment was repeated on three different days (blocks) and each treatment in a block contained three subset groups of egg mass (n ≈ 60). Total around 180 eggs were used for each treatment.

Dose-response study with *Heracleum platytaenium* extract

Based on the single dose screening ovicidal test, a dose-response bioassay was carried out with *H. platytaenium* extract, given it gave the greatest ovicidal effect. The plant extract was diluted with 70% acetone to give 3, 5 and 7.5% (w/v) plant extract in acetone. The application of plant extract and incubation of egg masses were conducted as described above. Randomized block design was used and all treatments were replicated three times with three replicates of each dose and the control groups.

Oviposition deterrent effects

Oviposition deterrent effects of *H. platytaenium* and *H. lupulus* extracts, the most bioactive extracts in the preliminary test, were tested against *L. decemlineata* adults. Plant extract suspensions were prepared as described in the single dose ovicidal effect tests. A 3-5-leaf stage potato plant was sprayed with one of the extracts until run off using a hand spray. Control plants were treated with 70% acetone. The treated potato plants were left to dry at room temperature for 30 min. A choice-test was used for this experiment. One treated plant and one control plant were placed inside a curtain net cage, 60 x 60 x 60 cm, and two *L. decemlineata* adults (one female and one male) were released inside the cage and these were then incubated at 27ºC and a 16:8 h L:D photoperiod for 7 d. Paired treatments were replicated nine times. The number of eggs laid on the plants were recorded.

Repellent effects

In the repellent effect tests, the six plant extracts were screened against *L. decemlineata* adults. The plant extracts were prepared at 5% (w/v) in 70% acetone. Similar sized potato leaves, consisted of six to eight leaflets, were sprayed with a hand spray until run off. After application of the extracts, the petioles were wrapped with cotton wool moistened with distilled water to prevent the leaves wilting and the leaves left to dry under a fume hood for 30 min. A choice-test was used in the experiment. Two potato leaves (one treated and one control) were placed in a 150 x 300 x 50 mm plastic container. Ten mixed-sexed *L. decemlineata* adults were released into each container. To test both repellence and desensitizing of the adult beetles, the number of adults on each leaf was recorded after 15 min and 1, 12 and 24 h after release. The trial was repeated nine times for each plant extract-control paired combination.

Statistical analysis

The ovicidal test results were converted into percentages and arcsine transformed. The transformed data was analyzed by analysis of variance (ANOVA) and the means compared by Tukey's multiple comparison test. The number of egg laid in the oviposition deterrent test were compared with the paired t-test ($P < 0.05$). The results obtained in the repellence tests were converted to percentages and arcsine transformed. The converted data were analyzed with the paired-t test ($P < 0.05$).

Results and discussion

Heracleum platytaenium and *A. millefolium* extracts had the greatest ovicidal effect when compared with other extracts 5 d after treatment (DAT) ($F = 53.3$; $df = 6,14$; $P < 0.05$). The other extracts caused no significant reduction in hatching rate with 84.7% egg hatch rates for *H. lupulus*, 99.7% for *P. tuberose,* and 100% for both *B. radians* and *A. dioscoridis* 5 DAT (Table 2). *Heracleum platytaenium* and *A. millefolium* extracts produced similar ovicidal effects 6 DAT and these extracts were significantly different from the other treatments ($F = 276$, $df = 6,14$; $P = 0.05$). The hatch rates were nearly 100% for extracts 6 DAT. At 7 DAT, *H. platytaenium* and *A. millefolium* extracts remained significantly different from other treatments ($F = 276$; $df = 6,14$; $P < 0.05$) with 3.7% and 18.6 % hatching rates, respectively (Table 2).

Table 2. Ovicidal effect of plant extracts on *Leptinotarsa decemlineata* eggs over time

Extracts	Egg hatch rate ± SEM* (%)		
	5 DAT**	6 DAT	7 DAT
Control	99.4±0.47 ab***	99.4±0.47 a	99.4±1.40 a
Heracleum platytaenium	2.1±1.05 c	3.7±0.84 c	3.7±0.84 c
Humulus lupulus	84.7±15.18 b	100.0±0.00 a	100.0±0.00 a
Achillea millefolium	15.1±0.46 c	18.7±0.52 b	18.7±0.52 b
Bifora radians	100.0±0.00 a	100.0±0.00 a	100.0±0.00 a
Acanthus dioscoridis	100.0±0.00 a	100.0±0.00 a	100.0±0.00 a
Phlomoides tuberosa	99.7±0.37 ab	99.7±0.37 a	99.7±0.37 a

* SEM: Standard error of the mean;
** DAT: Days after treatment;
*** Means in a column followed by the same letter are not statistical significantly different (ANOVA P < 0.05, Tukey's test).

The testing of different doses of *H. platytaenium* extract on *L. decemlineata* eggs showed that the ovicidal effect of different concentrations were statistically different from each other and the control, except at 3%. The data indicated that the increasing concentration of *H. platytaenium* significantly decreased the egg hatch of *L. decemlineata* over time (5 DAT, F = 37.7; 6 DAT, F = 39.5; 7 DAT, F = 39.5; df = 3,9; P < 0.05) (Table 3). The greatest ovicidal effect was seen at 7.5% concentration, and it was only 1% at this concentration during the incubation period. This was followed by the 5% concentration, for which relatively low rate of egg hatch (<44%) was observed compared to the other concentrations and the control.

Table 3. Ovicidal effect of *Heracleum platytaenium* concentrations on *Leptinotarsa decemlineata* eggs

Extract concentration (%)	Egg Hatch Rate ± SEM* (%)		
	5 DAT**	6 DAT	7 DAT
7.5	1.0±0.59 c***	1.0±0.59 c	1.0±0.59 c
5	26.9±1.53 b	43.4±3.01 b	43.3±3.01 b
3	82.6±9.56 a	88.5±7.40 a	88.5±7.40 a
Control	99.4±0.44 a	99.7±0.44 a	99.4±0.44 a

* SEM: Standard error of the mean;
** DAT: Days after treatment;
*** Means in a column followed by the letter are not statistical significantly different (ANOVA, P < 0.05, Tukey's test).

The *H. platytaenium* extract had the greatest ovicidal effect in the single dose ovicidal test. A previous study on chemical components of *H. platytaenium* essential oil revealed that its essential oil contains isopropyl butyrate, octanal, heptyl acetate, hexyl butyrate, octyl acetate, (Z)-4-hexenyl butyrate, decanal, octanol, hexyl hexanoate and octyl butyrate (Iscan et al., 2004). Among these components, octyl acetate was the most abundant (Kürkçüoğlu et al., 1995) and is well known for being detrimental to insects (Carroll & Berenbaum, 2002). The *H. platytaenium* extract caused a significant reduction in egg hatch of *L. decemlineata*. The ovicidal activity of *A. millefolium* extract was not as high as that of the *H. platytaenium* extract, and it was the second most toxic extract among those tested. The other plant extracts did not show any ovicidal effects against *L. decemlineata* eggs. This difference in ovicidal activity of the tested extracts is likely to be related to their chemical composition. As mentioned above, octyl acetate is one of the main chemical component of *H. platytaenium* essential oil and this compound is known to have detrimental effects on insects. The ovicidal activity of *H. platytaenium* extract could be

attributable to this compound but further studies are needed to characterize the actual active compound(s). Notably, *H. lupulus* extract did not have any ovicidal effect on *L. decemlineata* eggs even though this extract is known to have high contact toxicities against the larvae and adults of this pest (Gökçe et al., 2006). This could be due to the chemical differences between the cuticle of egg and larvae. Solvent systems used for extraction of plant species have effects on both yield and composition. This leads to differences in insecticidal activities of the extracts. Arivoli & Tennyson (2013) showed that the solvent used for the preparation of plant extracts also effect the ovicidal activity of the extracts. Additionally, the ovicidal activity of the same plant ranged from 6 to 66% depending on the extraction solvent used (Jeyasankar et al., 2013).

The data presented in Table 4 shows that extracts of both *H. platytaenium* and *H. lupulus* had an oviposition deterrent effect on the *L. decemlineata* females. The greatest oviposition deterrence was seen with *H. platytaenium* extract and this was statistically different from the control ($t = 4.66$; $P < 0.05$). At the end of the incubation period, there were no eggs deposited on plants treated with *H. platytaenium* extract. An average of 8.8 eggs were laid on the plants treated with *H. lupulus* extract, which was significantly different from the control ($t = 4.29$; $P < 0.05$) (Table 4). Although oviposition deterrent effects of different plant extracts have been documented, there is no reports on the activity of *H. platytaenium* extracts against any insect species (Rajkumar & Jebanesan, 2005; Rehman et al., 2009; Tomasek & Dvorak, 2009; Sezer & Özalp, 2011; Singh et al., 2014; Dehghani & Ahmadi, 2013). High oviposition deterrent activity of *H. platytaenium* can be due to presence of larger concentrations of octyl acetate and octyl butyrate (Carroll & Berenbaum, 2002). Various oviposition deterrent effect of *H. lupulus* against different insect species *L. decemlineata* was reported in previous studies (e.g., Gökçe et al., 2005) While *H. lupulus* extract did not display oviposition deterrent activity against *Choristoneura rosaceana* Harris, 1841 (Lepidoptera: Tortricidae) and *Argyrotaenia velutinana* Walker, 1863 (Lepidoptera: Tortricidae), it showed a strong effect against *L. decemlineata* (Gökçe et al., 2005). These differences in oviposition deterrent activity could be related to differences in the taxonomic group of the test insect or due to behavioral differences between insect species. For example, *C. rosaceana* and *A. velutinana* larvae feed on various fruit and forest trees as well as vegetables, so that their tolerance to any kind of odor may be higher than that of *L. decemlineata*.

Table 4. Oviposition deterrent effects of *Heracleum platytaenium* and *Humulus lupulus* extracts against *Leptinotarsa decemlineata*

Extracts	Number of laid eggs	
	Control	Extract
Heracleum platytaenium	86.3 a[1] (38.8-133.8)	0.0 b (0.0-0.00)
Humulus lupulus	59.2 a (34.1-84.3)	8.8 b (4.6-22.2)

[1]Means in a row followed by the same letter are not statistical significantly different (paired t-test, $P < 0.05$).

The plant extracts tested also showed significant repellence of *L. decemlineata* adults. Repellent effects of different plant extracts against insect pest species has been tested in many studies with some promising results (Schearer, 1984; Wyrostkiewicz, 1987; Chiasson et al., 1994; Mateeva, 1998; Przybylski, 2002; Pavela, 2004; Sarbu et al., 2004; Wawrzyniak & Lamparski, 2008). Among the plant extracts tested, the greatest repellency was observed with *A. millefolium* extract with 0.01% proportional repellency in the first 15 min. It was significantly different from the control ($t = 5.99$; $P = 0.00$). This was followed by *H. platytaenium* with 0.03% proportional repellency with most of the released adults moving to the control potato leaflets. Notably, neither *B. radians* nor *H. lupulus* extracts showed any significant repellency in the first 15 min. Desensitizing of insect species is one of the important factors affecting their successful use in pest control. However, in the present study the repellent activity of plant extracts was consistent over the entire incubation period (Figure 1). After 12 and 24 h, *A. millefolium*, *B. radians* and *A. dioscoridis* did not show any significant repellency when compared to the control. Notably, *H. lupulus* repellency increased as the incubation time was extended and the number of insect moving to the plant extract treated leaflets dropped to 0.03% proportional repellency after 24 h (Figure 1). A similar increase in activity of plant extracts with increasing incubation time was also reported by Alkan & Gökçe (2012),

who tested the contact toxicity and behavioral effects of *Tanacetum abrotanifolium* (L.) Druce, 1914 (Asteraceae) stem and flower extracts against *Sitophilus oryzae* (L.) (Coleoptera: Curculionidae) and *Sitophilus granarius* (L.) (Coleoptera: Curculionidae) and reported that the plant extract repellency activity increased in parallel to the incubation time.

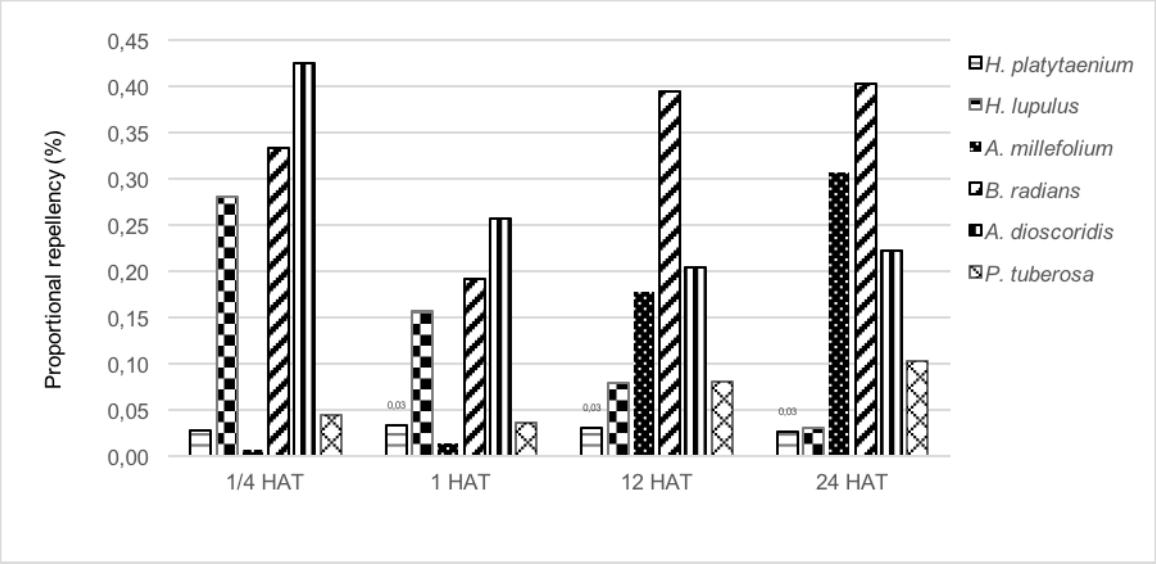

Figure 1. Proportional repellency of plant extracts against *Leptinotarsa decemlineata* at four time periods after treatment (HAT, Hours after treatment).

In the current study, ovicidal activity, oviposition deterrence and repellency of the extracts were tested against the *L. decemlineata*. Of the plant extracts tested, *H. platytaenium* extract showed the greatest ovicidal activity and repellency, and indicated that this extract has potential for development as biopesticide in a Colorado potato beetle control program. However, further studies are needed to elucidate the active compound(s) in this extract. Following the elucidation of active compound(s), further laboratory and field studies should be undertaken to examine the full potential of this plant extract in the control of *L. decemlineata*.

Acknowledgments

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Original article (Orijinal araştırma)

Evaluation of spermatheca morphology and systematics of some *Terellia* Robineau-Desvoidy, 1830 (Diptera: Tephritidae) species[1]

Bazı *Terellia* Robineau-Desvoidy, 1830 (Diptera: Tephritidae) türlerinin spermateka morfolojisi ve sistematik açıdan değerlendirilmesi

Murat KÜTÜK[2*] **Esra DOĞAN[3]** **Mehmet YARAN[4]**

Summary

Spermatheca structures of four species, *Terellia colon* (Meigen, 1826), *Terellia gynaecochroma* (Hering, 1937), *Terellia quadratula* (Loew, 1869) and *Terellia ruficauda* (Fabricius, 1794), belonging to the genus *Terellia* Robineau-Desvoidy, 1830 (Diptera: Tephritidae) were examined with scanning electron microscopy (SEM). The specimens were collected from various provinces in Turkey between 1999 and 2013. The samples were coated by gold/palladium with the Emitech SC 7620 Sputter Coater and examined with a Jeol 6390 LV SEM operated at 10 kV. The ultrastructure of the spermatheca for each of the studied species was described and differences between species were identified. The results add to the morphological variations recorded among these species.

Keywords: Fruit flies, SEM, spermatheca, Tephritidae, *Terellia*

Özet

Terellia Robineau-Desvoidy, 1830 (Diptera: Tephritidae) cinsine ait *Terellia colon* (Meigen, 1826), *Terellia gynaecochroma* (Hering, 1937), *Terellia quadratula* (Loew, 1869) ve *Terellia ruficauda* (Fabricius, 1794) dört türün spermateka yapıları taramalı elektron mikroskobu (SEM) ile incelenmiştir. Türler 1999-2013 yılları arasında Türkiye'nin çeşitli illerinden toplanmıştır. Örnekler Emitech SC 7620 Sputter Coater ile örneklere altın/paladyum kaplaması yapılarak Jeol 6390 LV SEM ile 10 kV' da incelendi. Her bir tür için ince spermateka yapıların tanımlamaları türler arasındaki morfolojik farklılıklar belirlendi. Sonuçlar, bu türler arasında kaydedilen morfolojik varyasyonlara katkıda bulunmaktadır.

Anahtar sözcükler: Meyve sinekleri, SEM, spermateka, Tephritidae, *Terellia*

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Introduction

The spermatheca is an accessory female reproductive organ that occurs in all orders of insects with the exception of the Protura and Collembola (Matsuda, 1976). It is a complex organ of the female reproductive system, and it varies greatly in shape and histology (Pendergrast, 1957). It serves for uptake and storage of the sperm from the time of mating until the time to fertilize the eggs (Lay et al., 1999).

The spermatheca (receptaculum seminis) is an ectodermal gland which opens to the anterior portion of the female oviduct. It has a significant role in sperm storage and egg fertilization (Kocorek & Danielczok-Demska, 2002). After mating, the spermatheca provides energy and nutrients to keep the sperm alive until a suitable time for egg fertilization and oviposition occurs (Pabalan et al., 1996). The period of sperm storage can range from hours to months in different insects, and for years in exceptional cases, such as honey bees (Candan et al., 2014).

The female genitalia tract in *Terellia* Robineau-Desvoidy, 1830 species (ovarian, spermatheca and auxiliary glands) extends over the entire abdomen and comprises two spermatheca, located within the fourth or fifth abdominal segments. Each spermatheca consists of three different parts: spermathecal bulb (reservoir), pumping region (intermediate part), spermathecal duct (ductus receptaculi) (Pluot-Sigwalt & Lis, 2008).

Spermathecal structure of *Terellia* has been examined using light microscope. Korneyev et al. (2013) has presented light microscope images of *Terellia virens* (Loew, 1846) group species and Zarghani et al. (2017) light microscope images of *Terellia amberboae* Korneyev & Merz, 1996 group species. In this study, the ultrastructure of the spermatheca in *Terellia* species (Tephritidae) is described for the first time using scanning electron microscopy (SEM).

Four species, *Terellia* (*Terellia*) *colon* (Meigen, 1826), *Terellia gynaecochroma* Hering, 1937, *Terellia quadratula* (Loew, 1869) and *Terellia ruficauda* (Fabricius, 1794), with similar wing pattern were selected for this study. Spermathecal structures, aspect ratio spermathecal bulb and spermathecal duct and the spicules on spermathecal bulb surface were defined. Also, similarities and differences between these species were determined. The structure of the spermatheca varies between species of Diptera and is useful as a systematic character.

Material and Methods

The terminology used for the spermathecal morphology was that of Mcalpine (1981). Spermathecal structure consists of spermathecal bulb, valve, pumping region and spermathecal duct. In addition, during the designation process, the aspect ratio was also used as a distinctive property.

Specimens examined: *Terellia* species which were collected between 1999 and 2014 from different regions of Turkey and preserved as museum material (Zoological Museum, Gaziantep University, Turkey).

Dissection of specimens: Specimens were treated with 10% potassium hydroxide solution for 3-4 days, then placed in Petri dishes containing 96% ethanol. Genital structures, other than the spermathecal structures, were removed using fine-tipped forceps. The isolated spermatheca were placed in glycerin. The preparation of the specimens followed Candan & Erbey, 2006. Observations were made using a stereomicroscope (Olympus SZX12, Olympus Optical Co. Ltd., Tokyo, Japan).

Preparing of specimens: For SEM. For SEM observation, spermathecal structures were dried with air for about 10 min and placed on SEM stubs. These samples were coated by gold/palladium with the Emitech SC7620 Sputter Coater (Quorum Technologies, Laughton, UK) and examined with a Jeol 6390LV SEM (JOEL Ltd., Tokyo, Japan) operated at 10 kV, in Gaziantep University Entomology Laboratory and Electron Microscopy unit.

Results and Discussion

Results

The ultrastructure of the spermatheca of *T. colon, T. gynaecochroma, T. quadratula* and *T. ruficauda* is shown in Figures 1-4, respectively.

***Terellia colon* (Meigen, 1826)**

Spermathecal bulb: width 62 µm, height 236 µm, aspect ratio 0.26; distinct corncob shaped (Figure 1a); apex swollen compared to base point; small fingerlike spicules on the bulb surface (Figures 1b, c); a cavity made by inward in the region close to apex; pores present both at the end of the fingerlike spicules and on the surface (Figure 1d); gland canaliculus stretched out from pores on the fingerlike spicules. Spermathecal duct: width 76 µm, height 257 µm, aspect ratio 0.29; distinctive; wide at apex, narrow at base; transverse muscle fibers densely present (Figure 1e).

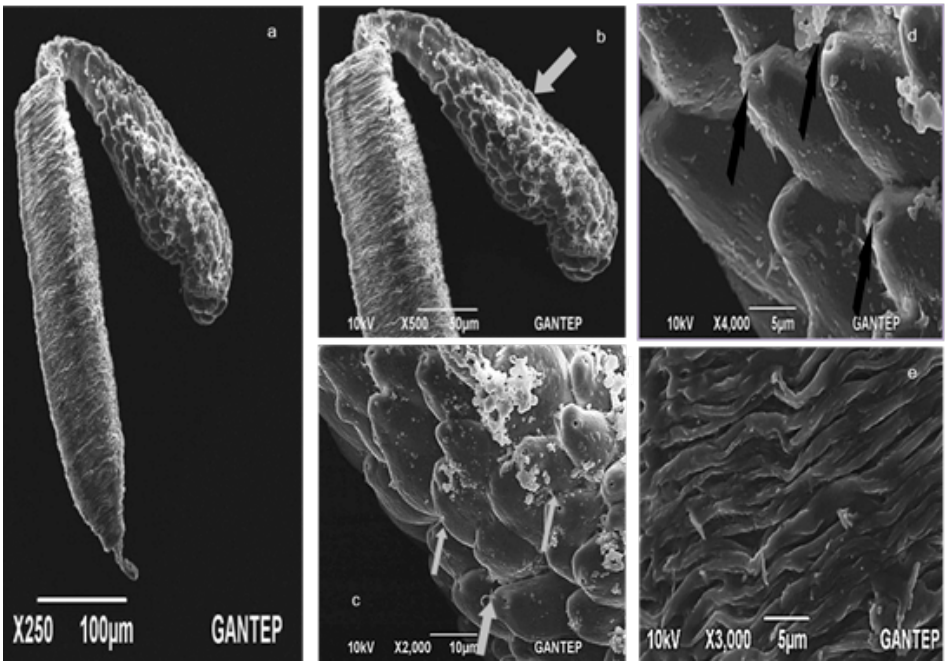

Figure 1. Spermatheca structures of *Terellia colon* (Meigen, 1826) a) general view of spermatheca, b) spermathecal bulb, c) pores, d) gland canaliculus, and e) muscle fibers.

***Terellia gynaecochroma* (Hering, 1937)**

Spermathecal bulb: width 24 µm, height 96 µm, aspect ratio 0.24; flattened and spatulate (Figure 2a); thick thorn-shaped spicules on the bulb surface; long and thick, directed upward spicules present densely at apex (Figure 2b); fewer on base; spicules directed upward on apex; point-shaped and fewer spicules present on the base; no pores and gland canaliculus present on bulb surface; pores in different sizes on base (Figure 2c). Spermathecal duct: width 37 µm, height 201 µm, aspect ratio 0.18; transverse muscle fibers densely present (Figure 2e); pumping region encloses apex of the duct like a cap.

***Terellia quadratula* (Loew, 1869)**

Spermathecal bulb: width 137 µm, height 279 µm, aspect ratio 0.49; elliptic; spicules thorn-shaped (Figure 3b); apex blunt; spicules directed upward on apex; fewer spicules present and towards duct on the base; fewer, almost none, small pores (Figure 3d); gland canaliculus present. Spermathecal duct: width 119 µm, height 217 µm, aspect ratio 0.55; transverse muscle fibers aligned regularly (Figure 3f); muscles in groove-shaped; pores present (Figure 3f); valve present on apex.

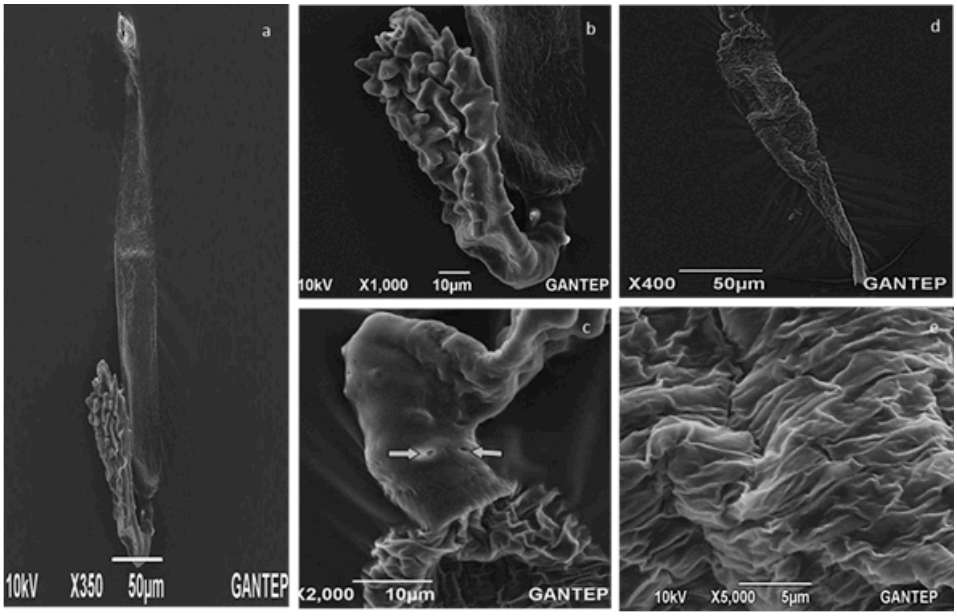

Figure 2. Spermatheca structures of *Terellia gynaecochroma* Hering, 1937 a) general view of spermatheca, b) spermathecal bulb, c) pores, d) spermathecal channel, and e) muscle fibers.

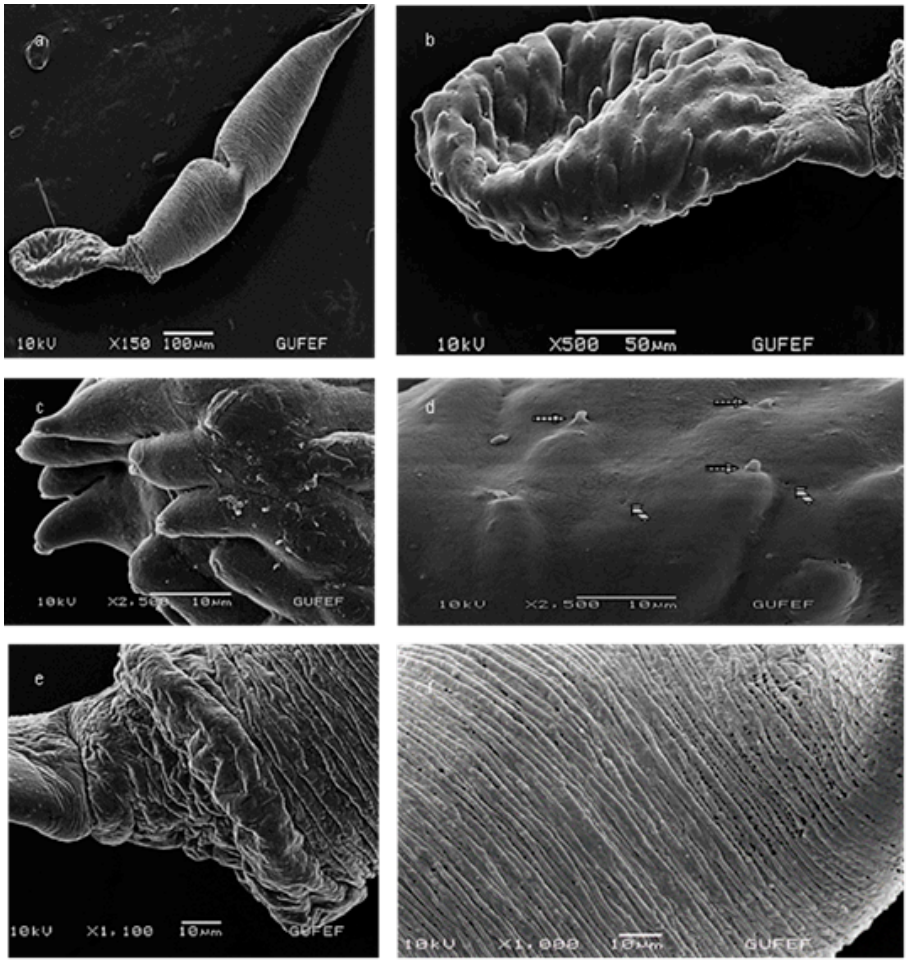

Figure 3. Spermatheca structures of *Terellia quadratula* (Loew, 1869) a) general view of spermatheca, b) spermathecal bulb, c) surface of bulb, d) gland canaliculus and pores, e) valve, and f) muscle fibers and pores.

***Terellia ruficauda* (Fabricius, 1794)**

Spermathecal bulb: width 59 µm, height 136 µm, aspect ratio 0.43; pear-shaped; long fingerlike spicules; denser on apex, few on base; pores in large numbers and of different sizes (Figure 4c); gland canaliculus densely present (Figure 4d). Spermathecal duct: width 28 µm, height 153 µm, aspect ratio-0.18; transverse muscle fibers aligned regularly (Figure 4f); muscles in groove-shaped; valve exists on apex (Figure 4e).

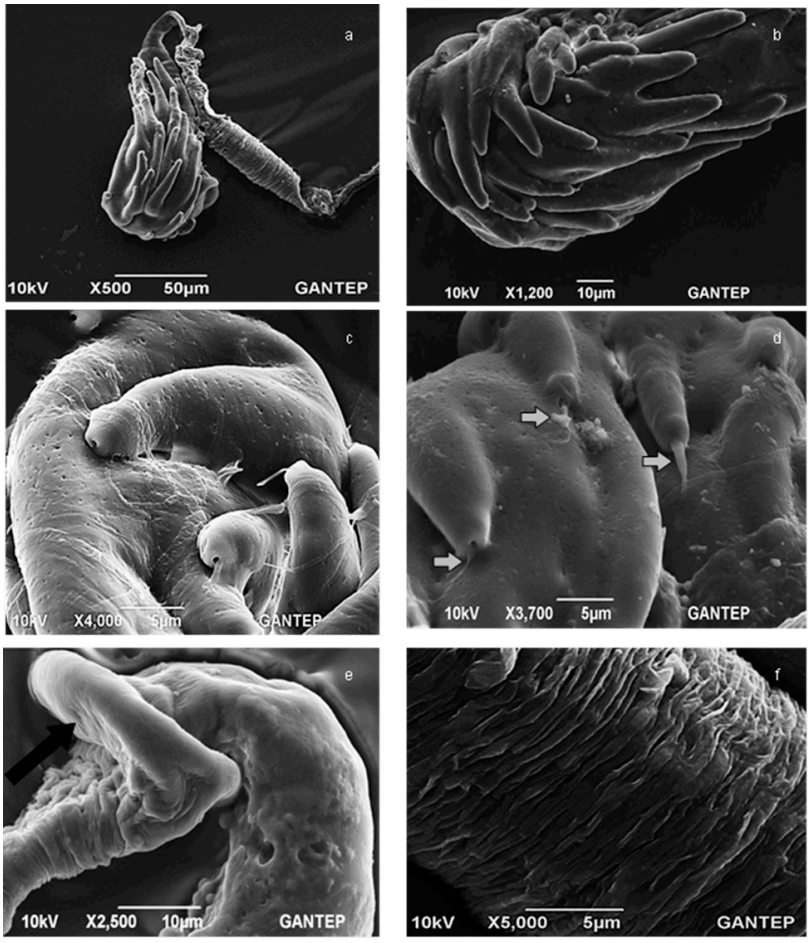

Figure 4. Spermatheca structures of *Terellia ruficauda* (Fabricius, 1794) a) general view of spermatheca, b) spermathecal bulb, c) pores, d) gland canaliculus, e) valve, f) muscle fibers.

Discussion

There are few published studies on the surface morphology of spermatheca compared to studies on egg morphology. There are some studies and descriptions about spermatheca structures of some *Terellia* species using light microscope (Korneyev, 2006; Korneyev et al., 2013; Zarghani et al., 2017). Surface morphology of spermatheca of genus *Terellia* has not been examined using electron microscope. Therefore, there is insufficient information on spermathecal structure of *Terellia*. In this report, we present descriptions and detailed images of spermathecal structure of some *Terellia* species.

Scanning electron microscopy revealed major differences in spermatheca ultrastructure of the four *Terellia* species studied. *Terellia colon* could be easily separated from the other species due to its corncob-shaped spermathecal bulb. In *T. gynaecochroma,* the spermathecal bulb was spatulate with thick thorn-shaped spicules. Moreover, pores were not evident on the bulb surface, unlike in the other *Terellia*

spp. Whereas, *T. quadratula* could be distinguished by its elliptically shaped spermathecal bulb with a blunt apex. Only in this species were pores have been found in the channel structure. *Terellia ruficauda* was also differentiated by its pear-shaped bulb, larger number and different sizes of pores, gland canaliculus being densely present. A valve structure was not evident on the apexes of the spermathecal ducts of *T. quadratula* and *T. ruficauda*.

The aspect ratio of the spermathecal bulbs was also differed between the four *Terellia* species. The aspect ratios were 0.49 and 0.24 for *T. quadratula* and *T. gynaecochroma,* respectively. Aspect ratios of spermathecal ducts were 0.18 in both *T. gynaecochroma* and *T. ruficauda*. This ratio (0.55) was greatest in *T. quadratula*. Transverse muscle fibers aligned regularly in the ducts of *T. quadratula* and *T. ruficauda*.

Consequently, it is clear that spermatheca structure is particularly useful for the systematics of *Terellia*. Also, spermatheca morphology of species could be used for species definition just as other morphological properties. Also, it is likely that more accurate identification and classification can be made using this morphological criterion, especially in otherwise similar species. Spermatheca morphology will provide an extra criterion for reliable identification of new species. Consequently, the results of this study make a significant contribution by demonstrating the value of spermatheca morphology as a diagnostic character for distinguishing similar species.

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Original article (Orijinal araştırma)

Detection and identification of citrus long-horned beetle, *Anoplophora chinensis* (Forster, 1771) (Coleoptera: Cerambycidae) a new pest in Antalya Province, Turkey by sequencing of mtCOI region[1]

Antalya ilinde yeni bir zararlı Turunçgil uzun antenli böceği, *Anoplophora chinensis* (Forster, 1771) (Coleoptera: Cerambycidae)'in tespiti ve mtCOI gen bölgesi sekansına göre tanımlanması

Nurdan TOPAKCI[2] **Utku YÜKSELBABA[3]*** **Hüseyin GOCMEN[3,4]**

Summary

In 2016, *Anoplophora chinensis* (Forster, 1771) (Coleoptera: Cerambycidae) was recorded for the first time on *Acer negundo* L. in Antalya Province, Turkey. Genetic identification of adult samples was made based on approximate alignment of a 729-bp sequence of the mitochondrial cytochrome oxidase I gene region. The sequence alignment in the BLAST analysis from the NCBI Genbank showed 99% similarity with *A. chinensis*. The sequence data was deposited to NCBI Genbank. A phylogenetic analysis was performed by using the sequence data belonging to *A. chinensis* and some species of same genus obtained from the NCBI. According to the phylogenetic analysis, populations of *A. chinensis* from Antalya and China were placed in the same subgroup and therefore it is postulated that the origin of the Antalya population is China.

Keywords: *Anoplophora chinensis*, DNA barcoding, mtCOI, phylogenetic analysis

Özet

Bu çalışma ile *Anoplophora chinensis* (Forster, 1771) (Coleoptera: Cerambycidae) Antalya ilinde ilk kez 2016 yılında *Acer negundo* L. bitkisi üzerinde tespit edilmiştir. Bununla birlikte, çalışmada *A. chinensis*'e ait ergin bireylerden DNA ekstraksiyonu yapılmıştır. Elde edilen DNA örneklerinin mitokondrial sitokrom oksidaz I gen bölgesinin amplifikasyonu sonucunda yaklaşık 729 bç sekans dizilimi elde edilmiştir. Elde edilen veriler, NCBI Genbank' da BLAST analizine tabi tutulmuş ve *A. chinensis* ile %99 benzerlik gösterdiği tespit edilmiştir. Sekans dizilimi NCBI'a kaydedilmiş, Genbank'ta *A. chinensis* ve aynı cinse ait bazı türlerin sekans verilerinden yararlanılarak filogenetik analiz yapılmıştır. Filogenetik analize göre Antalya *A. chinensis* popülasyonu Çin popülasyonu ile aynı alt grupta yer almış ve Antalya popülasyonunun kökeninin Çin olabileceği ağırlık kazanmıştır.

Anahtar sözcükler: *Anoplophora chinensis*, DNA barkodlama, mtCOI, filogenetik analiz

[1] Abstract of the study were presented as poster in The Eurasian Agriculture & Natural Sciences Congress (20-23 September 2017) Bishkek- Kirghizstan.

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Introduction

Being one of the largest families within the Coleoptera, the Cerambycidae includes over 11 subfamilies with more than 35,000 species in 4,000 genera (Lawrence, 1982). The citrus long-horned beetle, *Anoplophora chinensis* (Forster, 1771), a species in the Cerambycidae family, is native to China, Japan and North Korea (Ge et al., 2014). In June 2014, the pest was detected in Şile-Istanbul, which was the first record for Turkey (Hızal et al., 2015).

Adults are black with females (about 40 mm) larger than males (about 25 mm). The antennae have 11 segments, with white or light blue bands. The elytra are narrow in males, wider and rounded in females and there are many irregular white spots on the elytra. The legs are a light blue color (Figure 1).

Figure 1. Adults of *Anoplophora chinensis* (Forster, 1771).

The citrus long-horned beetle is distinguished by tubercles on the elytra from the Asian long-horned beetle, *Anoplophora glabripennis* (Motschulsky, 1853), which has a similar geographic distribution and causes similar damage (Hu et al., 2009). *Anoplophora malasiaca* (Thomson, 1865) is a synonym of *A. chinensis* (Lingafelter & Hoebeke, 2002).

The eggs of *A. chinensis* are on average 5 mm, elongate and creamy white. They turn yellowish brown when ready to hatch (Lieu, 1945). The larvae are legless and full-grown larvae are about 50 mm long. They are creamy white and with a yellowish chitinized pattern on the prothorax. The pupae are 27-38 mm long (Gyeltshen & Hodges, 2005) (Figure 2).

Figure 2. *Anoplophora chinensis* (Forster, 1771): a) Egg, b) larva and c) pupa (Haack et al., 2010).

Anoplophora chinensis commonly feeds on *Acer* spp., *Aesculus hippocastanum* L., *Alnus* spp., *Betula* spp., *Carpinus* spp., *Citrus* spp., *Cornus* spp., *Corylus* spp., *Cotoneaster* spp., *Crataegus* spp., *Fagus* spp., *Lagerstroemia* spp., *Malus* spp., *Platanus* spp., *Populus* spp., *Prunus laurocerasus* L., *Pyrus* spp., *Rosa* spp., *Salix* spp. and *Ulmus* spp. with *Casuarina* spp., *Cryptomeria* spp., *Ficus* spp., *Hibiscus* spp., *Litchi* spp., *Mallotus* spp., *Melia* spp. and *Morus* spp. among the others also recorded as hosts (Anonymous, 2013).

Females feeding and mating on young twigs on the upper parts of host plants move towards the bottom of the tree to search for a suitable place to deposit their eggs. T-shaped cracks are made in the bark during egg deposition with the ovipositor. The females make 3-4-mm long cuts in the bark with their mandibles. Only one egg is deposited into each wound. Eggs are usually deposited at the base of the trunk, root collars or exposed roots (Maspero et al., 2007). It can take one or two years to complete their life cycle under natural conditions and they adults usually live for about 1-3 months between May and August (Anonymous, 2013).

Adults cause damage by feeding on thin branches and leaves. The greatest damage is caused by the feeding of larvae. The freshly-hatched larvae feed on primarily cambium layers, and in time larvae feed on xylem tissues. Since damage to the xylem inhibits the flow of water and nutritions in the host tree, they cause death of branches and ultimately death of the whole tree. Through the attacks of the larvae, trees are weakened and become more susceptible to disease and wind damage (Sjöman et al., 2014).

The objectives of the present study were to identify the Antalya population of *A. chinensis* by molecular methods and to determine its evolutionary relationship with the sibling groups from different countries deposited in the NCBI Genbank database based on mtCOI sequences through phylogenetic analysis. To the best of our knowledge, this was the first study on the identification of *A. chinensis* based on mtCOI region in Turkey. The sequence information of the Antalya population was added to Genbank. Thus, this will contribute to genetic information about the insect and provide a reference source in different studies. In addition, brief information about the detection of the pest in Antalya is been provided.

Material and Methods

Determination of pests and obtaining pest samples

Upon the detection of adult pests on the Akdeniz University Campus on 3 June 2016, trees in the region were examined. Sampling was done randomly at irregular intervals. Male and female of the pest were identified mostly at the lower portions of the trunk and rarely at the top. Adult samples were collected, put into plastic bags, labeled and immediately brought to the laboratory for examination. Some of the samples were kept at -20ºC for a short time before molecular analysis.

DNA extraction and PCR amplification

In DNA extraction studies, leg parts belonging to live or just dead insects were used as described by Muraji et al (2011). DNA was extracted in accordance with the CTAB protocol for invertebrates (Folmer et al., 1994) from three individuals. The isopropanol step in the CTAB protocol was modified to allow samples to be kept overnight before being dissolved in 40 µl distilled water.

For the amplification of the mitochondrial cytochrome oxidase I (mtCOI) gene, CI-J-2195 and TL2-N-3014 universal primers reported to amplify this gene in coleopteran insects (Simon et al., 1994) were used. In accordance with to Yükselbaba & Göçmen (2016), the conditions specified were modified (0.5 µl DNA in 24 µl). The PCR cycling protocol for mtCOI was 94ºC for 5 mins, followed by 35 cycles (94ºC for 50 s, 50ºC for 50 s and 72ºC for 45 s) and with a final extension at 72ºC for 5 mins.

The PCR products obtained were sequenced in both directions by IONTEK (Istanbul, Turkey). Data acquired were inspected and the best sequence registered with NCBI (KX530204).

Phylogenetic analysis

The phylogenetic analyses were done by distance matrix using DNAdist program (Felsenstein, 1993). BLAST analysis for identification of species and seeking homologous *A. chinensis* sequence alignments was done with the NCBI database (http://www.ncbi.nlm.nih.gov). The multiple alignment analysis was done online by M-Coffee (http://www.tcoffee.org). To reconstruct the phylogenetic tree from evolutionary distance data, a phylogenetic tree was constructed according to the neighbor-joining method and Kimura two-parameter model (Saitou & Nei, 1987). Bootstrap was performed with 1000 repetitions to produce the phylogenetic tree. Species belonging to *Anoplophora* in the genbank used for phylogenetic analysis are detailed in Table 1.

Table 1. Organisms used from Genbank for sequence alignment

Genbank Accession No	Organism	Origin
KX530204	*Anoplophora chinensis*	Antalya, Turkey
AB500900	*Anoplophora chinensis*	Oita, Japan
AB533639	*Anoplophora chinensis*	Japan
AB500897	*Anoplophora chinensis*	Shizuoka, Japan
AB500901	*Anoplophora chinensis*	Hiroshima, Japan
AB500896	*Anoplophora chinensis*	Ibaraki, Japan
AB500902	*Anoplophora chinensis*	Kagoshima, Japan
KT726932	*Anoplophora chinensis*	Xiamen, China
AB533640	*Anoplophora macularia*	Japan
GU003937	*Anoplophora horsfieldi*	China
DQ768215	*Anoplophora glabripennis*	unknown
KF737826	*Anoplophora lurida*	unknown
EU599206	*Monochamus galloprovincialis*	unknown

Results

During sampling of damaged trees, both females and males were detected, however, no eggs or larvae were found. *Anoplophora chinensis,* native to China, Japan, and North Korea (Ge et al., 2014), was first recording in Antalya in this study. While adults and evidence of their feeding were rarely observed on the upper parts of trees, both females and males were mostly detected around the exit holes (6-10 mm diameter) near the base of the trunk (Figure 3); and it was observed that these adults could fly short distances when disturbed. Similarly, Haack et al. (2010) observed that exit holes were found within 50 cm of the base of the tree and in exposed roots.

Figure 3. Damage to *Acer negundo* L. by larvae and adults of *Anoplophora chinensis* (Forster, 1771).

Molecular studies

The amplified PCR products from the mtCOI gene were approximately 800 bp as expected for the primers used (Figure 4).

From the sequence analysis, an approximate 729 bp nucleotide alignment was obtained and registered to the NCBI Genbank (KX530204). The BLAST analysis showed 98-99% similarity with *A. chinensis* sequences, 96-97% with *Anoplophora macularia* (Thomson, 1865) and 93% with *A. glabripennis*.

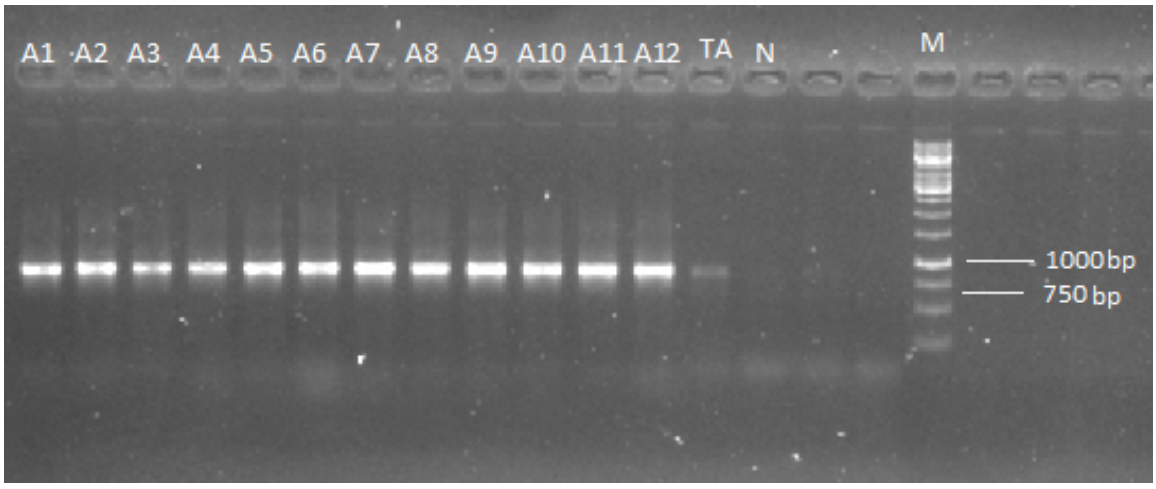

Figure 4. *Anoplophora chinensis* (Forster, 1771) PCR reaction image (1% TAE gel). A1-A12, *A. chinensis*; TA, *Tuta absoluta* (Meyrick, 1917) (for positive control); N, negative control; M, 1 kb marker.

According to the sequence alignments obtained from the Genbank, a phylogenetic tree was constructed (Figure 5). According to the neighbor-joining phylogenetic tree *A. macularia* and *A. chinensis* are in separate groups. Likewise, the Chinese and Japanese populations of *A. chinensis* are in different subgroups. The specimens from Antalya are placed in the same subgroup as those from China. The Japanese populations fall into two separate subgroups.

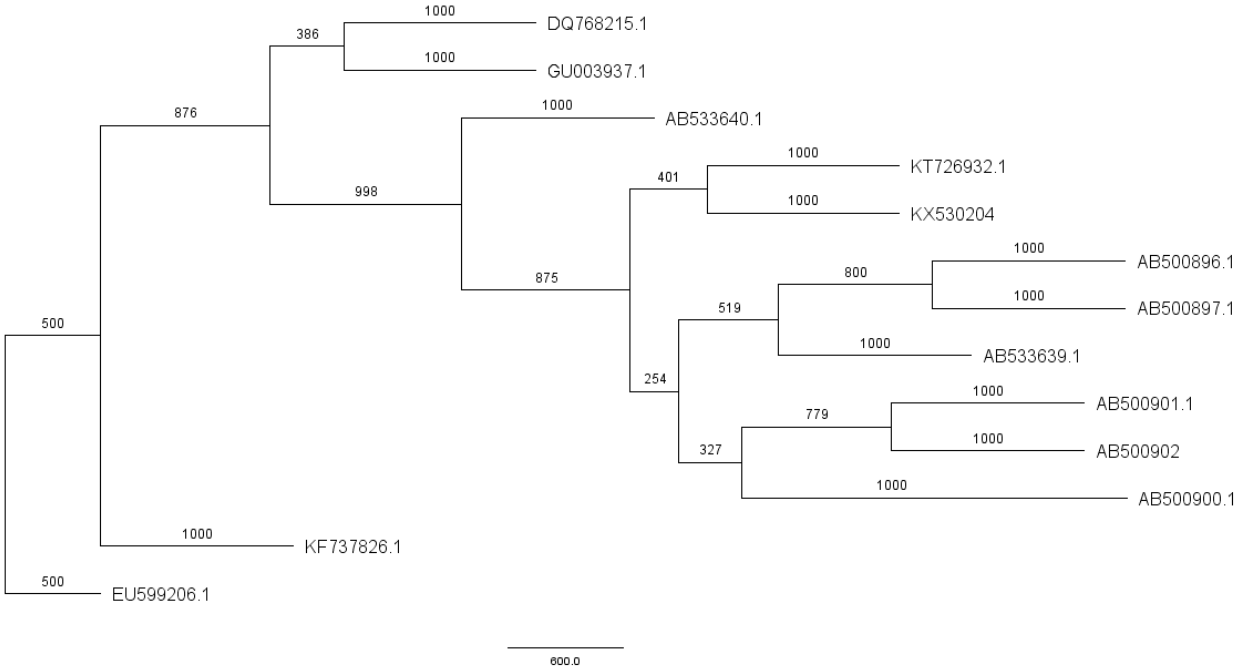

Figure 5. Phylogenetic tree from genebank sequences of *Anoplophora chinensis* (Forster, 1771) populations. The phylogenetic tree was constructed according to the neighbor-joining method and the Kimura two-parameter model with 1000 bootstrap replicates. The Genebank accessions numbers for each sample are as in Table 1.

Discussion

In this study, even though there are a variety of tree species (*Acer* spp*., Albizia julibrissin* Durazz*., Jacaranda mimosifolia* D. Don*, Melia azedarach* L*., Ailanthus* spp. and *Pinus* spp.) on the Akdeniz University Campus where the pest was detected, adult individuals and adult exit holes were only detected in *Acer negundo* L. In observations made in different areas of the province on the same dates, adult exit holes were also mostly found in *Acer* spp. (data not shown). These findings suggest that of the available tree, the first preference of the pest was *Acer* spp.. Similarly, in Europe, in some of the recorded cases, *A. chinensis* seems to attack *Acer* trees first (Herard et al., 2006, Peverieri & Roversi, 2010). Eradication methods for this new pest, *A. chinensis,* for our country are important. It is necessary to determine whether *A. chinensis* has caused any damage, particularly in locations from where plants are imported, and to avoid imports of plant, wooden material and timber from these locations. It is also important to eradicate adults when first detected, and to cut and remove trees intensely damaged by the pest. Potential host plants around trees on which the pest was detected should be examined carefully and any suspected ones should be removed (Hızal et al., 2015). Signs of the infestation include traces of egg laying in the bark (T-shaped cracks), existence of excrements from larvae activity and wood dusts, color change on the bark, deformations, bark grooves, galleries made by larvae, adult exit holes, damage from adult feeding, upward deaths in tree branches and death of whole trees. Adult exit holes occur in the lower 50 cm of the tree trunk, mostly at 15-20 cm (Anonymous, 2013, Maspero, 2015). Strangi et al. (2013) indicated that it would be useful for plant health research to be able to reliably detect *A. chinensis* infestation by molecular analysis of larval excrement and that this method could be used as a detection tool for plant inspection.

In their study on the mitochondrial genome of *A. chinensis*, Li et al. (2015) reconstructed the phylogenetic relations of 12 Lamiinae species based on mtCOI nucleotide sequences using the Bayesian inference method. They determined that the phylogenetic tree was divided into three major clades and that *Anoplophora* [*A. glabripennis, A. chinensis* and *Anoplophora lurida* (Pascoe, 1857)] and *Monochamus* [*Monochamus alternatus* Hope, 1842*, Monochamus urussovi* (Fisch., 1806) and *Monochamus sutor* (L., 1758)] were placed in the third lineage. Apart from such molecular studies supporting morphological studies in the Lamiinae, in another study, Muraji et al. (2011) determined that the genetic variation of species belonging to the *Anoplophora* in Japan was identified by mtDNA sequence and the species was split into two main groups. In the same study, they noted that a sample taken in East Honshu was the same as *A. chinensis* from China. Ohbayashi et al. (2009) studied the phylogenetic analysis of *Anoplophora* species and their relatives in the Lamiinae with respect to mtCOI gene region.

This study registered with Genbank the first mtCOI region sequence of *A. chinensis* from Turkey for a population from Antalya. Data recorded in Genbank can be used as a reference for further studies. Phylogenetic analysis was performed to determine that the Antalya population is likely to have originated from China not Japan. More complete knowledge of the dispersal mechanisms and genetic phylogeographical structure of *A. chinensis* is essential for developing effective management strategies.

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Original article (Orijinal araştırma)

Armored scale insects (Hemiptera: Sternorrhyncha: Diaspididae) on ornamental plants in Adana, Turkey[1]

Adana ili süs bitkilerinde zararlı Kabuklubit (Hemiptera: Sternorrhyncha: Diaspididae) türleri

Asime Filiz ÇALIŞKAN KEÇE[2*] **Mehmet Rifat ULUSOY[2]**

Summary

A study in 2011-2013 to identified armored scale insect species (Hemiptera: Diaspididae) feeding on ornamental plants in Adana, Turkey. Scale insect samples were collected from leaves, stems and fruits from different host plants and examined in the laboratory. Twenty-three species of Diaspididae were determined from 180 samples: *Aonidia lauri* (Bouché, 1833), *Aonidiella aurantii* (Maskell, 1879), *Aspidiotus hedericola* Leonardi 1920, *Aspidiotus nerii* (Bouché, 1833), *Carulaspis juniperi* (Bouché, 1851), *Carulaspis minima* (Signoret, 1869), *Chrysomphalus dictyospermi* (Morgan, 1889), *Comstockaspis perniciosa* (Comstock, 1881), *Diaspis echinocacti* (Bouché, 1833), *Diaspidiotus pyri* (Lichtenstein, 1881), *Diaspidiotus uvae* (Comstock, 1881), *Epidiaspis leperii* (Signoret, 1869), *Hemiberlesia cyanophylli* (Signoret, 1869), *Hemiberlesia lataniae* (Signoret, 1869), *Lepidosaphes conchiformis* (Gmelin, 1790), *Lepidosaphes gloverii* (Packard, 1869), *Leucaspis pusilla* Löw, 1883, *Melanaspis inopinata* (Leonardi, 1913), *Unaspis euonymi* (Comstock, 1881), *Parlatoria oleae* (Colvée, 1880), *Parlatoria pergandii* (Comstock, 1881), *Pseudaulacaspis pentagona* (Targioni Tozzetti, 1886) and *Torosaspis farsianus* (Balachowsky & Kaussari, 1955). Among these species, *D. uvae* and *T. farsianus* are new records for Turkey.

Keywords: Armored scale insects, new records, Turkey

Özet

Bu çalışma, 2011-2013 yılları arasında Adana İli park ve süs bitkileri üzerindeki zararlı kabuklubit türlerinin saptanması amacıyla yürütülmüştür. Yapılan sürvey çalışmalarında farklı konukçu bitkilerden, kabuklubitin üzerinde bulunduğu yaprak, dal ve meyve örnekleri alınarak laboratuvarda incelenmiştir. Toplanan 180 örneğin teşhis edilmesi sonucunda Diaspididae familyasına ait 23 tür saptanmıştır. Bu türler; *Aonidia lauri* (Bouché, 1833), *Aonidiella aurantii* (Maskell), *Aspidiotus hedericola* Leonardi 1920, *Aspidiotus nerii* (Bouché, 1833), *Carulaspis juniperi* (Bouché, 1851), *Carulaspis minima* (Signoret, 1869), *Chrysomphalus dictyospermi* (Morgan, 1889), *Comstockaspis perniciosa* (Comstock, 1881), *Diaspis echinocacti* (Bouché, 1833), *Diaspidiotus pyri* (Lichtenstein, 1881), *Diaspidiotus uvae* (Comstock, 1881), *Epidiaspis leperii* (Signoret, 1869), *Hemiberlesia cyanophylli* (Signoret, 1869), *Hemiberlesia lataniae* (Signoret, 1869), *Lepidosaphes conchiformis* (Gmelin, 1790), *Lepidosaphes gloverii* (Packard, 1869), *Leucaspis pusilla* Löw, 1883, *Melanaspis inopinata* (Leonardi, 1913), *Unaspis euonymi* (Comstock, 1881), *Parlatoria oleae* (Colvée, 1880), *Parlatoria pergandii* (Comstock, 1881), *Pseudaulacaspis pentagona* (Targioni Tozzetti, 1886) ve *Torosaspis farsianus* (Balachowsky & Kaussari, 1955). Bu türler arasında, *D. uvae* ile *T. farsianus* ülkemiz için yeni kayıt niteliğindedir.

Anahtar sözcükler: Kabuklubit, yeni kayıtlar, Türkiye

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Introduction

In recent decades, plant pests have been spreading very quickly because the trade of live plant material has increased sharply. Scale insects (Hemiptera: Sternorrhyncha: Coccoidea: Diaspididae) are often cryptic in habit and can escape detection during quarantine inspection of plants; they have become one of the most important invasive pest groups worldwide (Miller et al., 2002; Kaydan et al., 2013a; Ülgentürk et al., 2014). Scale insects are important pests, especially on perennial plants such fruit and nut trees, ornamental shade trees and shrubs and forest trees, as well as on plants grown in greenhouses and indoors (Kosztarab & Kozár, 1988). They cause serious damage to plants, such as by direct feeding on phloem sap. Due to sap depletion, the plant vigor is reduced and chlorotic areas may develop at the feeding sites. Moreover, premature leaf drop, and distortion of the stems and bark can be damage caused by scale insects. Also, some scale insect species can transmit viral diseases (Sforza et al., 2003).

The armored scale insects, Diaspididae is the largest family of scale insects including 2400 species in 380 genera (Miller & Davidson, 2005). Of these, 638 species in 118 genera occur in the Palearctic Region. Members of this family are highly specialized, such as having legless, wingless, eyeless sap-feeding females covered by a waxy shield, and unusual life histories with males that do not feed in the adult stage (Rosen, 1990). In the countries where they cause damage, most armored scale insect pests are known to be invasive species (Miller & Davidson, 2005). Due to the increase in international trade in live plants, scale insects have started to become a serious threat to the agricultural economy. Armored scales are usually known as pests of perennial plants; Miller & Davidson (1990) compiled a list of 199 species considered as pests in some part of the world.

Armored scales have a rather unusual biology. Each female is enclosed beneath or within a separate, non-living scale cover constructed of wax and other compounds. This enclosed lifestyle has mandated that armored scales no longer produce honeydew waste from the anus, and has caused them to alter their feeding behavior to tap individual plant cells rather than feeding on phloem sap like other, related scale insects. Armored scales can feed on any part of the plant, including as leaves, fruits, stems, branches and roots (Miller & Davidson, 2005). Heavy infestations can become so dense that they cover the bark of twigs and branches and frequently are associated with plant dieback.

The first study of Diaspididae in Turkey was conducted by Bodenheimer (1941); many others have since studied the family in different regions of Turkey and on various host species (Çanakçıoğlu, 1977; Yaşar, 1995; Şengonca et al., 1998; Uygun et al., 1998; Karsavuran et al., 2001; Kaydan et al., 2001, 2005, 2009a, b; Yaşar et al., 2003; Tanyürek & Yaşar, 2005). More recently Kaydan et al. (2013b) reported that, among all the scale insect families in Turkey, Diaspididae is the most abundant, represented by 134 species in 42 genera.

Adana is one of the largest cities in southeastern Turkey, and has been developing for many years in terms of agriculture, industry and trade. The city has many parks and gardens, most of them well maintained, which in the warm climate are open all year long without the need of winter maintenance. The main woody trees in parks and streets are in the genera *Citrus* (Rutaceae), *Hibiscus* (Malvaceae), *Quercus* (Fagaceae), *Nerium* (Apocynoidae), *Fraxinus* (Oleaceae) and *Juniperus* (Cupressaceae). Many scale insect species are regarded as important pests in parks. Although several studies have been conducted to identify the pest species on ornamental plants in Adana (Çalışkan et al., 2015; Kaydan et al., 2013a), until now there has no specific and comprehensive study of armored scale insect species. This paper reports the survey and identification of armored scale insects in Adana, with data on their hosts and distribution.

Material and Methods

Armored scale insects were collected from woody ornamental plants and shrubs in parks and recreation areas in Adana during spring and summer in 2011 to 2013. Samples consisted of plant material infested by scale insects. Each sample was put into a labeled plastic bag and taken to the laboratory for examination. Samples were prepared for observation under the light microscope using the slide-mounting method described by Kosztarab & Kozár (1988). The morphological terminology used follows that of Miller & Davidson (2005).

All the samples in the 2011-2013 survey were collected by the first author. The collection data (host locality, collection date and phenological stages of the host plant) are given in the results section. Previously recorded distribution and host-plant data were taken from ScaleNet (García et al., 2016).

Dry material and permanent slide mounts are deposited at the collection of the Plant Protection Department, Agricultural Faculty, Çukurova University, Balcalı-Adana, Turkey.

Results and Discussion

The research material consisted of 180 adult samples from Adana. Among these samples, 23 species were determined, and are listed and discussed below.

Aspidiotinae

Aonidia lauri (Bouché, 1833) (Figure 1a)

Synonyms: *Aspidiotus lauri* Bouché, 1833; *Aonidia purpurea* Targioni Tozzetti 1868 (García et al., 2016).

Material examined: Atatürk Park, *Laurus nobilis* L. (Lauraceae), 04.II.2012, 4 ♀♀; Kurttepe, *L. nobilis*, 07.II.2012, 17 ♀♀; Adnan Menderes Avenue, *Ficus retusa* L. (Moraceae), 10.III.2012, 1 ♀; Sanatçılar Park, *L. nobilis*, 21.III.2012, 7 ♀♀; Cumhuriyet Park, *L. nobilis*, 04.IV.2012, 4 ♀♀; Kazım Karabekir Park, *L. nobilis*, 08.IV.2012, 1 ♀; Botanik Park, *L. nobilis*, 04.VII.2012, 1 ♀.

Comments: The species occurs mainly in the Palearctic Region; there are only two records from the Nearctic (García et al., 2016). It has been recorded in Turkey previously, from the Mediterranean, Southeastern Anatolia and Marmara Regions, on *L. nobilis* (Kaydan et al., 2013b).

Aonidiella aurantii (Maskell, 1879)

Synonyms: *Aspidiotus aurantii* Maskell, 1879; *Chrysomphalus coccineus* (Maskell, 1879) Lindinger (García et al., 2016).

Material examined: Kenan Evren Avenue, *Citrus aurantium* L. (Rutaceae), 23.XI.2011, 13 ♀♀; Balcalı, *Euonymus japonicus* Thunb. (Celastraceae), 15.I.2012, 2 ♀♀; Atatürk Park, *E. japonicus*, 04.III.2012, 3 ♀♀; Merkez Park, *E. japonicus*, 09.III.2012, 3 ♀♀; Reşatbey, *C. aurantium*, 09.III.2012, 3 ♀♀; Adnan Menderes Avenue, *L. nobilis*, 10.III.2012, 16 ♀♀; Çoban Dede, *C. aurantium*, 11.III.2012, 2 ♀♀; Sakıp Sabancı Natural Park II, *Rosa* spp. (Rosaceae), 25.III.2012, 3 ♀♀; Cumhuriyet Park, *L. nobilis*, 04.IV.2012, 7 ♀♀; Kurttepe, *Acacia saligna* (Labill.) H. L. Wendl. (Fabaceae), 07.IV.2012, 6 ♀♀; Barış Park, *L. nobilis*, 22.IV.2012, 5 ♀♀; Barış Park, *Ceratonia siliqua* L. (Fabaceae), 22.IV.2012, 2 ♀♀; Mavi Avenue, *C. siliqua*, 22.IV.2012, 10 ♀♀; Sinanpaşa Park, *L. nobilis*, 06.V.2013, 4 ♀♀; Beyazevler, *Rosa* spp., 06.VIII.2013; 6 ♀♀; Hayal Park, *C. aurantium*, 18.I.2013, 6 ♀♀.

Comments: *Aonidiella aurantii* has a relatively cosmopolitan distribution (García et al., 2016). It has been recorded on 263 plant host species belonging to 87 families (García et al., 2016). In Turkey, it has been recorded only in the Mediterranean and Aegean Regions, on *Acacia* spp*., Citrus* spp., *Rosa* spp. and *Amaranthus viridis*, and (Kaydan et al., 2013b). The species is regarded as one of the most important pests of *Citrus* worldwide.

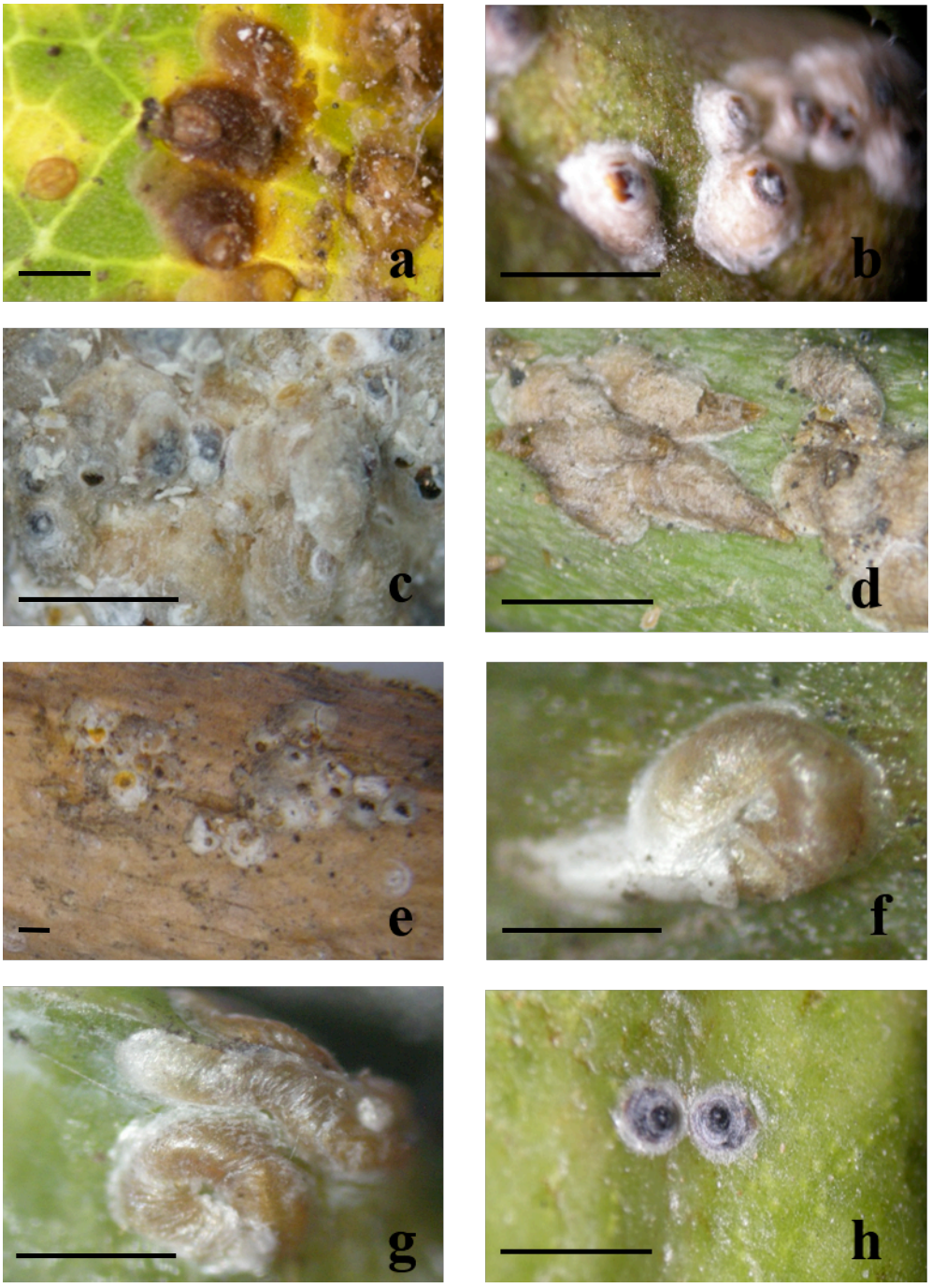

Figure 1. a. *Aonidia lauri*; b, c. *Diaspidiotus uvae*; d. *Unaspis euonymi*; e. *Pseudaulacaspis pentagona*; f, g. *Torosaspis farsianus*; h. *Parlatoria oleae*. (The scale bar in each figure = 0.5 cm).

Aspidiotus hedericola Leonardi, 1920

Synonym: *Aspidiotus hedericola* Leonardi, 1920 (García et al., 2016).

Material examined: Atatürk Park, *Hedera helix* L. (Araliaceae), 29.III.2012, 9 ♀♀; Balcalı, *H. helix*, 16.VII.2013, 3 ♀♀; Beyazevler, *H. helix*, 06.VIII.2013, 2 ♀♀.

Comments: This Palearctic species was recorded previously on *H. helix* and *L. nobilis* and in the Mediterranean, Aegean and Marmara Regions of Turkey (Kaydan et al., 2013b).

Aspidiotus nerii (Bouché, 1833)

Synonyms: *Diaspis obliquum* Costa, 1829; *Aspidiotus vagobundus* (Bouché, 1833) Tao, 1999 (García et al., 2016).

Material examined: Çoban Dede, *A. saligna*, 11.III.2012, 1 ♀; Çoban Dede, *Nerium oleander* L. (Apocynaceae), 11.III.2012, 9 ♀♀; Şehit Tolga Kargıoğlu Park, *A. saligna*, 21.IV.2012, 13 ♀♀; Galeria, *N. oleander*, 26.V.2012, 1 ♀; Balcalı, *N. oleander*, 02.VI.2012, 5 ♀♀; Botanik Park, *C. siliqua*, 04.VII.2012, 1 ♀; Botanik Park, *A. saligna*, 04.VII.2012, 4 ♀♀; Botanik Park, *Punica granatum* L. (Lythraceae), 04.VII.2012, 2 ♀♀; Balcalı, *Melia azedarach* L. (Meliaceae), 04.VII.2012, 2 ♀♀; Aytaç Durak Rest Area, *Elaeagnus pungens* var. *aurea* L. (Elaeagnaceae), 18.VII.2012, 3 ♀♀; Adnan Menderes Avenue, *N. oleander*, 20.VII.2012, 10 ♀♀; Adnan Menderes Avenue, *Cercis siliquastrum* L. (Fabaceae), 20.VII.2012, 1 ♀; Yalçın Park, *Robinia pseudoacacia* L. (Fabaceae), 16.VIII.2012, 2 ♀♀; Balcalı, *Ligustrum japonicum* L. (Oleaceae), 21.IX.2012, 1 ♀; Balcalı, *H. helix*, 01.VI.2013, 5 ♀♀; Balcalı, *Jasminum officinale* L. (Oleaceae), 16.VII.2013, 4 ♀♀.

Comments: This species has a cosmopolitan distribution and has been recorded on 546 plant species belonging to more than 100 families (García et al., 2016). In Turkey, it has been recorded in the Mediterranean, Aegean, Black Sea and Marmara Regions, on *Acacia* spp. (Kaydan et al., 2013b).

Chrysomphalus dictyospermi (Morgan, 1889)

Synonyms: *Aspidiotus dictyospermi* Morgan, 1889; *Chrysomphalus jamaucebsis* (Morgan, 1889) Chou, 1985 (García et al., 2016).

Material examined: Merkez Park, *F. retusa*, 03.III.2012, 3 ♀♀.

Comments: This polyphagous species has a cosmopolitan distribution (García et al., 2016). It has been recorded previously in the Mediterranean, Aegean and Black Sea Regions of Turkey on *Aralia* spp., *Buxus microphylla* Siebold & Zucc., *C. siliqua, Citrus* × *aurantium* L., *Citrus limon* (L.) Osbeck, *Citrus* × *sinensis* (L.) Osbeck, *Dracena* spp., *Dracena deremensis* (L.) Ker Gawl., *Eriobotrya japonica* (Thunb.) Lindl. (Rosaceae), *Euonymus japonicus* Thunb., *Ficus carica* L. (Moraceae), *Jacobaea maritima* (L.) Pelser & Meijden and *Taxus* sp. (Kaydan et al., 2013b).

Comstockaspis perniciosa (Comstock, 1881)

Synonyms: *Aonidia fusca* Maskell, 1895; *Quadraspidiotus perniciosus* (Comstock, 1881) Ferris, 1938 (García et al., 2016).

Material examined: Balcalı, *Pittosporum heterophyllum* Franch. (Pittosporaceae), 15.II.2012, 2 ♀♀; 75th Year Park, *Pyracantha coccinea* M. Roem. (Rosaceae), 06.IV.2012, 2 ♀♀; Huzurevleri 3rd Park, *P. coccinea*, 21.IV.2012, 3 ♀♀.

Comments: This species has a cosmopolitan distribution and quite a wide range of host species worldwide (García et al., 2016). *Comstockaspis perniciosa* has been recorded previously as a polyphagous species on many ornamental and fruit trees in the Mediterranean, Aegean, Black Sea and Middle Anatolia Regions of Turkey (Kaydan et al., 2013b).

Diaspidiotus pyri (Lichtenstein, 1881)

Synonyms: *Aspidiotus pyri* Lichtenstein, 1881; *Diaspidiotus pyri* (Lichtenstein, 1881) Danzig & Pellizzari, 1998 (García et al., 2016).

Material examined: Sinanpaşa Park, *Viburnum opulus* L. (Adoxaceae), 06.V.2013, 1 ♀.

Comments: *Diaspidiotus pyri* is a Palearctic species that has been recorded on 22 plant host species belonging to the families Betulaceae, Fabaceae, Hippocastanaceae, Carpinaceae, Moraceae, Oleaceae, Platanaceae, Rosaceae and Salicaceae. It has been recorded on *Malus sylvestris* (L.) Mill., *Salix* spp. in the East Anatolian, Black Sea, Marmara and Middle Anatolia Regions of Turkey (Kaydan et al., 2013b).

Diaspidiotus uvae (Comstock, 1881) (Figures 1b,c and 2)

Synonyms: *Aspidiotus uvae* Comstock, 1881; *Aspidiotus uvaspis* Lindinger, 1937 (García et al., 2016).

Material examined: Atatürk Park, *L. nobilis*, 04.III.2012, 1 ♀; Adnan Menderes Avenue, *L. nobilis*, 10.III.2012, 2 ♀♀; Adnan Menderes Avenue, *A. saligna*, 10.III.2012, 1 ♀; Çoban Dede, *Berberis thunbergii* DC. (Berberidaceae), 11.III.2012, 2 ♀♀; Adnan Menderes Avenue, *Fraxinus excelsior* L. (Oleaceae), 21.III.2012, 31 ♀♀; Adnan Menderes Avenue, *Morus alba* L. (Moraceae), 21.III.2012, 6 ♀♀; Şehit Ast. Ahmet Umut Kahya Park, *Schinus molle* L. (Anacardiaceae), 04.IV.2012, 5 ♀♀; Belediye Evleri District, *A. saligna*, 06.IV.2012, 7 ♀♀; Zübeyde Hanım Park, *Prunus cerasifera* Ehrh. (Rosaceae), 08.IV.2012, 4 ♀♀; Mehmet Akif Ersoy Park, *Forsythia* × *intermedia* Zabel (Oleaceae), 08.IV.2012, 1 ♀; Kurttepe, *A. saligna*, 12.IV.2012, 12 ♀♀; Güney Yıldız Park, *F. excelsior*, 15.IV.2012, 3 ♀♀; Güney Yıldızı Park, *Duranta erecta* L. (Verbenaceae), 15.IV.2012, 5 ♀♀; Barış Park, *P. cerasifera*, 22.IV.2012, 3 ♀♀; Dilberler Park, *Celtis caucasica* Willd. (Cannabaceae), 02.VI.2012, 1 ♀; Balcalı, *B. thunbergii*, 23.VI.2012, 1 ♀; Mahfesığmaz District, *Platanus orientalis* L. (Platanaceae), 04.VII.2012, 1 ♀; Dilberler Park, Merkez Park, *R. pseudoacacia*, 20.V.2012, 2 ♀♀; Merkez Park, *Paulownia tomentosa* (Thunb.) Steud. (Paulowniaceae), 26.V.2012, 2 ♀♀; Turgut Özal Avenue, *P. coccinea*, 30.V.2012, 2 ♀♀; Çoban Dede, *Philadelphus coronarius* L. (Hydrangeaceae), 18.VII.2012, 2 ♀♀; Yüreğir, *M. alba*, 20.VII.2012, 12 ♀♀; Adnan Menderes Avenue, *C. siliquastrum*, 20.VII.2012, 1 ♀; Çınarlı Park, *Jacaranda mimosifolia* D. Don (Bignoniaceae), 27.VII.2012, 3 ♀♀; Doğal Park, *R. pseudoacacia*, 02.VIII.2012, 3 ♀♀; Atatürk Avenue, *H. helix*, 08.IX.2012, 2 ♀♀; Karacaoğlan, *R. pseudoacacia*, 09.IX.2012, 5 ♀♀; *C. siliquastrum*, 08.V.2013, 2 ♀♀; Kenan Evren Avenue, *M. alba*, 27.VII.2013, 9 ♀♀.

Field characters: Adult female scale cover with white or grayish white, flat, circular or slightly elongate felted scale with central or subcentral exuviae yellow or orange. The small yellow/orange parts are the cast skins (exuviae) and the larger gray part is the felted secretions produced by the insect.

Adult female body pear shaped, head and thorax often sclerotized in older adult females. Eyes usually each represented by sclerotized spur or dome on prothorax near the intersegmental line with mesothorax, rarely absent. Antennae each with one seta. Median lobes developed, second and third pairs of lobes absent or rarely represented by small, unsclerotized points. Median lobes separated by narrow space, medial margins of lobes usually slightly divergent apically, lateral margins converging towards midline, each with one lateral notch and one medial notch. Lobes 2 and 3, when present, simple, each represented by unsclerotized point, without lateral notches. Plates between median lobe and lobe 2, and between lobes 2 and 3, usually with noticeable tines; plates between median lobes absent. Paraphyses as follows: each median lobe with small paraphysis on medial margin; paraphysis present in each space between position of lobe 2 and median lobe, on medial margin of each of lobes 2 and 3, and in space between positions of lobes 2 and 3. On dorsum of pygidium, anal opening located between median lobes and level of vulva. Long dorsal seta situated laterad of each lobe. Macroducts of two sizes: larger size present on pygidium between median lobes and on segments V to VII in marginal and submarginal areas. Pygidial macroducts absent; prepygidial macroducts of two sizes, large size in submedial areas of any or all of metathorax to abdominal segment IV, smaller size in submarginal areas of head or prothorax to segment II or III. On venter, pygidial microducts present in submarginal and marginal areas of segment V, sometimes on VI; prepygidial ducts of one size in submarginal and marginal areas of segments III and IV, present submedially near mouthparts and spiracles. Prevulvar pores in five indefinite clusters (median cluster composed of one or two pores only) (based on Miller & Davidson, 2005).

Comments: *Diaspidiotus uvae* has been recorded on 12 different host species belonging to the Agavaceae, Betulaceae, Juglandaceae, Moraceae, Platanaceae, Rosaceae and Vitaceae in the Nearctic Region, and in the Azores, Canary Islands, Italy, Madeira Islands, Portugal and Spain in the Palearctic Region (García et al., 2016).

This is the first record of *D. uvae* in Turkey. The species is regarded as a plant pest in vineyards in the USA. (Hollinger, 1923; Johnson et al., 1999; Miller & Davidson, 2005; Zimmer, 1912). Further studies are needed to understand its distribution and host-plant diversity in Turkey and other Mediterranean countries.

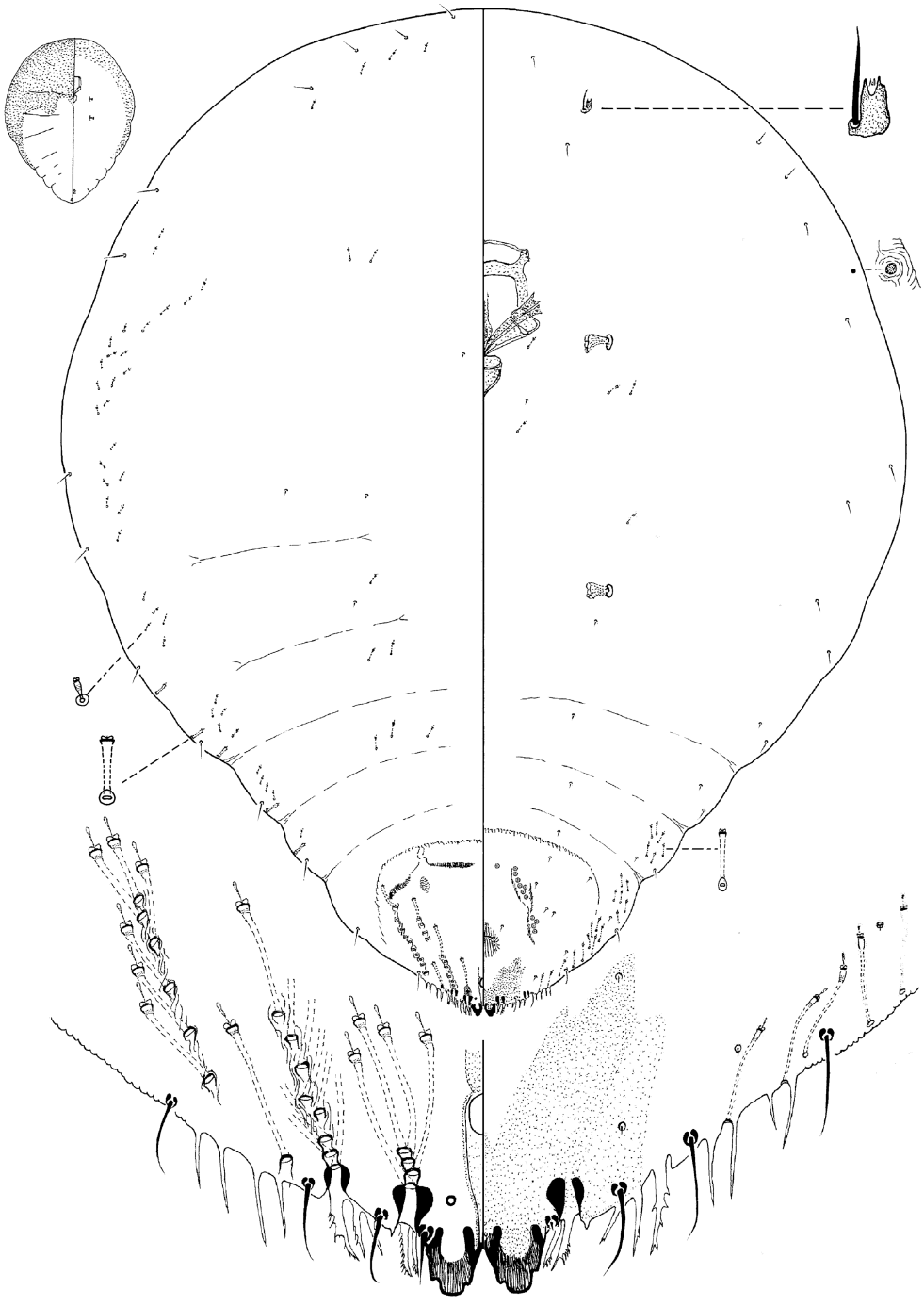

Figure 2. *Diaspidiotus uvae* after Miller & Davidson (2005), with modifications.

Hemiberlesia cyanophylli (Signoret, 1869)

Synonyms: *Aspidiotus cyanophylli* Signoret, 1896; *Aspidiotus cyanophlli* (Signoret, 1896) Chou, 1985 (García et al., 2016).

Material examined: Balcalı, *Gazania rigens* (L.) Gaertn var. *rigens* (Asteraceae), 13.X.2012, 7 ♀♀; Barajyolu, *G. rigens* var. *rigens*, 31.VII.2013, 3 ♀♀.

Comments: *Hemiberlesia cyanophylli* feeds on more than 180 plant species belonging to 72 families, with a worldwide distribution (García et al., 2016). It has been recorded previously in the Aegean, Marmara and Middle Anatolia Regions of Turkey on *Brasiliopuntia brasiliensis* (Willd.) A.Berger*, Cactus* spp., *Echinopsis chamaecereus* H.Friedrich & Glaetzle*, Dianthus caryophyllus* L.*, Gasteria bicolor* (L.) Haw.*, Gasteria carinata verrucosa* (Mill.) van Jaarsv. and *J. maritima* (Kaydan et al., 2013b).

Hemiberlesia lataniae (Signoret, 1869)

Synonyms: *Aspidiotus lataniae* Signoret, 1869; *Aspidiotus implocatus* (Signoret) Chou, 1985 (García et al., 2016).

Material examined: Hayal Park, *Albizia julibrissin* Willd. (Leguminosae), 18.I.2014, 2 ♀♀.

Comments: This cosmopolitan and polyphagous species has been recorded previously in the Aegean and Marmara Regions of Turkey (Kaydan et al., 2013b).

Melanaspis inopinata (Leonardi, 1913)

Synonyms: *Aonidiella inopinata* Leonardi, 1913; *Pelomphala inopinata* (Leonardi, 1913) Lupo, 1954 (García et al., 2016).

Material examined: Sanatçılar Park, *Chaenomeles speciosa* (Sweet) Nak. (Rosaceae), 21.III.2012, 3 ♀♀; Adnan Menderes Avenue, *F. excelsior*, 25.III.2012, 3 ♀♀; Balcalı, *P. coccinea*, 15.V.2012, 7 ♀♀; Balcalı, *Pistacia atlantica* Desf. (Anacardiaceae), 04.VII.2012, 1 ♀.

Comments: *Melanaspis inopinata* has a Palearctic distribution, with 42 host records from 13 different families (García Morales et al., 2016). The species has been recorded previously in the Aegean, Mediterranean and Middle Anatolian Regions of Turkey on *Arbutus unedo* L.*, Bauhinia* sp.*, Celtis* sp.*, C. siliquastrum, Malus pumila* Miller*, Prunus* sp.*, P. avium, Pyrus communis* L. and *Astragalus* sp. (Kaydan et al., 2013b).

Diaspidinae

Carulaspis juniperi (Bouché, 1851)

Synonyms: *Aspidiotus juniperi* Bouché, 1851; *Diaspis taxicola* (Bouché, 1851) Baccetti, 1960 (García et al., 2016).

Material examined: Yüreğir, *Platycladus orientalis* (L.) Franco (Cupressaceae), 18.VII.2012, 1 ♀.

Comments: *Carulaspis juniperi* has a wide range of distribution all over the world (García Morales et al., 2016). Juniper scale is restricted to conifers, but is most commonly collected on *Juniperus* spp. (Miller & Davidson, 2005). It has been recorded previously in the Aegean and Mediterranean Regions of Turkey on *Cupressus sempervirens* L.*, Juniperus excelsa* and *Platy. orientalis* (Kaydan et al., 2013b).

Carulaspis minima (Signoret, 1869)

Synonyms: *Diaspis carueli* Signoret, 1869; *Carulaspis carueli* (Signoret, 1969) Borchsenius, 1966 (García et al., 2016).

Material examined: Merkez Park, *Cupressus arizonica* Greene (Cupressaceae), 09.III.2012, 1 ♀; Balcalı, *Platy. orientalis*, 04.VII.2012, 5 ♀♀; Balcalı, *C. sempervirens*, 04.VII.2012, 2 ♀♀; Balcalı, *C. sempervirens* var. *pyramidalis*, 02.VIII.2012, 2 ♀♀.

Comments: *Carulaspis minima* has a cosmopolitan distribution (García et al., 2016). It is restricted to conifers but, like *C. juniperi*, is most commonly collected on *Juniperus* spp. (Miller and Davidson, 2005). The species has been recorded previously in the Marmara and Mediterranean Regions of Turkey on *Juniperus drupacea* Labill*., Chamaecyparis lawsoniana* (A. Murray) Parl*., C. arizonica, Juniperus communis* L. and *Platy. orientalis* (Kaydan et al., 2013b).

Diaspis echinocacti (Bouché, 1833)

Synonyms: *Coccus luteus* Lancry, 1791; *Carulaspis calyptroides* (Bouché, 1833) Bodenheimer, 1953 (García et al., 2016).

Material examined: Reşatbey, *Dypsis lutescens* (H. Wendl.) Beentje & J. Dransf. (Arecaceae), 15.XII.2012, 10 ♀♀; Garden Koala, *Ferocactus macrodiscus* (Mart.) Britton & Rose (Cactaceae), 28.II.2013, 5 ♀♀.

Comments: *Diaspis echinocacti* occurs wherever cacti are grown, including on indoor plants in a continental climate globally (García et al., 2016). It has been recorded previously in Turkey in the Mediterranean Region (under natural conditions) and the Middle Anatolia Region (indoors) on *Cactus* spp. and *Opuntia ficus-indica* (L.) Mill.(Kaydan et al., 2013b).

Epidiaspis leperii (Signoret, 1869)

Synonyms: *Diaspis leperii* Signoret, 1869; *Epidiaspis peperii* (Signoret, 1869) Schvester, Milaire & Gireau, 1955 (García et al., 2016).

Material examined: Balcalı, *P. atlantica*, 04.VII.2012, 2 ♀♀.

Comments: *Epidiaspis leperii* is a polyphagous species recorded on 48 species from 13 plant families such as Juglandaceae, Moraceae, Lauraceae and Rosaceae (García et al., 2016). The species has been recorded in almost all regions of Turkey on *Cactus* spp*., Opuntia ficus-indica, Pistacia* sp*., Prunus* sp., *P. domestica* and *Aesculus hippocastanum* L. (Kaydan et al., 2013b).

Lepidosaphes conchiformis (Gmelin, 1790)

Synonyms: *Coccus conchiformis* Gmelin, 1790; *Insulaspis minima* (Gmelin, 1790) Borchsenius, 1963 (García et al., 2016).

Material examined: Mavi Avenue, *F. carica*, 18.I.2014, 2 ♀♀.

Comments: Fig scale occurs in Argentina, Chile, Europe, Iran, Iraq, Israel, North Africa, Pakistan, the former USSR and Syria. *Lepidosaphes conchiformis* has been recorded previously on *F. carica,* Lamiaceae, *Rhamnus* spp. and *Ulmus* spp. in the Mediterranean and Aegean Regions of Turkey (Kaydan et al., 2013b).

Lepidosaphes gloverii (Packard, 1869)

Synonyms: *Aspidiotus gloverii* Packard, 1869; *Cornuaspis gloverii* (Packard, 1869) Alayo Soto, 1976 (García et al., 2016).

Material examined: Mavi Avenue, *C. aurantium*, 26.VII.2012, 7 ♀♀.

Comments: *Lepidosaphes gloverii* is widely distributed in tropical and subtropical parts of the world and is often found on *Citrus* spp. (Miller & Davidson, 2005). The species has been recorded previously in Turkey in the Aegean, Marmara and Mediterranean Regions on *Citrus* spp. (Kaydan et al., 2013b).

Pseudaulacaspis pentagona (Targioni Tozzetti, 1886) (Figure 1e)

Synonyms: *Diaspis pentagona* Targioni Tozzetti, 1886; *Diaspis gerannii* (Targioni Tozzetti, 1886) Borchsenius, 19966 (García et al., 2016).

Material examined: Merkez Park, *Morus alba*, 09.III.2012, 19 ♀♀; Adnan Menderes Avenue, *M. alba*, 21.III.2012, 10 ♀♀; Atatürk Park, *M. alba*, 21.III.2012, 58 ♀♀; Doğal 2 Park, *M. alba*, 25.III.2012, 6 ♀♀; Şehitler Park, *M. alba*, 29.III.2012, 42 ♀♀; Cumhuriyet Park, *M. alba*, 04.IV.2012, 20 ♀♀; Ulus Park, *M. alba*, 08.IV.2012, 8 ♀♀; Mehmet Akif Ersoy Park, *F. excelsior*, 08.IV.2012, 1 ♀; Merkez Park, *P. tomentosa*, 26.V.2012, 4 ♀♀; Dilberler Park, *E. pungens*, 23.VI.2012, 4 ♀♀; Dilberler Park, *R. pseudoacacia*, 23.VI.2012, 9 ♀♀; Çoban Dede District, *A. saligna*, 08.VIII.2012, 14 ♀♀; Balcalı, *M. alba*, 09.XI.2012, 12 ♀♀; Merkez Park, *M. alba*, 14.III.2013, 25 ♀♀; Doğal Park, *M. alba*, 11.IV.2013, 2 ♀♀; Kazım Karabekir Park, *M. alba*, 02.V.2013, 12 ♀♀.

Comments: White peach scale is one of the most polyphagous armored scale insect species, feeding on at least 393 plant host species belonging to 90 families (García et al., 2016). Reportedly it is one of 43 principal armored scale pests worldwide (Beardsley & Gonzalez, 1975). It has been recorded in all regions of Turkey as polyphagous, and is regarded as an important pest (Kaydan et al., 2013b).

Torosaspis farsianus (Balachowsky and Kaussari, 1955) (Figures 1f,g and 3)

Synonym: *Acanthomytilus farsianus* Balachowsky & Kaussari, 1955 (García et al., 2016).

Material examined: Merkez Park, *C. arizonica*, 09.III.2012, 2 ♀♀; Balcalı, *C. sempervirens* var. *horizantalis*, 05.V.2012, 4 ♀♀; Hayal Park, *C. sempervirens* var. *horizantalis*, 18.I.2014, 8 ♀♀; Türkmenbaşı Avenue, *C. sempervirens* var. *pyramidalis*, 18.I.2014, 1 ♀.

Live appearance: scale cover of adult female flat, elongate, oyster-shell shaped, broadest posteriorly, light brown, with two larval exuviae pale yellow and transparent. Live female beneath scale cover cream-colored, pygidium darker. Test of second-instar male yellowish-brown, parallel-sided, narrower and shorter than that of female.

Adult female: Body elongate oval, widest across abdominal segment I; membranous, except for sclerotized pygidium. Each antenna with two large flagellate and two short conical setae. Anterior spiracles each with one associated trilocular disc pore. Pygidium rounded, slightly sclerotized, with two pairs of lobes. Median lobes prominent, rounded, with space between as wide as one lobe. Lobe 2 unilobular, similar in shape to median lobe but smaller; lobe 3 barely perceptible, unilobular, triangular and finely pointed. Gland spines each twice the length of the median lobe. Dorsum with marginal macroducts on pygidium singly present on each of segments IV-VII (formula 1-1-1-1). Anal opening situated near anterior margin of pygidium on abdominal segment V. Venter with macroducts smaller than dorsal macroducts, present in a submarginal group on abdominal segments I and II, and present on the submargin of prothorax and mesothorax. Posterior spiracles each associated with three to five glandular tubercles and five to eight macroducts in a transverse band extending to margin. Microducts few, present on the head and thorax.

Comments: *Torosaspis farsianus* has only been recorded previously in Iran on *Cupressus* sp. and *C. sempervirens* (Balachowsky & Kaussari, 1951; Moghaddam, 2013). Its detection in Turkey is a new country record. It is considered that this scale is an element of the local fauna, rather than a recent introduction.

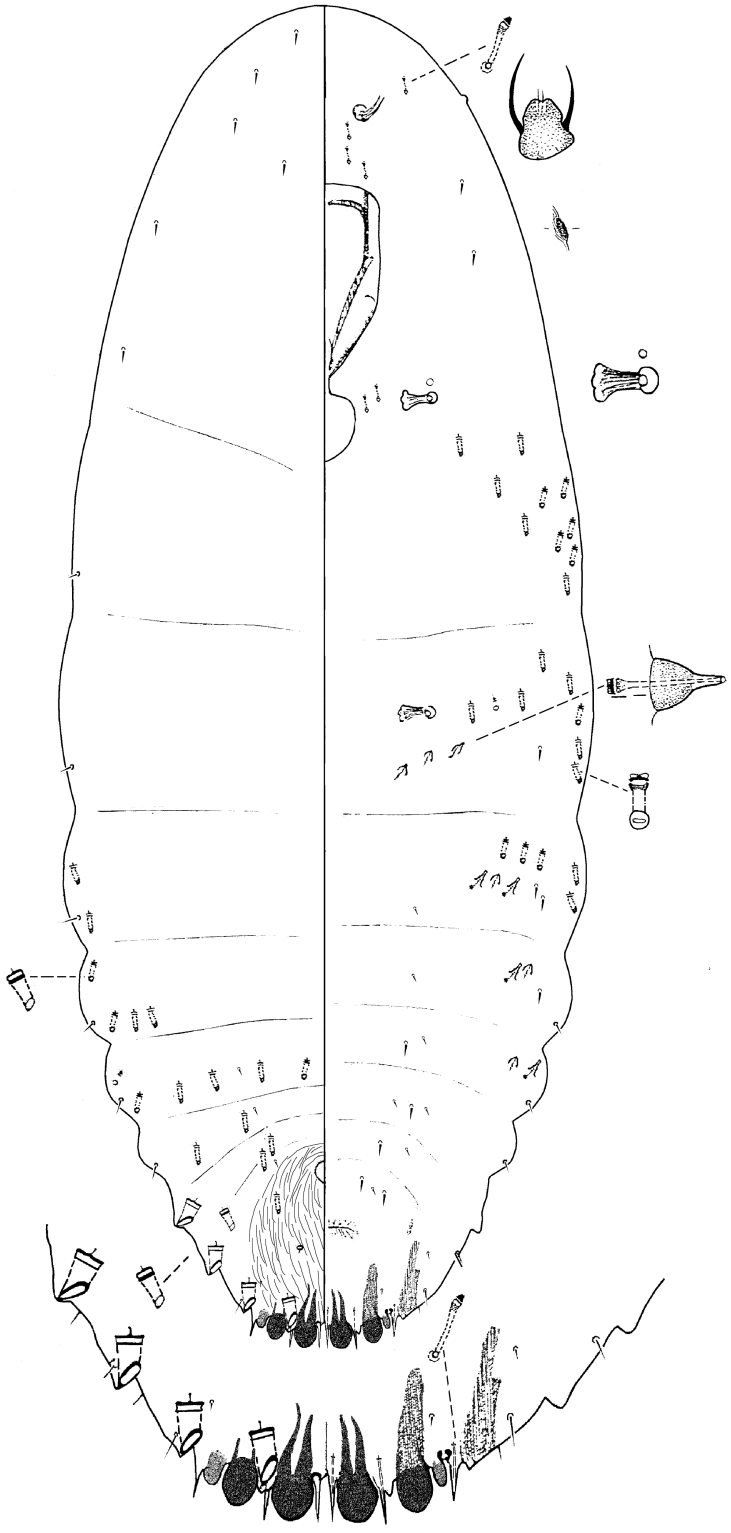

Figure 3. *Torosaspis farsianus* after Balachowsky & Kaussari (1955), with modifications.

Unaspis euonymi (Comstock, 1881) (Figure 1d)

Synonyms: *Chionaspis euonymi* Comstock, 1881; *Unaspis nakayamai* Takahashi & Kanda, 1939 (García et al., 2016).

Material examined: Uğur Mumcu Avenue, *E. japonicus*, 20.IV.2011, 5 ♀♀; Balcalı, *E. japonicus*, 15.XI.2011, 1 ♀; Mahfesığmaz District, *E. japonicus*, 04.III.2012, 4 ♀♀; Adnan Menderes Avenue, *E. japonicus*, 10.III.2012, 19 ♀♀; Çoban Dede District, *E. japonicus*, 11.III.2012, 14 ♀♀; Doğal Park, *E. japonicus*, 25.III.2012, 2 ♀♀; Balcalı, *E. japonicus*, 26.IV.2012, 18 ♀♀; Dilberler Park, *E. japonicus*, 23.VI.2012, 1 ♀; Süleyman Demirel Avenue, *E. japonicus*, 04.VIII.2012, 10 ♀♀; Doğal Park, *E. japonicus*, 02.VIII.2012, 8 ♀♀; Merkez Park, *E. japonicus*, 14.III.2013, 15 ♀♀; Doğal Park, *E. japonicus*, 11.IV.2013, 5 ♀♀.

Comments: *Unaspis euonymi* has a cosmopolitan distribution and has been found on 43 plant species belonging to 20 families. It has been recorded in almost all regions of Turkey on *Buxus sempervirens, Rosa* spp*., Euonymus fortunei* (Turcz.) Hand.-Maz. and *E. japonicus* (Kaydan et al., 2013b), and is regarded as a pest species on ornamental Japanese spindle in many cities in Turkey.

Leucaspidinae

Leucaspis pusilla (Löw, 1883)

Synonyms: *Leucaspis pusilla* Löw, 1883; *Pusillaspis pusilla* (Löw, 1883) Lindinger, 1957 (García et al., 2016).

Material examined: Yurt Park, *Pinus pinaster* Aiton (Pinaceae), 30.V.2012, 3 ♀♀; Balcalı, *P. pinea* L., 04.VII.2012, 1 ♀.

Comments: *Leucaspis pusilla* has a Palearctic distribution and has been recorded on 25 plant species belonging only to the family Pinaceae (García et al., 2016). The species has been found in almost all regions of Turkey, on *Cedrus* spp*., Pinus* sp., *Pinus brutia* Tenore*, Pinus halepensis* Miller and *Pinus pinea* L. (Kaydan et al., 2013b).

Parlatoria oleae (Colvée, 1880) (Figure 1h)

Synonyms: *Diaspis oleae* Colvée, 1880; *Parlatoria morrisoni* Bodenheimer, 1944 (García et al., 2016).

Material examined: Atatürk Park, *L. nobilis*, 04.III.2012; 2 ♀♀; Stadium District, *E. japonica*, 09.III.2012, 8 ♀♀; Çoban Dede District, *B. thunbergii*, 11.III.2012, 9 ♀♀; Sanatçılar Park, *C. speciosa*, 21.III.2012, 4 ♀♀; Adnan Menderes Avenue, *F. excelsior*, 25.III.2012, 20 ♀♀; Çoban Dede District, *Cotoneaster franchettii* Bois. (Rosaceae), 02.VIII.2012, 4 ♀♀; Doğal Park, *Acacia homalophylla* A. Cunn. ex. Benth. (Fabaceae), 02.VIII.2012, 7 ♀♀; Pınar 2 Park, *Lagerstroemia indica* (L.) Pers. (Lythraceae), 12.VIII.2012, 4 ♀♀.

Comments: *Parlatoria oleae* is known from Asia, Australia, Mexico, North Africa, South America, southern Europe and southern former USSR. The species is regarded as a polyphagous pest on more than 200 plant species belonging to 56 families (García et al., 2016). It occurs in almost all regions of Turkey, feeding on *Eriobotrya* sp*, Fraxinus* sp*., Rosa* spp., *M. sylvestris, Prunus* spp. and *Syringa vulgaris* L. (Kaydan et al., 2013b).

Parlatoria pergandii (Comstock, 1881)

Synonyms: *Parlatoria sinensis* Maskell, 1897; *Parlatoreopsis pergandii* (Comstock, 1881) Kawai, 1972 (García et al., 2016).

Material examined: Balcalı, *Hoya carnosa* (L. f.) R. Br. (Apocynaceae), 15.IV.2011, 3 ♀♀; 5 Ocak Lions Park, *C. aurantium*, 03.III.2012, 6 ♀♀; Mahfesığmaz District, *E. japonicus*, 04.III.2012, 4 ♀♀; Adnan Menderes Avenue, *L. nobilis*, 10.III.2012, 2 ♀♀; Turgut Özal Avenue, *P. coccinea*, 30.V.2012, 4 ♀♀;

Dilberler Park, *B. thunbergii*, 23.VI.2012, 2 ♀♀; Balcalı, *C. siliqua*, 04.VII.2012, 1 ♀; Sanatçılar Park, *C. aurantium*, 16.V.2013, 6 ♀♀.

Comments: Miller and Davidson (1990) listed this insect as a serious and widespread pest of citrus, although some researchers regard it as a relatively minor citrus pest (Rosen & DeBach, 1978). The species is a very important pest in southern Japan and Italy, and an important pest in Spain, Lebanon, Israel, Southeast Asia, Central America, Mexico, and USA. (García et al., 2016). *Parlatoria pergandii* was recorded previously in the Mediterranean Region of Turkey on *Citrus* spp. and *M. sylvestris* by Kaydan et al. (2013b).

Due to the geographical location of Turkey, different climates in different regions, as well as different altitudes from sea level, cause different and rich in plant and animal ecosystem. For this reason, the insect species richness of Turkey is always dynamic and it is thought that it will continue to increase in future.

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Original article (Orijinal araştırma)

Evaluation of diatomaceous earth formulations for the control of rice weevil, *Sitophilus oryzae* L., 1763 (Coleoptera: Curculionidae) in stored rice[1]

Depolanmış çeltikte pirinç biti, *Sitophilus oryzae* L., 1763 (Coleoptera: Curculionidae)'nin mücadelesinde diyatom toprağı formülasyonlarının değerlendirilmesi

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Summary

A study was conducted between 2012 and 2014 on the protective efficacy of diatomaceous earth (DE) formulations against *Sitophilus oryzae* L., 1763 (Coleoptera: Curculionidae) (rice weevil), a major pest of the stored rice. Biological tests were carried out at 30°C and 75% RH on rice treated with DE formulations. In the biological tests, different concentrations of Protector® (DE formulation) and DEA-P [mixture of natural DE (83%) and 0.25% abamectin (w:w)] were used. After DE treatment, dead adults were counted once a week for a month and percentage mortalities were determined. In order to determine progeny production (F_1), rice was also incubated under the same conditions for a 60-day period. Protector® gave 100% mortality to *S. oryzae* adults at 1000 ppm after 14 d, and DEA-P gave the same mortality rate at 75 ppm at the same time. In studies to determine progeny (F1) emergence, Protector® resulted in 100% mortality at 1750 ppm after 60 d of storage, while DEA-P caused 100% mortality at 50 ppm concentration. In conclusion, the protective effect of both DE formulations was confirmed, however DEA-P was more effective against *S. oryzae* at lower concentrations than Protector®.

Keywords: Diatomaceous earth, physical control, *Sitophilus oryzae*, stored rice

Özet

Bu çalışma 2012-2014 yılları arasında laboratuvar koşullarında yürütülmüştür. Bu çalışma ile diyatom toprağı (DT) formülasyonlarının, önemli bir depo zararlısı olan *Sitophilus oryzae* L., 1763 (Coleoptera: Curculionidae) (Pirinç biti)'ye karşı depolanmış çeltikte koruyucu etkisi araştırılmıştır. Çalışmalar 30°C sıcaklık ve %75 orantılı nemde, DT uygulanmış çeltikte yürütülmüştür. Denemelerde Protector® (DT formülasyonu) ve DEA-P (DT ile abemectin karışım formülasyonu) formülasyonlarının farklı konsantrasyonları kullanılmıştır. Çalışmalarda DT uygulamasının ardından birer hafta ara ile 4 kez ölü ergin birey sayımı yapılmış ve ölüm oranları belirlenmiştir. Ayrıca *S. oryzae*'nin F_1 çıkışını (birinci nesil erginleri) belirlemek amacı ile çeltik tekrar aynı koşullarda 60 gün süre ile bekletilerek yeni nesil ergin sayıları hesaplanmıştır. Protector®, *S. oryzae* erginlerinde 1000 ppm konsantrasyonunda 14. günde %100 ölüm sağlarken DEA-P aynı ölüm oranını 75 ppm konsantrasyonda aynı sürede sağlamıştır. F_1 çıkışlarını belirlemek için yapılan çalışmalarda ise Protector® 60 günlük depolama süresi sonunda 1750 ppm konsantrasyonda %100 etkili olurken, DEA-P ise 50 ppm konsantrasyonda %100 ölüme neden olmuştur. Sonuçlar değerlendirildiğinde, her iki diyatom toprağı formülasyonunun ürünü koruyucu etkisinin olduğu, ancak DEA-P' nin *S. oryzae*'ye karşı Protector®'unkilerden daha düşük konsantrasyonlarda etkili olduğu saptanmıştır.

Anahtar sözcükler: Diyatom toprağı, fiziksel mücadele, *Sitophilus oryzae*, depolanmış çeltik

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Introduction

Rice, *Oryza sativa* L. is a staple food for 1.5 billion people around the world. Global rice production is around 741 Mt annually, of which Turkey produces 830 kt (FAO, 2014). Rice has an important place in human nutrition due to its high protein, starch, vitamin (B1, B3, B6 and E) and mineral (K, P, Fe and Mg) (Özder et al., 2013) content. To maintain its nutritive quality, rice, like other cereal grains, should be dried and stored under the proper conditions, and special precautions against the pests must be taken. For the latter, continuous pest monitoring and timely applications of control measures are very important.

About 10-30% of annual global grain production is damaged by storage insect pests (Singh et al., 2009). Chemical insecticides are currently the main way of managing insect pests of stored grains. However, environmental, ecological and health effects of pesticides have lead researchers to seek safe alternatives (Zettler & Keever, 1994; Benhalima et al., 2004; Isikber & Oztekin, 2009; Pimentel et al., 2010; Alkan & Gökçe, 2012; Kepenekçi et al., 2013; Alkan et al., 2015). One of these alternatives is diatomaceous earth (DE), which is used as a physical control measure against stored grain insects. Diatomite rocks (kieselgur) that are used for a wide range of purposes from filter aid to filling materials and from food additives to refractories, are the fossilized silica remains of one-celled microscopic algae which are known as diatoms (Özbey & Atamer, 1987). DE is the only mineral that is of organic origin. In USA, silica-based DEs are rated by the Food and Drug Administration as GRAS (generally recognized as safe) for human consumption and are also registered as animal feed additives (Banks & Fields, 1995). DEs are currently used against stored product pests in the EU. Water loss in the DE-exposed insects is the main cause of mortality (Ebeling, 1971).

Due to their low mammalian toxicity (in rats, oral LD_{50} >5000 mg/kg body weight) (Subramanyam et al., 1994), stability, efficacy and lack of toxic residues, DEs have been the subject of considerable research (Ebeling, 1971; Banks & Fields, 1995; Golob, 1997; Korunic, 1998; Fields & Korunic, 2000; Subramanyam & Roesli, 2000). DEs can be used alone or in combination with insecticides of various origin (Athanassiou et al., 2005; 2006). DEA-P, for example, used in the current study is a mixture of DE and abamectin, an insecticidal/acaricidal toxin produced by fermentation by *Streptomyces avermitilis* (Burg et al., 1979) Kim and Goodfellow 2002, a soil-inhabiting bacterium (Athanassiou & Korunic, 2007).

Although numerous and versatile DE formulations have been tested against pests of various stored products (Korunic, 1998; Athanassiou et al., 2005; Ferizli & Beris, 2005; Ziaee & Khashaveh, 2007; Kavallieratos et al., 2007; Kostyukovsky et al., 2010; Doğanay, 2013; Ertürk & Emekci, 2014), there is no literature on the long-term efficacy of DEs against rice weevil, *Sitophilus oryzae* L., 1763 (Coleoptera: Curculionidae). Therefore, this study aimed to evaluate the efficacy of two DE formulations, Protector® and DEA-P, against rice weevil.

Material and Methods

Insect rearing

Sitophilus oryzae were reared in 1-L glass jars on whole grain soft wheat in incubators (Nüve ID 501, Ankara, Turkey) maintained at about 30°C and 75% RH. The mouth of each jar was covered with a perforated lid with inner surface were with US standard sieves mesh #120 to facilitate ventilation and prevent escape of insects. To obtain 1-2-wk-old adults in sufficient numbers, about 700-1000 adults were transferred into glass jars having approximately 500 g of wheat and left to oviposit for 48 h. After oviposition, adults were removed and after 30 d newly emerged adults were sieved off every 3-4 d to use in the experiments.

Diatomaceous earth formulations

Two DE formulations, DEA-P (supplied by C.G. Athanassiou, University of Thessaly, Greece) and Protector® (Intrachem Bio Italia, Grassobbio, Bergamo, Italy) were used in the experiments. DEA-P is a DE formulation composed of a mixture of natural DE (83%) and 0.25% abamectin (w/w). Abamectin is a mixture of avermectins containing >80% avermectin B1a and <20% avermectin B1b. These two components have very similar biological and toxicological properties. The avermectins are insecticidal or anthelmintic compounds derived from the soil bacterium *S. avermitilis*. Abamectin is a natural fermentation product of this bacterium (Lankas & Gordon, 1989; Hayes & Laws, 1990). Its DE component is of freshwater origin and contains 89% amorphous SiO_2, 4% Al_2O_3, 1.7% Fe_2O_3, 1.4% CaO, <1% MgO and K_2O and 3% water (w/w) (Athanassiou & Korunic, 2007).

The other DE formulation was natural DE sold as Protector® and is composed of 69.7% SiO_2, 5.89% Al_2O_3, 0.414% CaO and 1.05% Fe_2O_3, and, half of its particles were below 9.46 µm. (Baldassari et al., 2008).

Experimental protocol

A Turkish rice variety, *Oryza sativa* cv. Osmancık-97, with 9.8% moisture content (Multi-Grain Moisture Tester, Dickey John, Auburn, IL, USA) was used in the experiments. DEA-P was used at the concentration of 25, 50, 75, 100, 150, 175 and 200 ppm, and Protector® at 250, 500, 750, 1000, 1500, 1750 and 2000 ppm (i.e., mg DE/kg rice). Untreated rice (0 ppm) was used as the control for both DEs. DE required for four replicates of each concentration was weighed and added to 280 g of rice in 1-L plastic bags. The plastic bags were sealed inflated, then thoroughly shaken by hand for 4-5 min to ensure even distribution, and left for 10 min to allow dust settle before dividing into 225-mL PVC test vials (3 x 8 cm). Each vial was fitted with a plastic lid prepared as for the rearing jars. The vials were then filled with 70 g of DE treated rice and with 50 adult weevils each. Test vials were put into large PVC boxes containing KOH (22.25 g KOH, 77.75 g distilled water) solution to maintain the humidity at about 75%. The PVC boxes were closed tightly and then placed in an incubator (Binder KB 720, Tuttlingen, Germany) adjusted at 30±1°C. To determine mortality, live and dead adults were counted after 7, 14, 21 and 28 d. On day 28, all the live insects were removed. The vials were incubated for another 60 d and F_1 progeny counted.

Statistical analysis

Results were analyzed by factorial design repeated measures ANOVA (Gürbüz et al., 2003) using Statistica 8 (Weiß, 2007), with observation time as the repeated-measures factor and DE formulation and DE concentration as categorical predictor variables. Mortality data were arcsine transformed before analysis. The differences among the treatment means were analyzed by Tukey's HSD test at 5% significance level (Sokal & Rohlf, 1995).

Results and Discussion

Studies with Protector®

Mortality of rice weevil increased with the increase of both concentration and exposure time, and complete mortality had occurred by 14 d at 1000 ppm ($F = 23.6$; $df = 21,576$; $P < 0.05$) (Table 1). Similar results for rice weevil were reported by other researchers using various DEs. Kostyukovsky et al. (2010), reported complete mortality after 21 d in wheat treated with DDDE (Detia Degesch diatomaceous earth, Laudenbach, Germany) at 1000 ppm at 28°C and 65% RH. Athanassiou et al. (2004), who worked with various cereals treated with Insecto, SilicoSec, and PyriSec, obtained complete mortality of *S. oryzae* after 7 d. Matti & Awaknavar (2009) reported that Protect-It applied to sorghum at 1000 ppm for 7 d caused complete mortality of *S. oryzae* adults at 30°C with RH up to 90%; however, decreased temperatures of 25 and 20°C restricted the RH range at which complete mortality was observed, to 50 and 30%, respectively. The differences in the reported times to reach complete mortality is considered to be due to be the differences in experimental conditions, such as DE, crop/cultivar, temperature and RH (Korunic, 1998; Fields & Korunic, 2000; Baldassari et al., 2008; Matti & Awaknavar, 2009).

Table 1. Mortality of *Sitophilus oryzae* adults exposed to rice treated with Protector® at 30°C and 75% RH

Concentration (ppm)	Mortality±SE (%)			
	Exposure time (day)			
	7	14	21	28
Control	2.0±0.23 aC*	3.1±0.24 aB	4.1±0.30 aC	6.5±0.45 aC
250	1.5±0.27 cC	24.5±2.57 bcB	53.0±2.97 abB	80.0±0.27 aB
500	23.0±1.54 cBC	80.5±0.83 bA	97.5±1.81 aA	100.0±0.00 aA
750	74.5±2.93 bA	98.0±1.20 aA	100.0±0.00 aA	100.0±0.00 aA
1000	78.0±1.80 bA	100.0±0.00 aA	100.0±0.00 aA	100.0±0.00 aA
1500	93.5±0.27 aA	100.0±0.00 aA	100.0±0.00 aA	100.0±0.00 aA
1750	99.0±0.16 aA	100.0±0.00 aA	100.0±0.00 aA	100.0±0.00 aA
2000	96.0±0.80 aA	100.0±0.00 aA	100.0±0.00 aA	100.0±0.00 aA

*Means followed by the same lowercase letter within a row or the same uppercase letter within a column are not significantly different ($P \leq 0.05$).

F_1 progeny suppression is as important as the immediate mortality in population suppression of the pests. Fewer adults than that of the beginning of the experiment developed with both DEs applied at ≥750 ppm (Table 2). Similarly, Ferizli & Beris (2005) reported that in *Rhyzopertha dominica* (F., 1792) (Coleoptera: Bostrichidae) increase in dosage resulted in fewer F_1 progeny than at the start of exposure. Kavallieratos et al. (2007) also reported enhanced and natural DEs, such as Pyrisec, Insecto and Protect-It, had a similar effect in decreasing in population growth in *Tribolium confusum* Jacqueli du Val, 1863 (Coleoptera: Tenebrionidae) with increasing concentration from 500 to 1000 ppm due to the increased effects on larvae.

Table 2. Mean number and mortality of F_1 progeny of *Sitophilus oryzae* exposed to rice treated with Protector® at 30°C and 75% RH

Concentration (ppm)	F_1 Progeny (Mean)	Mortality±SE (%)
Control	49.00	26.0±0.41 d*
250	62.75	47.6±3.17 cd
500	54.5	46.5±1.95 cd
750	43.25	49.6±1.52 cd
1000	19.75	82.4±2.16 bc
1500	22.75	90.1±1.19 ab
1750	11.00	100.0±0.00 a
2000	5.25	100.0±0.00 a

*Means followed by the same lowercase letter are not significantly different ($P \leq 0.05$).

Studies with DEA-P

As with Protector®, adult mortality increased with the increase of concentration and exposure time, but complete mortality occurred after 7 d at ≥100 ppm ($F = 6.09$; $df = 21,288$; $P < 0.05$) (Table 3). Since DEA-P is an enhanced DE formulation combined with abamectin, complete mortality was obtained at lower concentration and shorter time than with Protector®. This can be helpful to overcome obstacles

associated with natural DEs applied at higher concentrations. Athanassiou et al. (2006), in order to eliminate decrease in bulk density and flowability caused by high concentrations of DEs, used DEA-P against *Prostephanus truncatus* (Horn, 1878) (Coleoptera: Bostrichidae) and *R. dominica* at a concentration of 75 ppm and obtained complete mortality after 14 d in corn and wheat. The differences in time to achieve complete mortality in various reports is thought to be a consequence to different pest species investigated. Among insect pest species, there are differences in tolerance to DEs, from least to most tolerant being *Cryptolestes* spp., *Sitophilus* spp., *Oryzaephilus* spp., *R. dominica, Tribolium* spp. and *P. truncatus* (Maceljski & Korunic, 1971; Desmarchelier & Dines, 1987; Subramanyam et al., 1998; Fields & Korunic, 2000).

Table 3. Mortality rate of *Sitophilus oryzae* adults exposed to rice treated with DEA-P at 30°C and 75% RH

Concentration (ppm)	Mortality±SE (%)			
	Exposure time (day)			
	7	14	21	28
Control	26.5±0.20 bB*	45.0±0.34 aB	51.8±0.21 aB	54.0±0.29 aB
25	91.5±0.50 aA	78.1±1.61 aA	75.0±6.12 aA	75.0±6.12 aA
50	95.8±0.12 aA	100.0±0.00 aA	100.0±0.00 aA	100.0±0.00 aA
75	99.8±0.12 aA	100.0±0.00 aA	100.0±0.00 aA	100.0±0.00 aA
100	100.0±0.00 aA	100.0±0.00 aA	100.0±0.00 aA	100.0±0.00 aA
150	100.0±0.00 aA	100.0±0.00 aA	100.0±0.00 aA	100.0±0.00 aA
175	100.0±0.00 aA	100.0±0.00 aA	100.0±0.00 aA	100.0±0.00 aA
200	100.0±0.00 aA	100.0±0.00 aA	100.0±0.00 aA	100.0±0.00 aA

*Means followed by the same lowercase letter within a row or uppercase letters within a column are not significantly different (P ≤ 0.05).

Progeny studies showed that complete F_1 mortality was obtained at all concentrations except 25 ppm ($F = 23.1$; $df = 7,96$; $P < 0.05$) (Table 4). Similarly, Athanassiou et al. (2006), using the same DE, reported that 125 ppm is required to get a complete mortality of F_1 progeny of *S. oryzae* at 27°C and 65%. The difference between the two studies probably due to the differences in temperature and RH.

Table 4. Mean number and mortality of F_1 progeny of *Sitophilus oryzae* exposed to rice treated with DEA-P at 30°C and 75% RH

Concentration (ppm)	F_1 Progeny (Mean)	Mortality±SE (%)
Control	44.50	27.2±1.87 b*
25	13.75	57.0±2.38 b
50	5.25	100.0±0.00 a
75	2.25	100.0±0.00 a
100	1.50	100.0±0.00 a
150	0.75	100.0±0.00 a
175	2.25	100.0±0.00 a
200	0.25	100.0±0.00 a

*Means followed by the same lowercase letter are not significantly different (P ≤ 0.05).

This study aimed to introduce DEs to Turkish rice sector as efficient and safe protectants posing the least risks for human health and the environment. Both DE formulations, Protector® (natural DE) and DEA-P (enhanced DE) were shown to be effective in protecting rice against rice weevil. As reported by several authors, natural DEs when used at high concentrations have some disadvantages, such as reduced grain flow ability and bulk density, abrasion of machines parts and workplace health concerns (Subramanyam et al., 1994; Golob, 1997). Therefore, enhanced DEs can address these limitations by using lower concentrations and thus broaden the adoption of DEs for the control of storage pests (Subramanyam & Roesli, 2000; Athanassiou et al., 2006; Athanassiou & Korunic, 2007; Kavallieratos et al., 2007; Vayias & Stephou, 2009; Wakil et al., 2010). As exemplified in the present study, DEs hold considerable promise for reducing the need for synthetic pesticides by effectively suppressing pest populations. However, to encourage their use more works are needed regarding the efficacy of DEs in other situations, such as with other pest species, temperature, moisture content and crop/cultivar.

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