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Original article (Orijinal araştırma)

Control of *Fusarium oxysporum* f. sp. *radicis lycopersici* Jarvis & Shoemaker (Ascomycota: Hypocreales) and *Meloidogyne incognita* (Kofoid & White, 1919) Chitwood, 1949 (Nemata: Meloidogynidae) with *Aspergillus niger* Tiegh. (Ascomycota: Eurotiales) culture filtrate on tomato[1]

Domateste *Fusarium oxysporum* f. sp *radicis lycopersici* (Jarvis & Shoemaker) (Ascomycota: Hypocreales) ve *Meloidogyne incognita* (Kofoid & White, 1919) Chitwood, 1949 (Nemata: Meloidogynidae)'nın *Aspergillus niger* Tiegh. (Ascomycota: Eurotiales) kültür filtratı ile kontrolü

Fatma Gül GÖZE ÖZDEMİR[2*] **Şerife Evrim ARICI[2]**

Abstract

The effects of *Aspergillus niger* Tiegh. (Ascomycota: Eurotiales) culture filtrate on *Meloidogyne incognita* (Kofoid & White, 1919) Chitwood, 1949 (N) and *Fusarium oxysporum* f. sp. *radicis lycoper*sici Jarvis & Shoemaker (Ascomycota: Hypocreales) (FORL) were investigated under controlled conditions on tomato between April and August 2022 in the Isparta province. The study consisted of 8 treatments; 1: N, 2: FORL, 3: N+*A. niger*, 4: FORL+*A. niger*, 5: N+FORL, 6: N+FORL+*A. niger*, 7: N+FORL+nematicide, 8: N+FORL+fungicide. In inoculation, 1000 *M. incognita* second juvenile larvae/1ml and $3X10^6$ spore/ml FORL were used for each seedling according to treatment. Two days after inoculation, 10 ml of undiluted *A. niger* culture filtrate was applied to each potting soil. After 60 days, 0-9 gall and egg mass index, and 0-4 disease severity scale were evaluated. While the suppressive effect of *A. niger* culture filtrate on the gall and egg mass of *M. incognita* was found over 55%, disease severity was found to be over 25%. The highest suppressive effect on gall and egg mass was determined in N+FORL+nematicide, followed by N+FORL+*A. niger*. The disease severity of N+FORL+*A. niger*, N+FORL+nematicide, and N+FORL+fungicide has been determined to be lower than N+FORL and FORL.

Keywords: *Aspergillus niger*, fermentation fluid, fungicidal effect, Fusarium wilt, nematicidal effect

Öz

Aspergillus niger Tiegh. (Ascomycota: Eurotiales) kültür filtratının *Meloidogyne incognita* (Kofoid & White, 1919) Chitwood, 1949 (N) ve *Fusarium oxysporum* f. sp. *radicis lycopersici* Jarvis & Shoemaker (Ascomycota: Hypocreales) (FORL) üzerindeki etkileri Isparta ilinde Nisan-Ağustos 2022 tarihleri arasında domateste kontrollü koşullarda araştırılmıştır. Çalışma 8 uygulamadan oluşmaktadır; 1: N, 2: FORL, 3: N+*A. niger*, 4: FORL+*A. niger*, 5: N+FORL, 6: N+FORL+*A. niger*, 7: N+FORL+nematisit, 8: N+FORL+fungusit. İnokulasyonda her fide için uygulamaya göre 1000 *M. incognita* ikinci dönem larva/1ml ve $3X10^6$ spor/ml FORL kullanılmıştır. İnokulasyondan iki gün sonra, her saksı toprağına 10 ml seyreltilmemiş *A. niger* kültür filtratı uygulanmıştır. Altmış gün sonra 0-9 gal ve yumurta paketi indeksi ve 0-4 hastalık şiddeti skalası değerlendirilmiştir. *Aspergillus niger* kültür filtratı uygulamasının *M. incognita*'nın gal ve yumurta paketi üzerindeki baskılayıcı etkisi %55'in üzerinde bulunurken, hastalık şiddeti üzerindeki baskılayıcı etkisi %25'in üzerinde bulunmuştur. Gal ve yumurta paketi üzerindeki baskılayıcı etki en yüksek N+FORL+nematisit'de belirlenmiştir, ardından N+FORL+*A. niger* uygulamasının geldiği belirlenmiştir. N+FORL+*A. niger*, N+FORL+nemasit ve N+FORL+fungisit uygulamalarının hastalık şiddeti N+FORL ve FORL uygulamalarına göre daha düşük saptanmıştır.

Anahtar sözcükler: *Aspergillus niger*, fermente sıvı, fungisidal etki, Fusarium solgunluğu, nematisidal etki

[1] Part of this study presented as a poster presentation at 8th International Entomopathogens and Microbial Control Congress (October 06th-08th, 2022-Antalya, Türkiye).

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Introduction

Tomato, *Solanum lycopersicum* L. is an important Solanaceae species and is widely grown in tropical and temperate regions of the world. Many diseases and pests, including plant-parasitic nematodes, damage tomato plants and cause yield losses (Ji et al., 2019). Root-knot nematodes feed on roots and vascular tissues, disrupting water and nutrient flow, and cause symptoms such as stunning, slow growth, yellowing of leaves, wilting and early plant death in infected plants (Asaturova et al., 2022). In addition, root-knot nematodes suppress the host plant's defense mechanism, making the plant more susceptible to attacks by other plant pathogens (Goverse & Smant, 2014). In presence of nematodes, pathogens appear earlier in plants, disease effect increases and the plant dies completely (Lobna et al., 2016, 2017; Göze Özdemir et al., 2022a). *Meloidogyne incognita* (Kofoid & White, 1919) Chitwood, 1949 is the most important root-knot nematode species due to its aggressive, wide host spectrum which may infect almost all plants, causing significant economic damage in all subtropical-tropical regions and can be seen in high prevalence in the world and including Türkiye (Sikora & Fernández, 2005; Uysal et al., 2017; Gürkan et al., 2019; Aslan & Elekçioğlu, 2022; Evlice et al., 2022; Tapia-Vázquez et al., 2022). It is stated that the damage by the root-knot nematodes and symptoms of large galls in the roots are more severe in tropical areas than in temperate areas. It is reported that crop loss in different tomato varieties is between 25 and 100% in the world (Kaşkavalcı, 2007; Seid et al., 2015; Aydınlı & Mennan, 2019). It is seen that nematicides and resistant varieties are generally used to control root-knot nematodes in tomatoes (Hajihassani et al., 2022). Although nematicides can effectively suppress nematodes, their use is limited due to their short-term effects. In addition, high costs, resistance development in nematodes, health and environmental hazards, residues, adverse effects on soil fauna and beneficial microflora, and phytotoxic effects on plants limit the use of nematicides (Haydock et al., 2013; da Silva et al., 2019). With the widespread use of resistant varieties, *Mi* virulent root-knot nematode populations have been reported in many countries (Devran & Söğüt, 2010; Aydınlı & Mennan, 2019; Hajihassani et al., 2022).

Fusarium oxysporum f. sp. *radicis lycopersici* Jarvis & Shoemaker (Ascomycota: Hypocreales) (FORL), which causes tomato root rot, is an important pathogen species that causes more than 60% yield loss in an open field and greenhouse tomato production (Can et al., 2004; Özbay et al., 2004; Hibar et al., 2007; Arıcı et al., 2013; Manzo et al., 2016). While it causes stunting and yellowing in tomato seedlings, root rot, wilting and death occur in plants in later periods. Rot in the root area and necrosis in the stem vascular bundles are around 15-30 cm from the soil surface at most (Singh et al., 2022). FORL has been reported from the Mediterranean region, Europe (the UK, the Netherlands, Belgium and France), where the tomato is intensively grown, USA, Japan (Katan & Katan, 1999; Baysal et al., 2008). First detected in Türkiye by Can et al. (2004), this pathogen causes significant yield losses in tomato growing regions (Baysal et al., 2008; Çolak & Biçici, 2013). The resistant varieties as well as cultivation methods and chemical control programs are used in *Fusarium* diseases (Aydın, 2019; Bilici et al., 2021). However, cultural methods are insufficient due to the saprophytic viability and limited development of resistant varieties (Çolak & Biçici, 2011; Jiménez-Díaz et al., 2015). In some studies, root-knot nematodes were found to break the plant's resistance even in resistant cultivars developed against Fusarium wilt (Lobna et al., 2016; Colak-Ates et al., 2018; Göze Özdemir et al., 2022a). Some chemicals such as metam sodium, and dichloro-propene used as soil fumigants are effective and recommended against soil-borne pathogens to alleviate disease severity before planting in infested areas. However, the uninformed chemical application against soil-borne pathogens causes environmental pollution and toxic effects on human health, as well as the possibility for pathogens to develop resistance to chemicals (Baysal et al., 2008). Soil fumigation with methyl bromide has been used successfully and extensively to control FORL in tomatoes for several years. However, methyl bromide has been phased out in developed countries (Myresiotis et al., 2012).

Management of root-knot nematode and FORL disease complex in tomatoes has proved to be difficult. Therefore, attention has been focused on its biological control through beneficial microorganisms

that can act on both factors. Numerous reports of several fungal and bacterial antagonists suppressing their reproduction and growth have been reported in separate studies on root-knot nematodes and FORL (Omar et al., 2006; Baysal et al., 2008; Myresiotis et al., 2011; Arici, 2015; Moosavi & Zare, 2020; Göze Özdemir et al., 2022b, c). Important species belonging to the genus Aspergillus are included in toxin-producing fungi (Sandoval et al., 2020). *Aspergillus niger* Tiegh. (Ascomycota: Eurotiales) produced extracellular enzymes like citric acids, amylases, lipases, cellulases, xylanases and proteases. It is also used for waste management and transformations (Patil et al., 2017). Also, *A. niger* causes black mould in onions and certain fruits like grapes, peanuts, vegetables etc. (Sharma, 2012). Therewithal, researchers report that different *Aspergillus* species have nematicidal effects on root-knot nematodes (Bhat & Wani, 2012; Devi & Bora, 2018; He et al., 2020; Xiang et al., 2020; Naz et al., 2021). *Aspergillus niger* LOCK 62 produces an antifungal chitinase enzymes (Brzezinska & Jankiewicz, 2012). Application of dextrose base formulation of *A. niger* significantly reduced Fusarium wilt of brinjal (Patil et al., 2017). The extracts of *Aspergillus fumigatus* Fresenius, 1863 and *Rhizopus oryzae* Went & H. C. Prinsen Geerligs, 1895 exhibited promising antifungal activity against *F. oxysporum in vitro* (Attia et al., 2022). Alwathnani & Perveen (2012) reported that the effectiveness of the *A. niger* was 35.6% to *F. oxysporum* f. sp. *lycopersici* (FOL).

Göze Özdemir et al. (2022b) investigated the inhibitory activity of different concentrations of culture filtrates of some fungi against *M. incognita in vitro* and found a high inhibitory activity of *A. niger*. At 100% concentration of the culture filtrate of *A. niger*, it suppressed the hatching from the egg masses of *M. incognita* by 81%, while mortality rate on second juvenile larvae (J2) was found to be 85. 3%. Subsequently, the same concentrations of *A. niger* in tomatoes and peppers were investigated under controlled conditions. The percent control effect on gall, egg mass number, and soil J2 density of *M. incognita* at 100% and 75% concentrations of *A. niger* culture filtrate was determined to be over 70% in tomatoes and peppers (Göze Özdemir et al. 2022c).

There are limited studies using *A. niger* against root-knot nematodes and FORL disease. The suppression of at least one or both of these interacting organisms is of great importance in terms of preventing the formation of the disease complex. Therefore, it is important to study the effect of *A. niger* in controlling *M. incognita* and FORL disease complex. This study aims to investigate the culture filtrate of *A. niger* local isolate which is isolated from Türkiye for antinematicidal and antifungal effects in tomatoes under controlled conditions.

Materials and Method

Material

The FORL isolate used in this study was isolated from a tomato plant in Antalya / Serik district in Türkiye, and its diagnosis was made according to Gerlach & Nirenberg (1982) and Davis & Raid (2002) (Göze Özdemir et al., 2022a). *Meloidogyne incognita* isolate DR17 was used (Uysal et al., 2017), whose mass production continues under climate room conditions (24±1°C, 60%±5% humidity). The local *A. niger* culture used in this study was obtained from Isparta University of Applied Sciences (ISUBU), Faculty of Agriculture, Biotechnology and Tissue Culture Laboratory (Arıcı & Tuncel, 2020). Isolate of *Aspergillus niger* (AnIB18) was isolated from vermicompost using red California worms, *Eisenia fetida* (Savigny, 1826) (Annelida: Lumbricidae) in the Isparta province, Türkiye. The study was carried out on a tomato cultivar Gulizar F1, which is known to be susceptible to root knot nematode and FORL (Göze Özdemir et al., 2022a). Two positive controls were used in the study, one chemical nematicide (Velum®, Fluopyram, Bayer Crop Production Inc., Türkiye) and one fungicide (Cebir®, Fludioxonil + Metalaxyl, Hektaş Crop Protection Inc., Türkiye). The maximum field recommendation doses of Velum and Cebir were used 0.16 ml/L and, 0.25 ml/L, respectively. Only plants with simultaneous application of *M. incognita* and FORL were evaluated as the negative control.

Methods

Preparation of culture filtrate of *Aspergillus niger*

Aspergillus niger isolate (AnIB18) was cultured on Potato Dextrose Agar (PDA) medium in 6 cm diameter petri dishes at 27°C for 7 days. Three mycelial discs (5 mm in diameter) of this isolate were transferred into 50 mL of Potato Dextrose Broth in 250 mL Erlenmeyer flask and incubated for 15 days at 27±1°C. In this way, 5 erlenmeyer flasks were prepared to be used in the study. At the end of this period, the fungal cultures were filtered twice through Whatman filter paper (Naz et al., 2021). The culture filtrate was used at 100% concentration without dilution in the study (Göze et al., 2022b, c) and stored at 4°C.

Preparation of *Meloidogyne incognita* inoculum

Since root-knot nematodes are obligate parasites, their mass production on live plants was continued and renewed every 2-3 months in the Tueza F1 tomato variety. Mass production of *M. incognita* was carried out on the Tueza F1 tomato variety with 20 replicates under climatic room conditions (24±1°C, 60±5% humidity-RH). After mass-produced tomato roots of the Tueza F1 tomato variety were washed in tap water, egg masses were removed from the roots under a stereomicroscope and incubated in water at 25±2ºC for three days in a petri dish containing a sterile sieve of 3 cm diameter. After three days, the J2s hatched from the eggs were counted under a light microscope and placed in 1 ml tubes, adjusted to the number to be used in the experiment. Approximately 1000 J2 of *M. incognita* were used as the nematode inoculum (Lobna et al., 2017).

Preparation of *Fusarium oxyspo*rum f. sp. *radicis lycopersici* Inoculum

FORL isolate was incubated at 25°C for 7 days in sterile petri dishes (9 cm) containing PDA. Then, 5 fungal disc pieces (1 cm^2) were cultured in autoclaved 250 ml flasks containing 50 ml of PDB (potato dextrose broth agar) and incubated at 25°C in the dark for 7 days. Handshaking was performed daily during the incubation period. After seven days, the culture filtrate was first filtered through two layers of filter paper (Whatman No.1) and then refiltered through a 0.45 µm pore size filter to remove fungal spores and mycelium. The filtrate was kept at +4°C until the experiment was established (Lobna et al., 2016).

Effect of *Aspergillus niger* culture filtrate on *Meloidogyne incognita* and *Fusarium oxysporum* f. sp. *radicis lycopersici* disease severity on tomato

In this study, the effects of undiluted culture filtrate of local *A. niger* isolate and positive controls of nematicide and fungicide on nematode and fungus in the simultaneous inoculation of *M. incognita* (N) and FORL in the Gülizar F1 tomato cultivar were investigated between April and August 2022 in Isparta province, Türkiye. Only plants with simultaneous application of *M. incognita* and FORL were evaluated as a negative control. The study was set up in a climate room under controlled conditions (24±1°C, 60%±5% humidity-RH) in plastic pots and in a randomized plot design for each treatment with 5 replications. A single tomato plant was used in each replication, and a total of five tomato plants were used in five replications. The study consists of 8 treatments; 1: N, 2: FORL, 3: N+*A. niger*, 4: FORL+*A. niger*, 5: N+FORL, 6: N+FORL+*A. niger*, 7: N+FORL+nematicide, 8: N+FORL+fungicide. Three-week-old tomato seedlings were transplanted into plastic pots with a diameter of 14 cm containing approximately 1500 g of sterile soil (68% sand, 21% silt and 11% clay). In the initial inoculum density per pot, 1000 *M. incognita* J2/1ml and $3X10^6$/10 ml FORL were used, and simultaneous inoculation was performed. The nematode inoculum was evenly dispersed with a pipette into three 2-3 cm deep holes drilled around the seedling stem and deep enough to contact the roots in the soil. FORL inoculum was poured into these holes and opened on the soil surface of the pots with the help of a measuring tape (Lobna et al., 2016, 2017). Two days after nematode and FORL inoculation, 10 ml of undiluted *A. niger* culture filtrate was applied to the holes drilled around the seedling in each potting of soil. The maximum field recommendation doses of Velum® and Cebir® were used at 0.16 ml/L and 0.25 ml/L, respectively.

The study was terminated 60 days after the culture filtrate applications of *A. niger*. Tomato plants related to each application were carefully removed from the soil and their soils were washed with tap water. Evaluation was 1-9 root gall scale for nematodes (1= no gall, 2= 5% root gall, 3= 6-10% root gall, 4= 11-18% root gall, 5= 19-25% root gall, 6= 26-50% root gall, 7= 51-65% root gall, 8= 66-75% root gall, 9= 76-100% root gall) and egg mass production rate scale (1= no egg mass, 2 = 1 or 2 egg masses, 3= 3-6 egg masses, 4= 7-10 egg masses, 5= 11-20 egg masses, 6= 21-30 egg masses, 7= 31-60 egg masses, 8= 61-100 egg masses, 9= more than 100 egg masses) (Mullin et al., 1991). The severity of disease caused by FORL was evaluated according to the 0-4 scale (0: No damage to the seedling, 1: Discoloration and small lesions at the junction of the seedling with the soil surface, 2: Larger lesions turned stem, 3: Large lesions surrounding the stem, resulting in a concave appearance, 4: Dead plant due to fungal damage) (Chandler & Santelman, 1968; Erberk, 2020). The percentages of suppressing gall, egg mass and disease severity were calculated with the formula % = (nematode or FORL alone – treatment/Nematode or FORL alone) X 100 (Xiang et al., 2020).

Statistical analyses

The SPSS Version 20 software (IBM Corporation, Armonk, New York, USA) was used for the statistical analysis. The results were presented as mean±standard error. All the data were checked for normality of distribution by using the Shapiro-Wilk tests. In the data conforming to normal distribution, one-way ANOVA and Tukey multiple comparison tests were performed ($p \leq 0.05$).

Result and Discussion

The highest gall and egg mass index were found in N and N+FORL treatments. These two treatments were followed by the N+FORL+fungicide treatments with 6.0 gall index and 6.2 egg mass indexes. The lowest gall (1.4) and egg mass (1.4) index was determined in the N+FORL+nematicide treatment. The gall and egg mass index of the N+*A. niger* treatment was lower than the N treatment. It was determined that the gall and egg mass index of the N+FORL+*A. niger* treatment was lower than the N+FORL+fungicide treatment, but higher than the N+FORL+nematicide treatment and the difference between these three treatments was found to be statistically significant ($p \leq 0.05$) (Table 1).

The suppressive effect of *A. niger* culture filtrate treatment on gall and egg mass of *M. incognita* was found to be 59.9% and 57.7%, respectively. In simultaneous treatments, the highest suppressive effect was determined in the N+FORL+nematicide treatment, followed by the treatment of N+FORL+*A. niger*. While the suppressive effect of the N+FORL+nematicide treatment on gall and egg mass was determined over 75%, of that N+FORL+*A. niger* treatment was found above 60%. On the other hand, the N+FORL+fungicide treatment showed a suppressive effect of 33.3% and 31.0% on gall and egg mass, respectively (Table 1).

While the disease severity was found to be highest in the N+FORL treatment with 3.6, it was found 3.0 in the FORL treatment. However, the difference between them is statistically insignificant ($p \geq 0.05$). The disease severity of the FORL+*A. niger* treatment (2.4) was determined to be lower than the FORL and N+FORL treatments. The FORL+*A. niger*, N+FORL+*A. niger*, N+FORL+nematicide and N+FORL+fungicide treatments were found to have similar disease severity and no statistical difference were observed between them ($p \geq 0.05$) (Table 1).

The suppressive effect of *A. niger* culture filtrate treatment on the disease severity of FORL was found to be 26.6%. The suppressive effect of the fungicide, nematicide and *A. niger* treatments on disease severity in simultaneous inoculations was found to be similar. Although the suppressive effect on disease severity in simultaneous inoculations was the highest at 33.3% in the N+FORL+*A. niger* treatment, the difference between this and the FORL+*A. niger*, N+FORL+nematicide (26.6%) and N+FORL+fungicide (26.5%) treatments were not statistically significant ($p \geq 0.05$) (Table 1).

Generally, the nematicidal and fungicidal effects of *A. niger* are studied by different researchers separately (Alwathnani & Perveen, 2012; Bhat & Wani, 2012; Devi & Bora, 2018; Arici &Tuncel, 2020; Attia et al., 2022). In the current study, it was revealed that *A. niger* has both nematicidal and fungicidal properties. In

simultaneous inoculations, the suppressive effect of *A. niger* on gall and egg mass was found to be lower than nematicide treatment, while *A. niger* culture filtrate partially suppressed FORL disease in the current study (Disease severity scale 2.4). Also, there are several studies on the biological efficacy of *A. niger* as Plant Growth-Promoting Fungi against Fusarium wilt in tomato. Similar results were obtained in earlier studies (Kerkeni et al., 2007; Nikhat et al., 2019; Abdel-Motaal et al., 2020; Jamil et al., 2021; Abd Alhakim et al., 2022).

Table 1. Effect of *Aspergillus niger* culture filtrate on *Meloidogyne incognita* and *Fusarium oxysporum* f. sp. *radicis lycopersici* disease severity on tomato

	Treatments	Gall index (1-9)[1]	Percent suppressive effect on gall (%)	Egg mass index (1-9)[2]	Percent suppressive effect on egg mass (%)	Disease severity scale[3]	Percent suppressive effect on disease (%)
1	N	9.0±0.0 a [4]	-	9.0±0.0 a	-	-	-
2	FORL	-	-	-	-	3.0±0.4 ab	-
3	N+*A. niger*	3.6±0.2 c	59.9±2.7 b	3.8±0.2 c	57.7±2.2 b	-	-
4	FORL+*A. niger*	-		-		2.4±0.2 b	26.6±6.6 a
5	N+FORL+*A. niger*	3.2±0.2 c	64.3±2.2 b	3.2±0.3 c	64.3±4.1 ab	2.0±0.3 b	33.3±10.5 a
6	N+FORL (negative control)	8.6±0.2 a	4.4±2.7 d	8.8±0.2 a	4.4±2.7 d	3.6±0.2 a	0.0±0.0 b
7	N+FORL+ Nematicide (Positive control)	1.4±0.2 d	84.3±2.7 a	1.4±0.2 d	77.7±6.0 a	2.2±0.2 b	26.6±6.6 a
8	N+FORL+Fungicide (Positive control)	6.0±0.4 b	33.3±4.9 c	6.2±0.3 b	31.0±4.1 c	2.2±0.2 b	26.5±6.6 a

[4] The lowercase letters in the same column indicate significant differences between treatments ($p \leq 0.05$).

Aspergillus niger shows that the disease severity can be reduced by suppressing the growth or reproduction of either nematode or fungus when inoculated at the same time. It was determined that disease severity was highest in the negative control. However, it was observed that disease severity decreased when nematode was controlled. Yeon et al. (2022) reported that *A. niger* F22 formulation (Nemafree, 20% SC) and oxalid acid can reduce nematode populations and promote tomato plant growth by increasing the activities of defense enzymes in tomato plants. Jin et al. (2019) reported that culture filtrate application of *A. niger* NBC001 isolate can control *Heterodera glycines* Ichinohe, 1952, cyst nematode in soybean seedlings, in both pot and field conditions. It has been stated that *A. niger* and *A. candidus* are potential fungal agents that can be used against plant parasitic nematodes (Khan & Anwer, 2008; Yin et al., 2015; Jang et al., 2016; Shemshura et al., 2016; Maishera et al., 2019).

Although the application of fungicide to N+FORL did not show a large suppressive effect on gall and egg mass, it reduced the disease severity. Additionally, the nematicidal and fungicidal effect of *A. niger* is higher than the fungicide against N+FORL. However, the lack of suppression of the nematode may result in the formation of new offspring and the emergence of new infections from these offspring to the plant. Since the exposure of the plant to nematode attack will increase the susceptibility of the plants to secondary microorganisms, the disease may recur in the plant or the severity of the disease may increase (Back et al., 2002; Göze Özdemir et al., 2022a). It has been reported in different studies that simultaneous infection with root-knot nematode and FORL causes more severe damage to the host plant than infection with each nematode and fungus alone (McGawely, 2001; Hajji et al., 2016). All these results show that nematode control is a priority in preventing disease formation. There are problems experienced in chemical control on both organisms and a lack of resistance is observed in some commercial tomato varieties. It is important to prioritize alternative control methods in the prevention of disease complexes. Antifungal and antinematicidal effects of *A. niger* against root-knot nematode and FORL were determined, and it provides disease control as a good biocontrol agent.

The fact that this isolate is native to Türkiye increases the importance of the study. Metabolite production may differ depending on the type of fungal isolates and culture medium or its composition (Wang et al., 2004; Mohanty et al., 2008; Kim et al., 2013). This effect may be caused by toxic compounds such as secondary

enzymes or toxins secreted by *A. niger* (Maria & Urszula, 2012; Patil et al., 2017; He et al., 2020; Xiang et al., 2020; Naz et al., 2021). It is thought that toxic enzymes and toxins are more in culture filtrates and nematicidal effect increases as a result of synergistic or antagonistic interactions with each other (Kim et al., 2013).

In previous studies, we applied *A. niger* separately only to fungi and nematodes. This is the first study conducted in Türkiye to evaluate the use of *A. niger* culture filtrate in the control of FORL on tomatoes. According to the results, undiluted *A. niger* culture filtrate were determined to be a highly promising potential source of microbial nematicide or fungicide on tomato. Also, its compounds will be a resource for the development of new chemicals to manage root-knot nematodes and FORL. For this reason, chemical compounds of *A. niger* isolate (AnIB18) should be identified. It is necessary to investigate the environmental conditions (temperature, RH, culture medium, etc.) that will affect the effectiveness of *A. niger*. Since this study was carried out in pots containing sterilized soil, the effectiveness of *A. niger* in field conditions needs to be investigated because when applied to the field, its interaction and competitiveness with other soil microorganisms are unknown. As a conclusion, it can be said that application of *A. niger* to the soil will be an effective alternative control method to reduce pesticide use and increase yield in root-knot nematode and FORL control.

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Original article (Orijinal araştırma)

The effects of different Charleston pepper cultivars on the demographic parameters and the antioxidant levels of *Myzus persicae* (Sulzer, 1776) (Hemiptera: Aphididae)[1]

Farklı Charleston biber çeşitlerinin *Myzus persicae* (Sulzer, 1776) (Hemiptera: Aphididae)'nin demografik parametreleri ve antioksidan seviyeleri üzerine etkileri

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Abstract

Host plant diversity causes differences in the biology and adaptation of insects. In this study, variations in some biological properties and adaptive antioxidative response of *Myzus persicae* (Sulzer,1776) (Hemiptera: Aphididae) on five Charleston pepper, *Capsicum annuum* L. (Solanaceae) cultivars were investigated under laboratory conditions (25±1°C, 60±5% RH). The lowest intrinsic rate of increase (r = 0.193 d^{-1}) of *M. persicae* was estimated in the tested cohort fed with the Kanyon cultivar, while the highest intrinsic rate of increase (r = 0.248 d^{-1}) was found on the Tufan cultivar. The cohort fed with Safkan cultivar exhibited the highest levels of GST-CDNB and EST-PNPA at 562.80 and 207.64 nmol/mg protein, respectively, whereas the cohort fed with Kanyon cultivar showed the lowest levels at 317.04 and 132.14 nmol/mg protein, respectively. Analysis of life table parameters and enzymatic/non-enzymatic antioxidant levels of *M. persicae* showed that among the cultivars we tested, the Tufan cultivar was the most preferred host by *M. persicae,* while Kanyon cultivar was a less suitable host.

Keywords: Age-stage two-sex life table, *Capsicum annuum*, enzymatic antioxidant, *Myzus persicae*

Öz

Konukçu bitki çeşitliliği, böceklerin biyolojisinde ve adaptasyonunda farklılıklara neden olur. Bu çalışmada, laboratuvar koşullarında (25±1°C, %60±5 orantılı nem) *Myzus persicae* (Sulzer,1776) (Hemiptera: Aphididae)'nin beş Çarliston biber, *Capsicum annuum* L. (Solanaceae) çeşidi üzerindeki bazı biyolojik özellikleri ve adaptif antioksidan tepkilerindeki değişimler incelenmiştir. *Myzus persicae*'nin en yüksek kalıtsal üreme oranı (r = 0.248 g^{-1}) Tufan çeşidi üzerinde, en düşük ise (r = 0.193 g^{-1}) Kanyon çeşidi üstünde beslenen test edilen grupta kaydedilmiştir. Safkan çeşidi üstünde beslenen grup, sırasıyla 562.80 ve 207.64 nmol/mg protein ile en yüksek GST-CDNB ve EST-PNPA seviyelerini sergilerken, Kanyon çeşidi üstünde beslenen grup sırasıyla 317.04 ve 132.14 nmol/mg protein düzeyleri ile en düşük seviyeleri göstermiştir. *Myzus persicae*'nin yaşam çizelgesi parametreleri ve enzimatik/enzimatik olmayan antioksidan düzeylerinin analizleri, test ettiğimiz çeşitler arasında *M. persicae* tarafından en çok tercih edilen konukçunun Tufan çeşidi olduğunu, Kanyon çeşidinin ise daha az uygun konukçu olduğunu göstermiştir.

Anahtar sözcükler: Yaş ve döneme özgü iki eşeyli yaşam çizelgesi, *Capsicum annuum*, enzimatik antioksidan, *Myzus persicae*

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Introduction

One of the most critical factors that determine the level of damage caused by herbivorous insects is the quality and suitability of the host plant (Hong et al., 2019). Depending on the physical differences and biochemical compounds of the host plants, the biology of the insect feeding on it is also affected in different ways. Even different cultivars of the same host plant species can have varying effects on the development, reproduction, and survival rates of the insect (La Rossa et al., 2013; Razazzian et al., 2015; Özgökçe et al., 2018).

Insects produce enzymatic antioxidants, which are proteins that help protect cells from oxidative stress by neutralizing harmful free radicals as a defense mechanism against their damaging effects. They include catalase (CAT), glutathione (GSH), superoxide dismutase (SOD), ascorbate peroxidase (APX) and guaiacol peroxidase (GPX) (Ologundudu, 2021). Non-enzymatic antioxidants work by interrupting free radical chain reactions. They include ascorbate, carotenoids, flavonoids, and other phenolics (Ologundudu, 2021). Lukasik et al. (2011) reported that changing the host plant affected the levels of enzymatic and non-enzymatic antioxidants in *Acyrthosiphon pisum* (Harris, 1776) (Hemiptera: Aphididae). This suggests that oxidative stress may play a significant role in the interactions between herbivorous insects and their host plants, as noted by Lukasik & Goławska (2013). Superoxide anion, hydroxyl radical, and hydrogen peroxide are typical reactive oxygen species (ROS), generally produced by oxidative metabolism in herbivorous insect cells (Lukasik & Golawska, 2013). Additionally, they are exposed to exogenous ROS, which is created by the defense mechanism of plants (Orozco-Cardenas & Ryan, 1999). Furthermore, the saliva and damage caused by aphids have been claimed to induce ROS production in the host plant cells, particularly in phloem cells (Lukasik & Golawska, 2013). ROS are crucial for the defense of host plant against insects that feed on plants.

Insects have detoxifying enzyme systems that include esterases (EST) and glutathione S-transferases (GST). These enzymes take a role in the metabolism of xenobiotic substances that could cause cellular and tissue damage, derived from oxidative stress (Konuş, 2014). Furthermore, insect cells have non-enzymatic ROS scavenger compounds such as ascorbate and glutathione (Kazek et al., 2020). Lastly, the total thiol content of insect cells depletes due to the enhanced metabolism of xenobiotics (Vontas et al., 2001).

The green peach aphid, *Myzus persicae* Sulzer, 1776 (Hemiptera: Aphididae) is a polyphagous pest that can infest over 400 host plant species from more than 40 families, including pepper, and can also transmit more than 100 plant viruses (Bass et al., 2014; van Emden & Harrington, 2017). Under favorable environmental conditions, aphids can quickly produce a large population, significantly damaging plant growth and development by feeding on the phloem sap, which contains important photosynthesis products. Aphids also cause indirect damage by secreting sooty mold during feeding, which reduces the photosynthetic capacity of the plant (Naranjo & Legg, 2010; Cameron et al., 2013). Additionally, direct damage from aphids can include dehydration, loss of flower buds, weakness, and an overall decrease in vegetative growth (La Rossa et al., 2013).

Türkiye is the world's third-largest producer of peppers after China and Mexico, with a production of 2.6 million tons (FAO, 2020). In 2021, the country's pepper production increased to 3 million tons (TUIK, 2022). Charleston peppers are an essential agricultural commodity due to their economic value and nutritional and medicinal benefits (Emmanuel-Ikpeme, 2014). *Capsicum* cultivars are known to contain substantial amounts of vitamins B, C, E, and provitamin A (carotene) (Bozokalfa & Eşiyok, 2010). Peppers are composed of various compounds, including oil, colors, resin, protein, cellulose, pentosa, and minerals. Peppers are high in vitamin C, with some cultivars containing up to 340 mg/100 g (Eşiyok, 2006).

The green peach aphid, *M. persicae*, is a significant threat to pepper crops in Türkiye, and conventional pest control methods rely heavily on the use of pesticides, both within the country and worldwide (Bass et

al., 2014; Özgökçe et al., 2018). However, the extensive use of pesticides over the years has resulted in widespread and varied forms of resistance developing (Bass et al., 2014). Hence, there has been a growing emphasis on alternative methods to chemicals, specifically on the use of resistant varieties in recent years (Silva et al., 2012).

The use of resistant varieties or unsuitable host plants in agricultural production offers significant advantages such as compatibility with other control techniques like biological pesticides or biological agents, environmental friendliness, affordability, and reduction of harmful pest populations (Stansly & Natwick, 2009; Vieira et al., 2011; Silva et al., 2012). This approach can be particularly effective in integrated pest management (IPM) strategies, where multiple methods are used in combination to control pests while minimizing their impact on the environment and human health. By incorporating resistant varieties or unsuitable host plants into IPM, producers can reduce their reliance on traditional chemical pesticides, which can be expensive and have negative environmental consequences. These methods can help to slow the development of resistance in pest populations, making them a valuable long-term solution for sustainable agriculture. In addition, the genetic diversity offered by resistant varieties and unsuitable host plants provides a valuable resource for modern genetic research (Panda & Khush, 1995; Stout, 2007; Smith & Clement, 2012).

Life tables are an effective tool to comprehend how host plants influence the biology of insects. Using a life table is the most effective way to describe the development, stage differentiation, survival, reproduction, and population growth of a species (Yang & Chi, 2006; Huang & Chi, 2013; Yin et al., 2013; Özgökçe et al., 2018; Chi et al., 2020). Utilized the age-stage two-sex life table to determine the population parameters of *M. persicae* on various Charleston pepper cultivars in this study. To further substantiate the life table parameters, the impact of enzymatic/non-enzymatic antioxidant levels of *M. persicae* on diverse pepper cultivars was also assessed. The data acquired from this study will furnish significant fundamental insights for both Charleston pepper production programs and genetic studies.

Materials and Methods

Plant materials

Five Charleston pepper cultivars (A.3055, Tufan, Kanyon, Maraton, and Safkan) were used in this study. Seedlings were obtained from commercial suppliers such as Rijk Zwaan (Bellisa), Yüksel Tohum (Cümbüş), Antema Tarım (Diyar), AG Tohum (Paşa), Nunhems Tohum (Sarp) and Mars Tohum (Serenat) in Antalya and Mersin, Türkiye. The cultivars were planted in 4-litter pots, and all the plants were kept in climatic rooms set to 25±1°C, 60±5%RH, and 16:8 (L:D) h periods.

Insects

Myzus persicae colonies were acquired from the Van Yüzüncü Yıl University Plant Protection Department's stock culture in 2019, and all studies were completed at Van Yüzüncü Yıl University, Faculty of Agriculture, Plant Protection Department laboratories in 2020. Aphids were reared on each cultivar plant for at least 3-4 generations to enable their adaptation to their new hosts in a climatic room set at the experimental conditions mentioned above.

Construction life table and analysis

To conduct the experiment, a single wingless adult green peach aphid was placed on the undersides of medium-sized leaves of each cultivar for each treatment. After 12 hours, only one of the newborn nymphs was kept in cylindrical Plexiglas cages with a height of 2 cm, a diameter of 2 cm and covered with cheesecloth for 24 hours. With daily observations, development and nymphal mortality data were recorded. Following the adult emergence, all adults' fecundity and survival rates were observed daily until their death. The study was conducted using 36-49 replicates for each cultivar.

The TWOSEX-MSChart (Chi, 2022a) computer software based on the concept of age-stage, two-sex table life was used to analyze the raw data which was obtained from development time, survival and fecundity of green peach aphid (Chi, 1988; Chi & Liu, 1985). The most important life table parameters [the intrinsic rate of increase (r), the finite rate of increase (λ), net reproductive rate (R_0), and mean generation time (T)], and some crucial population parameters [age specific survival rate (l_x), fecundity (m_x), age-stage-specific survival rate (s_{xj}; where x = age and j = stage), life expectancy (e_{xj}), and reproductive value (v_{xj})] were calculated (Goodman, 1982; Chi & Su, 2006; Huang & Chi, 2011; Tuan et al., 2014). By employing a paired bootstrap test with 100,000 resamples to get reliable estimates, it was possible to compare the life table and population characteristics of the green peach aphid that fed with different cultivars (Efron & Tibshirani, 1994; Özgökçe et al., 2018; Wei at al., 2020).

Population projection

The population development of *M. persicae* was simulated using the computer software TIMING-MSChart (Chi, 2022b), which is based on the concepts of Chi & Liu (1985) and Chi (1990), using life table data collected from the experiments. The population size that *M. persicae* could reach at day 60 was simulated based on the initial population of 10 nymphs.

Preparation of insect homogenates and protein determination

Insect samples were homogenized with a homogenizer on ice in 0.75 ml of 100 mM potassium phosphate buffer (pH 7.2), containing 1 mM dithiothreitol (DTT) and 1 mM ethylenediaminetetraacetic acid (EDTA). In the homogenization process, the samples were observed homogeneously for 20 seconds and kept on ice for 20 seconds. This process was repeated five times. After homogenization, the samples were centrifuged at +4ºC and 10,000 x g for 30 minutes. As the final step, non-specific esterase, glutathione S-transferase, and total thiol group determination tests were performed using supernatant as enzyme source. Bradford assay was used to measure protein quantities (Bradford, 1976).

Determination of total thiol groups

To determine the total thiol groups of insect homogenates, a modified version of Sedlak and Lindsay method was used (Sedlak & Lindsay, 1968). This method depends on reducing DTNB (5,5'-dithiobis-(2-nitrobenzoic acid) by the thiol groups present in the tested samples. In this assay, the reaction mixture contained 20 mM EDTA, 2 mM DTNB, methanol, 10µL homogenate and 200 mM Tris buffer (pH: 8.2) with a final volume of 200 µL. After the addition of the thiol group containing homogenates to each well, the plates were incubated at 25ºC for 30 minutes. Finally, the absorbance value of each well was measured at a wavelength of 405 nm. Utilizing reduced glutathione standard curves, the total amount of thiol groups was determined. The amount of total thiol groups was given as nmol/mg protein.

Analysis of the activity of the GST enzyme against 1-chloro-2,4-dinitrobenzene (CDNB)

Using the substrate 1-chloro-2,4-dinitrobenzene (CDNB), the modified Habig et al. (1974) technique was used to evaluate the glutathione S-transferase activity. There were 100 mM potassium phosphate buffer (pH 7.4), 1 mM GSH, and 1 mM CDNB in each reaction mixture. GST-CDNB activity measurements were performed at 37ºC for 10 minutes at 340 nm. The activities of GST-CDNB were measured as described by Konus et al. (2014). The expression for GST-CDNB activities was nmol/min/mg protein.

Activity of non-specific esterase (EST-PNPA) determination

Using p-nitrophenyl acetate, the non-specific esterase enzyme activities of the insect samples were assessed using the van Asperen technique (PNPA) (van Asperen, 1962). 200 L of the reaction mixture including 100 mM potassium phosphate buffer, pH 7.0, 0.05% Triton X-100, and 3.8 mM PNPA was used as the final volume. EST-PNPA activity was calculated as described by Konus et al. (2014). The expression for EST-PNPA activity was nmol/min/mg protein.

Statistics for thiol and enzyme assays

All measurements were conducted between 3-to 7 times, and for the comparison purposes, 100,000 bootstrap simulations were executed using the Twosex MSCHART (Chi, 2022a) software. The paired test was employed to estimate the difference between the means.

Results

Development survival and reproduction

All the nymphs that were tested on different Charleston pepper cultivars have become adult by completing their developmental periods, and then adults gave birth. No any individuals died during their development periods, so survival rates were 100% in all cohorts. The data on each nymphal stage and total preadult durations of *M. persicae* on five different Charleston pepper cultivars were presented in Table 1. The durations of developmental stages of *M. persicae* were found to be significantly affected by feeding on different host plants. It had the longest total preadult development time when fed with Kanyon cultivar (10.95 days), while the shortest development time was observed in the cohort fed with the Tufan cultivar (8.21 days) ($p < 0.05$).

Table 1. The development times, longevity, fecundity and oviposition of *Myzus persicae* on different Charleston pepper cultivars (A.3055, Tufan, Kanyon, Maraton, and Safkan) (mean±SE)

Parameters	n	A.3055	n	Kanyon	n	Maraton	n	Safkan	n	Tufan
Nymph1	36	1.47±0.109c*	41	2.27±0.16a	49	2.06±0.138a	40	1.5±0.08c	48	1.75±0.076b
Nymph2	36	2.89±0.186a	41	2.34±0.183a	49	2.59±0.167a	40	3.08±0.194a	48	2.5±0.133a
Nymph3	36	2.33±0.154a	41	2.8±0.216a	49	2.53±0.173a	40	2.33±0.173a	48	2.19±0.142a
Nymph4	36	2.58±0.230b	41	3.54±0.288a	49	2.21±0.179b	40	2.23±0.184b	48	1.77±0.124c
Preadult time	36	9.28±0.315b	41	10.95±0.413a	49	9.42±0.323b	40	9.12±0.218b	48	8.21±0.181c
Adult longevity	36	29.56±1.066a	41	25.32±0.926bc	49	23.96±0.83c	40	27.75±0.928ab	48	25.96±0.639bc
Total longevity	36	38.83±1.047a	41	36.27±0.974ab	49	32.84±1.029c	40	36.88±0.899a	48	34.17±0.653bc
Fecundity	36	67.92±5.01a	41	31.10±2.42c	49	49.62±3.01b	40	59.95±2.07a	48	62.21±2.71a
TPRP	36	9.28±0.32b	41	10.95±0.41a	49	9.5±0.32b	40	9.12±0.22b	48	8.21±0.18c
Oviposition	36	25.67±1.04a	41	17.93±0.96b	49	20.19±0.87b	40	24.88±0.77a	48	23.42±0.61a

* Differences between means signed in the same line with the same letters are not significantly important ($p > 0.05$).

All the tested individuals began reproducing the day they reached adulthood. Therefore, it was determined that the durations for total preadult time and TPRP (total pre-reproductive period, which is defined as the average duration from birth to the first reproduction) were the same. Significantly longest adult longevities were observed in the A.3055 (29.56 days) and Safkan (27.75 days) cultivars, while the shortest longevity was observed in the Maraton cultivar (23.96 days) ($p < 0.05$). The cohort of the Maraton cultivar displayed the shortest total longevity (32.84 days), while the longest total longevity was observed in the A.3055, Safkan, and Kanyon cultivars (38.83, 36.88, and 36.27 days, respectively) ($p < 0.05$). The fecundity of *M. persicae* was affected by the pepper cultivars fed with. Among the tested pepper cultivars, the A.3055, Tufan, and Safkan cultivars resulted in the highest fecundity of *M. persicae* with 67.92, 62.21, and 59.95 offspring, respectively, while the Kanyon cultivar had the lowest fecundity with 31.10 offspring ($p < 0.05$). Compared to the Maraton (20.19 days) and Kanyon (17.93 days) cultivars, the oviposition time of the *M. persicae* was significantly longer on the A.3055, Safkan, and Tufan cultivars (25.67, 24.88, and 23.42 days, respectively) (Table 1) ($p < 0.05$).

The detailed age-stage survival rates (s_{xj}) (it reflects the probability that a newborn survives to age x and j stage) of the five cohorts of *M. persicae* fed with different cultivars are demonstrated in Figure 1. In this study, the probability that the newly born nymph of the pest to reach the adult stage was 1.00 (100%) on all cultivars because of no death during the preadult duration. The survival curves displayed stage overlapping, owing to the different developmental periods of individuals across all cultivars. It was observed that the first adults emerged in 4-7 days in all cultivars.

The age-specific survival rate (l_x), illustrated in Figure 2 for the green peach aphid on different cultivars, represents the probability that a newborn individual will live to age x. In all cultivars, the green peach aphid seems to have a fairly high survival rate up to the conclusion of the adult stage. At the end of its lifetime, survival rates of *M. persicae* on the Tufan, and Safkan cultivars dramatically decreased with a sharper trend than other cultivars.

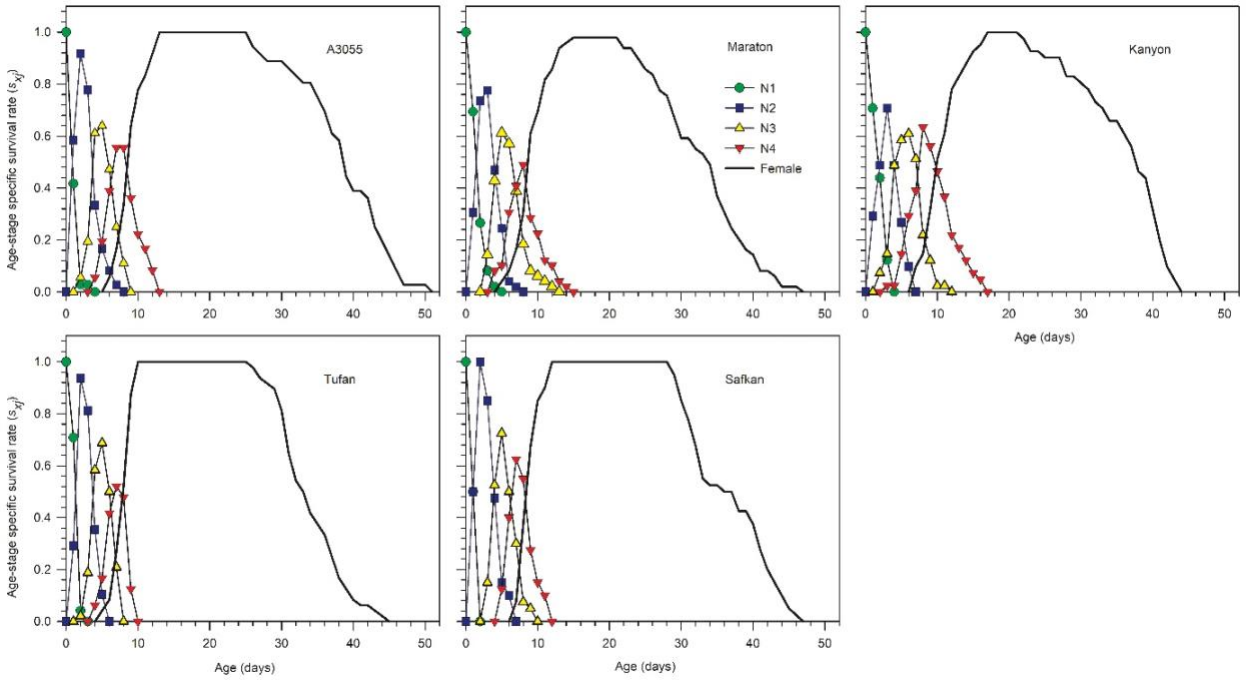

Figure 1. Age-stage specific survival rate (s_{xj}) of *Myzus persicae* reared on Charleston pepper cultivars (A.3055, Tufan, Kanyon, Maraton, and Safkan).

Based on the information provided, Figure 2 shows the age-specific rates of fecundity (m_x) and maternity ($l_x m_x$) of *M. persicae* on different cultivars. The curves indicate that the pest starts reproducing within 4-7 days of its lifetime on all the cultivars tested. The curves also show that the age-specific fecundity (m_x) and age-specific maternity ($l_x m_x$) peaks of *M. persicae* on the Kanyon cultivar were notably lower than those observed on the other cultivars. This suggests that the Kanyon cultivar may not be as suitable for the pest's reproduction as the other cultivars. Furthermore, the maternity curves were found to be very close and parallel to the fecundity curves on all cultivars tested until the end of the adult lifespan. This indicates that the percentage of adult females reproducing at each age was relatively constant, and that the reproductive output of the pest was primarily influenced by its age-specific fecundity.

The age-stage life expectancy (e_{xj}) curves of *M. persicae* were presented in Figure 3. The overall lifespan, which was previously described, is also the life expectancy of a newborn individual (e_{01}) (Table 1).

According to Yang & Chi (2006), life expectancy is an estimate of how long an individual would live under specific circumstances. For example, the expected life time of a newborn individual on the A.3055 cultivar will be 37.45 days, while on the Maraton cultivar it will be only 32.84 days.

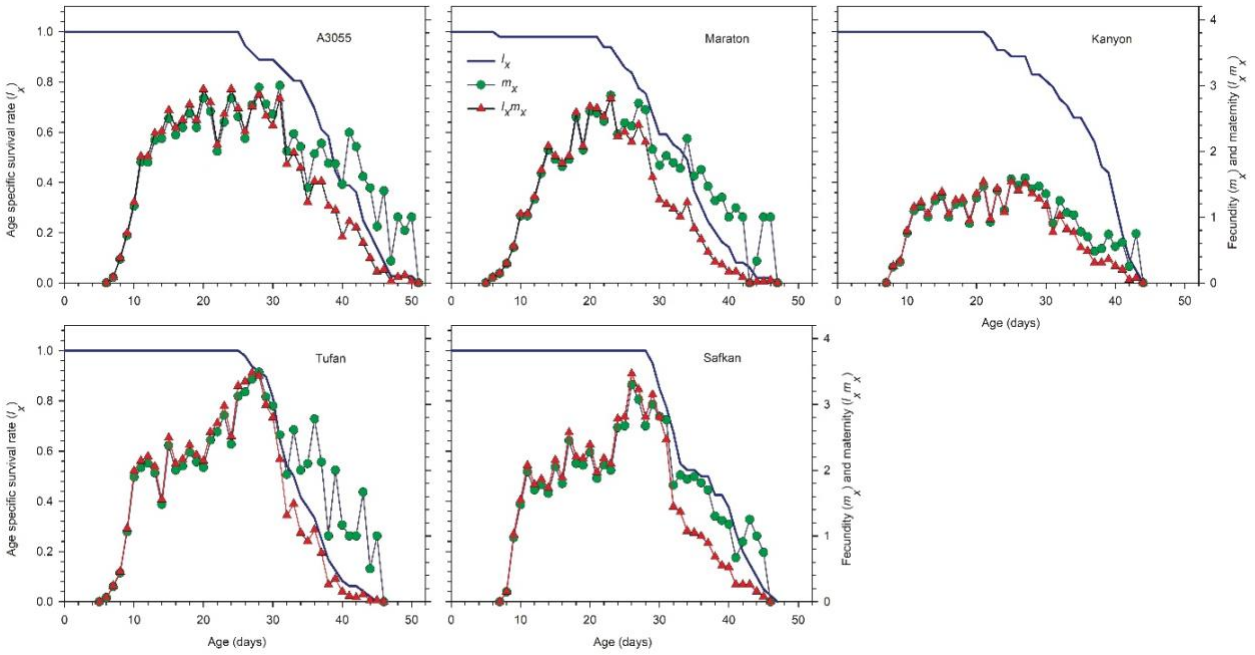

Figure 2. Age-specific survival rate (l_x), fecundity (m_x) and maternity ($l_x m_x$) of *Myzus persicae* reared on Charleston pepper cultivars (A.3055, Tufan, Kanyon, Maraton, and Safkan).

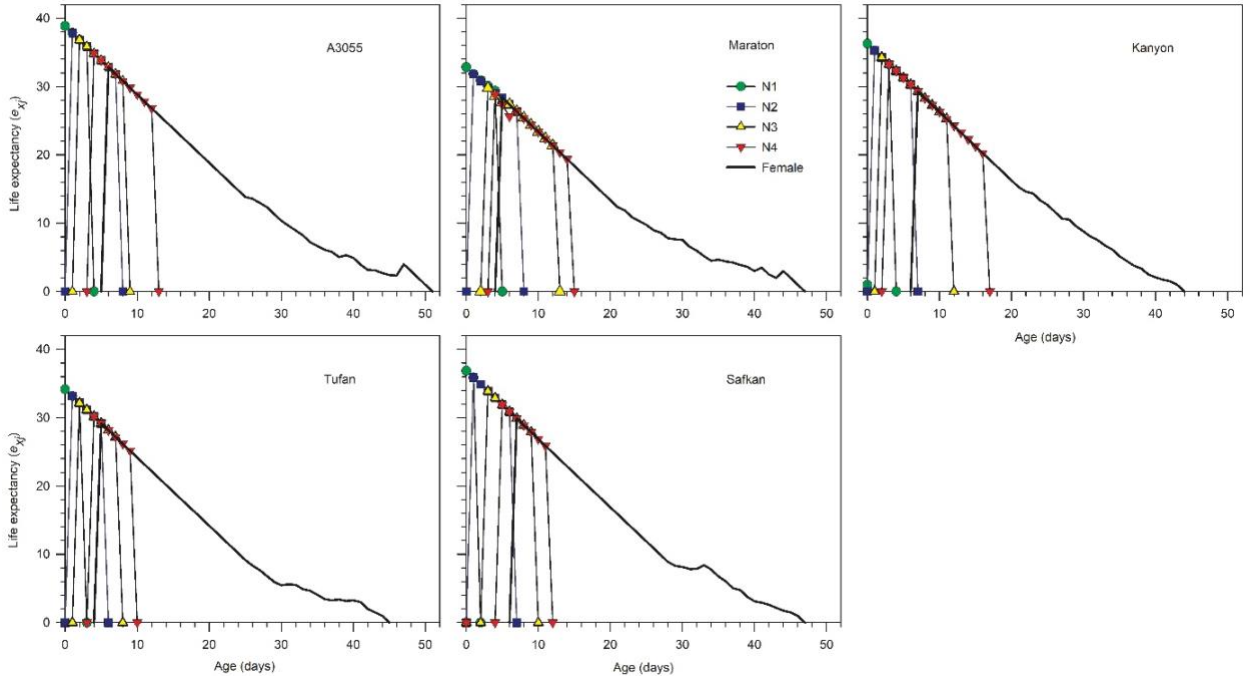

Figure 3. Age-stage-specific life expectancy (e_{xj}) of *Myzus persicae* reared on Charleston pepper cultivars (A.3055, Tufan, Kanyon, Maraton, and Safkan).

Fisher (1930) defined the term "reproductive value" as the value an individual has in terms of contributing to the future population. The Twosex life table theory, proposed by Chi in 1988, defines the age-specific and stage-specific reproductive value (v_{xj}) as an individual's contribution to the future population at a particular age x and stage j. It is equivalent to the finite rate of increase (λ) for a newborn individual (v_{01}). Age-stage reproductive value (v_{xj}) reached its highest level in the female stage in all cohorts,

with the following estimates: 11.2 at age 16 for A.3055, 12.19 at age 23 for Safkan, 10.76 at age 7 for Kanyon, 11.44 at age 17 for Maraton, and 12.2 at age 24 for Tufan (as shown in Figure 4).

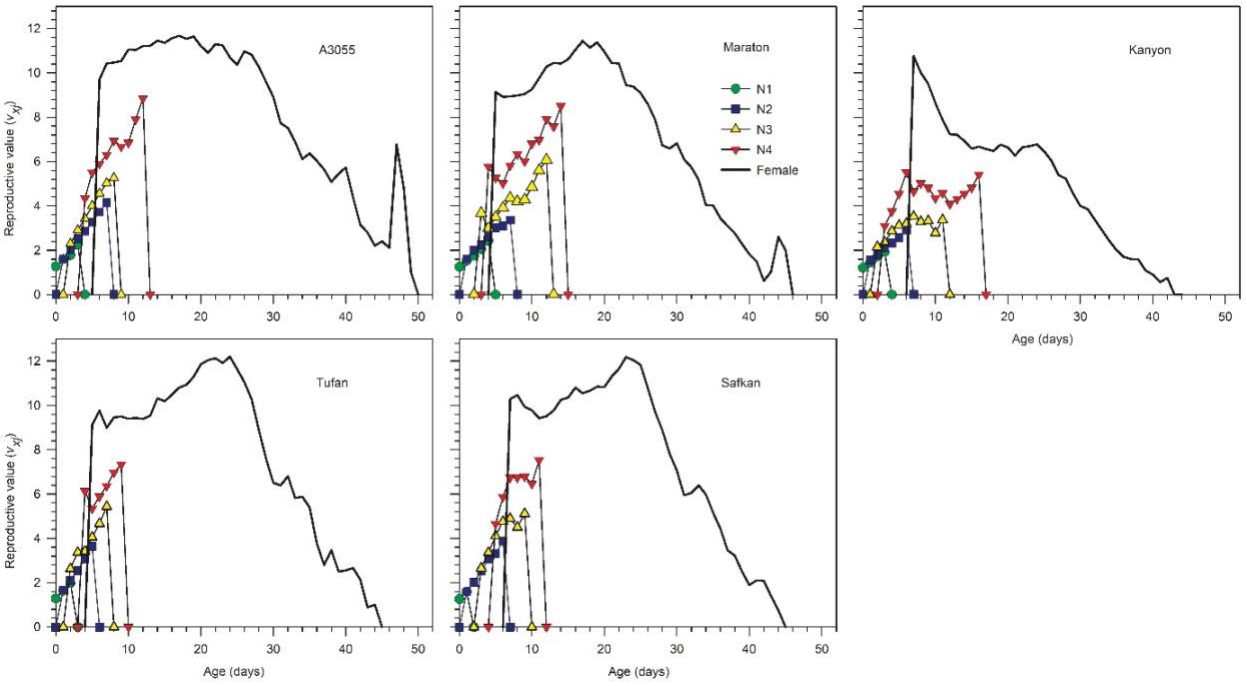

Figure 4. Age-stage-specific reproductive value (v_{xj}) of *Myzus persicae* reared on Charleston pepper cultivars (A.3055, Tufan, Kanyon, Maraton, and Safkan).

The life table parameters

The life table parameters of the green peach aphid, which include intrinsic rate of increase (r), net reproduction rate (R_0), mean generation time (T), and finite rate of increase (λ), were estimated on various Charleston pepper cultivars and are displayed in Table 2. The cohort of green peach aphid fed with the Kanyon cultivar exhibited significantly lower values of r, λ and R_0 (0.193 d^{-1}, 1.213 d^{-1}, and 31.09 offspring, respectively) compared to other cohorts ($p < 0.05$). Conversely, the Tufan cultivar had the highest values of r and λ (0.248 d^{-1}, 1.282 d^{-1}, respectively) among all the cultivars. No significant differences were observed in the mean generation time of *M. persicae* on different pepper cultivars, which ranged from 16.64 days to 17.84 days.

Table 2. The life table parameters of *Myzus persicae* on different Charleston peper cultivars (A.3055, Tufan, Kanyon, Maraton, and Safkan) (mean±SE)

Life table parameters	A3055	Kanyon	Maraton	Safkan	Tufan
The intrinsic rate of increase, r (d^{-1})	0.238±0.009ab	0.193±0.009c	0.222±0.007b	0.233±0.005b	0.248±0.006a
The finite rate of increase, λ (d^{-1})	1.269±0.012ab	1.213±0.011c	1.248±0.008b	1.263±0.006b	1.282±0.007a
Net reproductive rate, R_0 (offspring)	67.92±4.92a	31.09±2.38c	48.61±3.09b	59.95±2.04a	62.21±2.68a
Mean generation time, T (day)	17.72±0.61a	17.84±0.61a	17.51±0.44a	17.55±0.34a	16.64±0.38a

* Differences between means signed in the same line with the same letters are not significantly important ($p > 0.05$).

The intrinsic rate of increase, a key tool for summarizing an organism's physiological characteristics in relation to its growth potential, is widely used to compare the fitness of organisms in various climatic and nutritional settings (Andrewartha & Birch, 1954; Tsai & Wang, 2001; Hong et al., 2019). This parameter often provides important information independently from other life table parameters (Petitt et al., 1994). The intrinsic rate of increase is a single parametric value derived from variables of development, survivorship, fecundity and reproductive age of an organism kept under certain conditions. To use this value in comparisons, their pseudo values are derived by using Jacknife method in the classical method. However,

because the number of pseudo values derived from the Jacknife method depends on the number of repetitions in the trial and often does not show normal distribution, reliability in the comparison tests decreases. As a matter of fact, the Jacknife technique has proven to be inadequate in life table analysis (Huang & Chi, 2012, 2013; Yu et al., 2013). In this study, the 100,000 resampling bootstrap technique was used to obtain a precise estimate of population parameters.

Population projection

Using an initial population of 10 newborn nymphs, the age-stage population sizes were calculated for each cohort under the same experimental conditions, as shown in Figure 5. At the end of 60th days, the population size of green peach aphid was estimated as follows 10 654 406, 5 142 569, 4 264 705, 2 182 228, and 420 470 individuals on the Tufan, A.3055, Safkan, Maraton, and Kanyon cultivars, respectively (Table 3). The green peach aphid was able to produce a considerably lower population density on the Kanyon cultivar than others.

Table 3. Population size of *Myzus persicae* with 10 nymphs initial population on Charleston pepper cultivars (A.3055, Tufan, Kanyon, Maraton, and Safkan) after 60th days

Cultivars	N1	N2	N3	N4	Female	Total
A.3055	1 608 830	1 909 965	870 771	521 693	671 310	5 142 569
Kanyon	144 208	98 047	72 266	49 540	56 409	420 470
Maraton	775 706	613 216	322 507	175 410	295 389	2 182 228
Safkan	1 239 255	1 513 088	626 794	354 917	530 651	4 264 705
Tufan	3 694 497	3 151 651	1 562 075	788 511	1 457 672	10 654 406

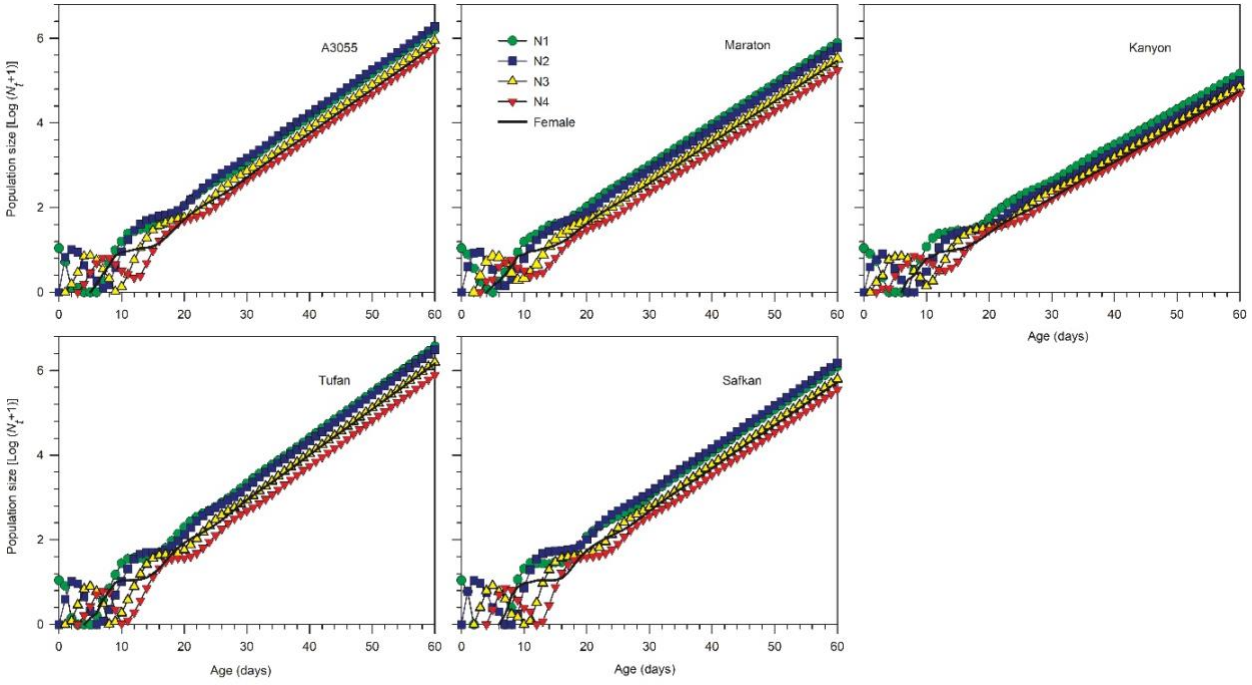

Figure 5. Population projection of *Myzus persicae* reared on Charleston pepper cultivars (A.3055, Tufan, Kanyon, Maraton, and Safkan).

Determination of total thiol groups

The results indicated that the highest amount of total thiol groups (15.59 nmol/mg protein) was obtained in the *M. persicae* individuals reared on the Safkan cultivar, however, the lowest amount was in individuals reared on the Kanyon and the Tufan cultivars as follows, 3.26, 3.53 nmol/mg protein, respectively ($p < 0.05$) (Table 4).

Table 4. Summary of overall enzyme activities and total thiol content results of *Myzus persicae* on different Charleston peper cultivars (A.3055, Tufan, Kanyon, Maraton, and Safkan) (mean±SD)

ASSAY	A.3055	Kanyon	Maraton	Safkan	Tufan
Total Thiol Content (nmol/mg protein)	4.24±0.5c	3.26±0.18d	5.12±0.27b	15.59±0.42a	3.53±0.23d
GST-CDNB (nmol/min/mg protein)	497.38±26.21b	317.04±11.98d	547.68±28.1a	562.8±21.91a	419.9±26c
EST-PNPA (nmol/min/mg protein)	196.33±36.21a	132.14±22.53b	204.23±15.19a	207.64±27.46a	154.94±9.55b

* Differences between means signed in the same line with the same letters are significantly not important ($P > 0.05$).

Analysis of the activity of the GST enzyme against 1-chloro-2,4-dinitrobenzene (CDNB)

Using CDNB substrate, the GST enzyme activity of insect samples was measured. GST enzyme activity of the pest reared on A.3055, Kanyon, Tufan, Maraton, and Safkan cultivars were given in Table 4. According to these findings, while the highest GST-CDNB activity (562.8±21.91 nmol/min/mg protein) was seen in the *M. persicae* individuals reared on the Safkan cultivar**,** and the lowest GST-CDNB activity (317.04±11.98 nmol/min/mg protein) was seen in the *M. persicae* individuals reared on the Kanyon cultivar ($p < 0.05$).

Activity of non-specific esterase (EST-PNPA) determination

The non-specific esterase enzyme activities of *M. persicae* samples were measured using the EST-PNPA test. The results showed that the samples of *M. persicae* reared on the Safkan, Maraton, and A.3055 cultivars had the highest EST-PNPA activity, whereas the individuals reared on the other two cultivars had the lowest EST-PNPA activity (Table 4).

Discussion

Plants have evolved diverse genetic variations over thousands of years, in response to both biotic and abiotic factors (Smith & Clement, 2012). Plant varieties exhibit varying pest-plant relationships, even within the same species, due to their wide range of genetic diversity. Plant growers have observed that certain varieties attract arthropods and cause substantial damage, while others are less preferred or even avoided. Early farmers engaged in agriculture thousands of years ago probably had knowledge of pest-resistant plants among those they grew (Smith & Clement, 2012). During the development of applied entomology in the eighteenth and nineteenth centuries, insect-resistant cultivars were frequently exploited (Smith & Clement, 2012).

Studies on the detection of insect-resistant varieties have become an area of interest, also in modern genetics, in recent years to discover resistant genes. In integrated pest management (IPM) programs, using resistant host plants is a crucial part of the control arthropod pests in modern agriculture. It provides primary data for genetic studies. Arthropod-resistant varieties provide economic benefits to producers by reducing or eliminating the need for pesticide applications (Smith, 2005; Smith & Clement, 2012). Arthropod-resistant genes used in global agriculture have an annual value of more than $ 2 billion (Smith, 2005; Smith & Clement, 2012).

The study findings indicated that the biology of the green peach aphid was affected by feeding on different Charleston pepper cultivars under laboratory conditions. Numerous studies have demonstrated that the biology of many aphid species can differ considerably based on the host plant, and even among different cultivars and varieties of the same host plant. These differences can be observed in terms of development time, survival rate, reproduction, and lifespan (Razmjou & Golizadeh, 2010; La Rossa et al., 2013; Özgökçe et al., 2018; Qayyum et al., 2018). Numerous studies have been carried out to demonstrate the significant impact of feeding on different pepper varieties on the population dynamics and biology of the green peach aphid. These studies include those conducted by Qing (2002), Luo et al. (2003), Nikolakakis et al. (2003), La Rossa et al. (2013), and Özgökçe et al. (2018). For example, Özgökçe et al. (2018) observed that the green peach aphid underwent development in 6.58-8.27 days and had an intrinsic rate of increase varying between 0.246-0.332 d^{-1} on different cultivars of *C. annuum* L. (Solanaceae), such as bell, sweet, or chili pepper, under similar laboratory conditions. These results closely resemble those of the present study.

Various factors can account for the differences in host preference among cultivars of the same plant species. Plant defense mechanisms against insect herbivores have been classified into three types: antixenosis (or non-preference), antibiosis, and tolerance (Painter, 1951; Kogan & Ortman, 1978). Antixenosis refers to the negative impact of the host plant on insect behavior, such as discouraging egg-laying, feeding, sheltering, and colonization processes (Kogan & Ortman, 1978; Cao et al., 2015; Sulistyo & Inayati, 2016; Stenberg & Muola, 2017). The non-preference behavior of an arthropod to a resistant plant is based on the host plant's morphological, biophysical, or allelochemical characteristics (Smith & Clement, 2012; Baldin et al., 2018). Antibiosis is the term used to describe how the biophysical or biochemical defense system of a resistant plant directly affects the physiology of harmful insects when they feed on the plant (Smith, 2005; Cruz & Baldin, 2017). The effects of insect antibiosis usually include a lengthening of immature stage duration, higher mortality rates, reduced fertility, and alterations to body size or weight (Panda & Khush, 1995; Smith, 2005). Tolerance, as defined by Smith (2005), is the capability of a plant to withstand or repair arthropod-induced damage while retaining its production capacity without altering the biology or behavior of the insect.

While host plants can produce ROS (especially in phloem cells) due to aphid injuries in host plants' cells, they may also cause the production of ROS in herbivorous insects by their allelochemicals (beta-carbonyl alkaloids, furanocoumarins, phenolic compounds, and thiophenes) (Lukasik & Golawska, 2013). These ROS may cause midgut cell breakdown and impair insect nutrition intake (Bi & Felton, 1995). Furthermore, ROS can interact with a wide range of intracellular biomolecules, including proteins, DNA, and lipids. Lipid peroxidation is harmful to herbivorous insects by changing the permeability of the cell membrane (Jamieson, 1989), decrease in the content of cuticle surface lipids, and juvenile hormone synthesis (Downer, 1985). Consequently, herbivorous insect performance on host plants directly affects the balance between the production and annihilation of ROS (Krishnan & Sehnal, 2006).

Levels of the GST-CDNB activities of *M. persicae* were ranked from highest to lowest as follows Safkan, Maraton, A3055, Tufan, and Kanyon, respectively. It has been proposed that increased GST activities might play an essential role in reducing oxidative stress (Konuş, 2014). Cells use modifying strategies such as glutathionylation of some important protein-based biomolecules to immediately cope with the oxidative stress that occurs during their natural metabolic activities (Musaoğulları et al., 2020). For example, caspase enzymes that might be essential in apoptosis are one of the protein groups that are inhibited due to the glutathionylation functions of GSTs (Huang et al., 2008; Singh & Reindl, 2021). According to these results, the increase in insect GST activity (1.8-fold) between Safkan and Kanyon cultivars shows the presence of oxidative stress and that it is an effective antioxidant enzyme in coping with this stress. According to these results, Safkan and Maraton cultivars could be suitable hosts because they confer high GST activities in *M. persicae*.

Our data on the total thiol content of tested samples of *M. persicae* fed with different Charleston pepper cultures is similar to the GST-CDNB activity results. As the cells use glutathione (GSH) both non-enzymatically and enzymatically (GST) while trying to cope with oxidative stress, the level of GSH in the cells decreases. As a result, the cells enter apoptosis. In other words, reducing the GSH level of cells predisposes those cells to apoptosis and/or directly induces cell death (Yılmaz et al., 2022). When we evaluated our results according to GST-CDNB, and total thiol (GSH) levels, the lowest total thiol, and the lowest GST activity were determined in *M. persicae* individuals fed with the Kanyon cultivar. Consequently, while the Kanyon cultivar may not be a suitable host for *M. persicae*, the Safkan and Maraton cultivars could be a good host because it confers a high level of GSH in *M. persicae*.

Esterases are enzymes that catalyze the conversion of ester-structured compounds to acids and alcohols (Konus, 2014). When the results of EST-PNPA activities are considered, Safkan Maraton, and A3055 showed higher esterase activities than the other two cultivars (Kanyon and Tufan). Furthermore, detoxification with esterases has been reported to be very important in the adaptation of *M. persicae* to cumin, anise, and coriander (Cabrera et al., 2010; Ramsey et al., 2010). Thus, it was concluded that Safkan, Maraton, and A3055 cultivars could be good hosts for the pest because they confer high esterase activities.

To summarize, based on life table parameters, the Kanyon cultivar is less favored by *M. persicae*, while Tufan and A3055 cultivars are deemed more suitable hosts. These findings were supported by enzymatic/nonenzymatic antioxidant levels, as determined by tests. However, as resistance mechanisms were not investigated in this study, the reasons for variation in biological parameters among insects cannot be fully explained, especially the first two resistance mechanisms often overlap and special tests required to distinguish between them (Smith, 2005). The study determined the most and least favored cultivars by *M. persicae* among all the tested cultivars. Conducting additional genetic research to identify the specific genes involved would be advantageous for the development of plant breeding programs and integrated pest management strategies.

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Original article (Orijinal araştırma)

Saproxylic beetles on oaks in a wooded pasture in the Eastern Mediterranean Region and contributions to Turkish entomofauna[1]

Doğu Akdeniz Bölgesinde ağaçlı otlak bir meşe ormanının saproksilik böcek türleri ve Türkiye entomofaunasına katkılar

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Abstract

This research was conducted to investigate the saproxylic Coleoptera fauna found in oak trees of different ages in Mersin/Gülnar in the Eastern Mediterranean Region in 2017. Fourty-five window traps were used to sample the beetles on the oak trees. Traps were checked five times, once a month, from June to October. In total, 13 217 individuals from the order Coleoptera were collected and 242 saproxylic beetle species belonging to 44 families were found. In the material, 33 beetle species were identified as new records for the Turkish fauna. Most beetle species were from the Buprestidae (24 species), Anobiidae (23 species) and Tenebrionidae (18 species) families. The highest numbers of individuals were from the Dermestidae (2 354), Elateridae (2 325) and Curculionidae (1 531) families. The highest number of individuals were *Xyleborus dryographus* (Ratzeburg, 1837) (Curculionidae) and *Cardiophorus* (*Cardiophorus*) *parvulus* Platia & Gudenzi, 2000 (Elateridae) with 1 109 individuals each. Three species were found; namely *Protaetia* (*Eupotosia*) *mirifica (*Mulsant, 1842), *Chromovalgus peyroni* (Mulsant, 1852) (Cetonidae) and *Propomacrus bimucronatus* (Pallas, 1781) (Euchiridae), which appear on the IUCN European and Mediterranean red list as Vulnerable (VU).

Keywords: Mersin, *Quercus* spp., saproxylic beetles, species diversity, wooded pasture

Öz

Doğu Akdeniz Bölgesinde Mersin/Gülnar'da yapılan bu çalışma meşe ormanlarında saproksilik Coleoptera türlerinin belirlenmesi amacıyla 2017 yılında yürütülmüştür. Alanda 45 pencere tipi böcek tuzağı asılmıştır. Tuzaklar haziran-ekim döneminde ayda bir kez olmak üzere beş kez kontrol edilmiştir. Coleoptera takımından 13 217 adet saproksilik böcek toplanmış ve 44 familyaya ait 242 adet tür saptanmıştır. Bu türlerden 33'ünün Türkiye saproksilik Coleoptera faunası için yeni kayıt olduğu belirlenmiştir. En fazla böcek türü sırasıyla Buprestidae (24 tür), Anobiidae (23 tür) ve Tenebrionidae (18 tür) familyalarından elde edilmiştir. En fazla birey ise sırasıyla Dermestidae (2 354 adet), Elateridae (2 325 adet) ve Curculionidae (1 531 adet) familyalarından tespit edilmiştir. Birey sayısı en fazla olan türler 1109 adet ile *Xyleborus dryographus* (Ratzeburg, 1837) (Curculionidae) ve *Cardiophorus (Cardiophorus*) *parvulus* Platia & Gudenzi, 2000 (Elateridae) olmuştur. *Protaetia (Eupotosia*) *mirifica* (Mulsant, 1842) *Chromovalgus peyroni* (Mulsant, 1852) (Cetonidae) ve *Propomacrus bimucronatus* (Pallas, 1781) (Euchiridae) IUCN'in Avrupa ve Akdeniz kırmızı listesinde nesli tehdit altında olan Duyarlı (VU) sınıfında yer almaktadır.

Anahtar sözcükler: Mersin, *Quercus* spp, saproksilik böcekler, tür çeşitliliği, ağaçlı otlak

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Introduction

In the forests of Türkiye, there are 17 oak species (*Quercus* spp.), four of which are endemic [*Q. vulcanica* (Boiss. Heldr. ex) Kotschy, *Q. macranthera* Fisch. & C. A. Mey. ex Hoh. subsp. *syspirensis* (C. Koch.) Menitsky, *Q. trojana* subsp. *yaltirikii* Ziel. et al. and *Q. aucheri* Jaub. et Spach.]. Including subspecies and varieties, there 24 taxa in total (Yılmaz, 2018). The broad-leaved forests of Türkiye cover an area of approximately seven million hectares (GDF, 2022).

An old oak tree can house hundreds of different species, especially fungi, insects and birds. As the tree ages, and the number of micro habitats, including dead twigs and branches, increase on the tree, additional species can colonize the tree. Initiation of decay by fungi starting in the tree trunk is an important factor in increasing the number of habitats. Wood, softened by fungal activities, becomes accessible to insect larvae and woodpeckers that create cavities. In the bottom of these cavities, which are usually sheltered from rain, organic matter from the tree accumulates and turns into compost. This material is often dominated by wood pieces and fungal remains, and over time becomes wood mould. The compost can also contain insect larva frass and waste from other insects and birds living in the cavities, thus the compost in different cavities may become varied and form unique mixtures (Dajoz, 1980; Micó, 2018). In hollow trees inhabited by saproxylic insects, there is a succession of fauna during the process of decay and decomposition (Speight, 1989), but the cavities in combination with this mixture can constitute a stable environment for many species in an old oak for hundreds of years (Ranius, 2002; Ranius et al., 2009). Oaks with decay cavities are used as indicators of high biological diversity and areas with a rich saproxylic beetle fauna often have a long historical continuity of old trees (Speight, 1989; Warren & Key, 1989; Buse, 2012). Saproxylic beetles are insects that depend on dead and decaying wood for at least part of their lifecycle and play important ecological roles in European habitats. Some species living in old and dead trees and in rotten wood in cavities are on the European and Mediterranean red list (Nieto & Alexander, 2010; Avgın et al., 2014; Calix et al., 2018; Garcia et al., 2018). The current IUCN European Red List provides an assessment of 693 species of saproxylic beetles. Overall, 17.9% and 21.7% of species are considered threatened in Europe and in the EU, respectively (Calix et al., 2018). 61 species of the 320 saproxylic beetles evaluated are threatened in the Mediterranean region, 29 species are Near Threatened and 131 species are Data Deficient (Garcia et al., 2018).

Saproxylic insect species living on old trees are considered an important group of highly endangered invertebrates in all of Europe, since their principal habitat has decreased. It is known that the presence of these insects in the forest directly and positively affects other forest species (McLean & Speight, 1993), but also provides important ecosystem services related to the decomposition of wood, nutrient cycling, forest pest control and pollination (Ulyshen, 2016; Micó, 2018; Ulyshen, 2018). In fact, some of these insects are considered indicator species during the establishment of protected areas. Studies conducted in the south of Türkiye also showed that the historical process of pruning these trees or their growth under natural conditions have no negative impact on the diversity of insects living in this mixture (Avcı et al., 2010a).

Saproxylic beetle communities in old oaks have also been examined in other Mediterranean countries in recent years. Several research programmes on ecology and biodiversity conservation have been conducted, and they have, in particular, focused on the saproxylic beetles guild (Buse et al., 2008; Sirami et al., 2008; Quinto et al., 2012, 2014, 2015; Ramírez-Hernández et al., 2014; Micó et al., 2015, 2020; Ramilo et al., 2017; Sanchez-Galvan, 2018; Della Rocca et al., 2022). There are indications that the beetle fauna of oak trees in Türkiye is richer than those occurring in many European countries and that it is also richer than all Northern European countries combined (Jansson et al., 2010). In recent studies of old oaks in the Aegean and Mediterranean part of Türkiye, overy 32 newly recognised species have been found (Schillhammer et al., 2007; Novak et al., 2011; Platia et al., 2011; Sama et al., 2011; Jansson, 2021). The most species-rich of the studied families are Elateridae, Buprestidae and Dermestidae with 51, 35 and

34 species respectively (Jansson, 2021). New beetle species were found living on old trees with dead branches and trunk cavities. These invertebrates are at the bottom of the food chain and are an important factor for many other species, especially birds such as woodpeckers (Sunnergren, 2008). Hollow trees have a range of crucial roles in society: they are valuable historically, culturally and aesthetically at a landscape level and for recreational purposes. People tend to be naturally attracted to old hollow trees, as demonstrated by the enhancement of monumental trees in different countries that are visited by thousands of tourists every year. These cultural and aesthetic values could be combined with the intrinsic biological importance of these trees that act as keystone structures for diversity conservation (Müller et al., 2013). Old oak trees in Türkiye are an important and unique heritage for the world. With increased knowledge of saproxylic beetles living in these trees, the importance of role of Turkish forests in global biodiversity will be enhanced.

The aim of the study was to examine and describe the saproxylic beetle fauna in oak-dominated wooded pastures in Mersin/Gülnar in the Eastern Mediterranean Region and to report scientific information useful for the motivation of the future preservation of this area.

Materials and Methods

Study area

The study area is located on the Taşeli Plateau, to the north and east of the Köseçobanlı village neighborhood, in the Gülnar district, Mersin Province (centre point: 36º27'53"N-33º09'40"E). The area of the plateau with similar habitat was approximately 4 400 hectares (ha) at an altitude of 1300-1602 meters (Figure 1). The whole area consisted of wooded pastures with scattered pollarded oaks (35-55 oaks/ha) mainly grazed by goats. The oaks form part of an ancient herding system with goat and sheep herding where oak foliage is an essential component of the fodder for the animals. In the Turkish forest management classification system, this type of habitat is classified as degraded oak (BM). The oak species in the area were mainly *Quercus ithaburensis* with some *Q. libani* and *Q. infectoria* and a few ash, *Fraxinus angustifolia* Reut. (Lamiales: Oleaceae). The total size of the studied areas was approximately 10.7 ha. Areas with almost pure oak forest were selected based on high abundance of old and hollow trees.

Figure 1. The location of study area.

Study material

Window traps were used to collect beetles (Figure 2). When setting up the traps, two one-meter-long wooden battens, one 30 x 50 cm transparent plexiglass sheet, one 15 x 15 x 30 cm metal container, nails, nylon rope and wire were used to attach each trap on the tree trunks (Jansson & Lundberg, 2000).

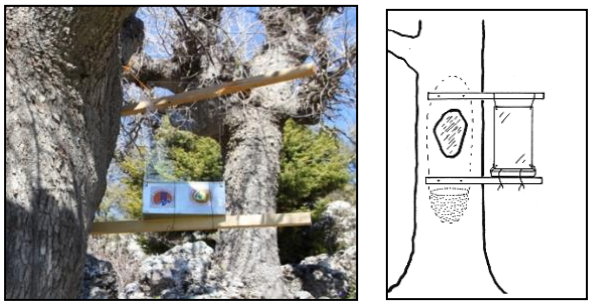

Figure 2. The window trap used to collect the saproxylic beetles.

In the laboratory, the beetles were separated in a 15 x 30 x 50 cm white plastic container into plastic cups, filtered with the help of a 15-cm-diameter wire strainer (0.5 mm mesh width), and were examined under a 16-40X magnification of Olympus SZ-CTV and Nikon C-LEDS microscopes. Insects were preserved in 70% ethyl alcohol in 1.5 ml Eppendorf tubes or 50 ml falcon tubes. Insect needles, forceps, Petri dishes, insect glue and insect sticking paper mounting boards were used.

Study design

In the study, 45 insect traps were set in oak stands containing trees of different ages. If the trees were hollow, traps were placed opposite the hollow entrance to the tree. While setting up the traps, attention was focused on keeping the trap parallel to the tree or vertical to ensure the stability of the trap. The trap was kept at an average of 1-3 meters above the ground.

After the traps were mounted on the trees, one liter of antifreeze and an average of one liter of water were added into each metal container. Subsequently, a small amount of detergent was added to reduce surface tension. The coordinates of the trapped trees were recorded with the help of a GPS device.

The traps were mounted on the trees on 28-29 April 2017 and were checked at monthly intervals until mid-October 2017. At each visit, the traps were examined individually, and the caught insects were filtered from the antifreeze water with a strainer and placed in plastic cups. At the end of this process, the liquid levels of the traps were checked, and the antifreeze and water levels were topped up to the original volume.

Separation of the insects in the laboratory, preparation, and the identification process

The insects collected from each sample were decanted into a white plastic tray (50 x 30 x 10 cm) after which water was added to the tray. Beetles were sorted without magnification under strong light according to their size and were stored in plastic tubes containing 70% alcohol. Labels with the date the insect was collected, the trap number and the coordinates were placed on each tube.

In the next step, separation into families was carried out using both an identification key and help from different experts. Subsequently, larger species were pinned, and small species were glued to paper mounting boards with water-based glue. After the preparation process, the general appearance was recorded along with identification photographs of the beetles produced with a LEICA Z16 APO binocular fitted in a Nikon D7000 camera.

The identifications of the specimens obtained in the study were partly made by the authors, but most were made by specialists.

In addition, the IUCN category classification of the species (Akçakaya & Ferson, 2001) was recorded. Classification of the saproxylic beetles according to their feeding patterns and their locations in the tree followed the methods of Carpaneto et al. (2015) with some minor adjustments (Table 1).

When examining if an identified species was previously recorded in Türkiye, the following publications were used: Löbl & Smetana (2006, 2007, 2008, 2010, 2011, 2013); Avgın et al. (2014); Koçak (2014); Löbl & Löbl (2015, 2016, 2017); Gülperçin & Tezcan (2016); Alonso-Zarazaga et al. (2017) and Tezcan (2020).

Table 1. Trophic categories of saproxylic insects (Carpaneto et al., 2015)

Abbreviations	Trophic category
CO	Commensal of SX/XY or of other saproxylic insects
MB	Mycetophagous on carpophora of macrofungi (mostly Polyporales) growing on veteran trees or on old stumps
MM	Myrmecophilous or melittophagous inside hollow trees or stumps hosting colonies of ants or other social Hymenoptera
MY	Mycophagous (on hyphae of saproxylic fungi or on micromycetes, yeasts and Myxomyceta)
NI	Commensal in bird or small mammal nests, feeding on parts of dead animals including other insects inside hollow trees or other cavities in dead wood
PR	Predator (as larvae or imagoes) of SX/XY or of other saproxylic insects
SF	Feeding on fermented sap and exudates (usually including a mixture of bacteria and yeasts) produced by trees attacked by XY, fungi or wounded by external physical agents
SP	Saprophytophagous on rotting vegetal matter associated with dead wood and wood debris
SX	Saproxylophagous in dead wood during the whole process of decomposition, including wood mould inside hollow trees
UN	Trophic category unknown
XY	Xylophagous (fresh wood or bark but also developing on healthy trees)

Results and Discussions

In total, 13 217 individuals of saproxylic beetles representing 242 species in 44 families were identified. Of these species, 33 species were identified as new records for the fauna of Türkiye. While most of the species were from the Buprestidae family with 24 species, the highest number of individuals were from the Dermestidae family (n=2 354) (Figure 3). The species found, numbers of individuals per species, IUCN (Europe and Mediterranean) red list category and the classification of the saproxylic beetles according to their feeding guilds (trophic strategy) are given in Appendix Table 1. Trophic categories of 228 of the 242 species obtained in the study were classified.

When comparing numbers of species per family, the three richest families were Buprestidae (n=24), Anobiidae (n=23) and Tenebrionidae (n=18) (Figure 4).

The families with the highest number of individuals in this study were Dermestidae with 2 354 individuals, Elateridae with 2 325 individuals and Curculionidae with 1 531 individuals (Figure 5).

When the number of individuals of the newly recorded species was compared, *Mordellistena neuwaldeggiana* (Panzer, 1796), of the Mordellidae family, was most abundant with 155 individuals. The second highest number of individuals was of *Clypeorhagus clypeatus* (Hampe, 1850) from the Eucnemidae family, followed by *Anthocomus semipolitus* (Abeille de Perrin, 1882) of the Malachiidae family, with 36 individuals.

Atay et al. (2012a) conducted a smaller study of saproxylic beetles (Coleoptera) on old oak trees in Adana-Kozan, Türkiye, and found 11 families, 32 genera and 40 species. Species richness, in terms of most represented family, was similar to the results reported in the present study (Elateridae, Buprestidae and Scarabaeidae). In other work, Atay et al. (2012b) identified 87 coleopteran species from 18 families in the same region. The families with most species were Elateridae, Anobiidae and Tenebrionidae, which were among the first five families with the highest numbers of species identified in the present study.

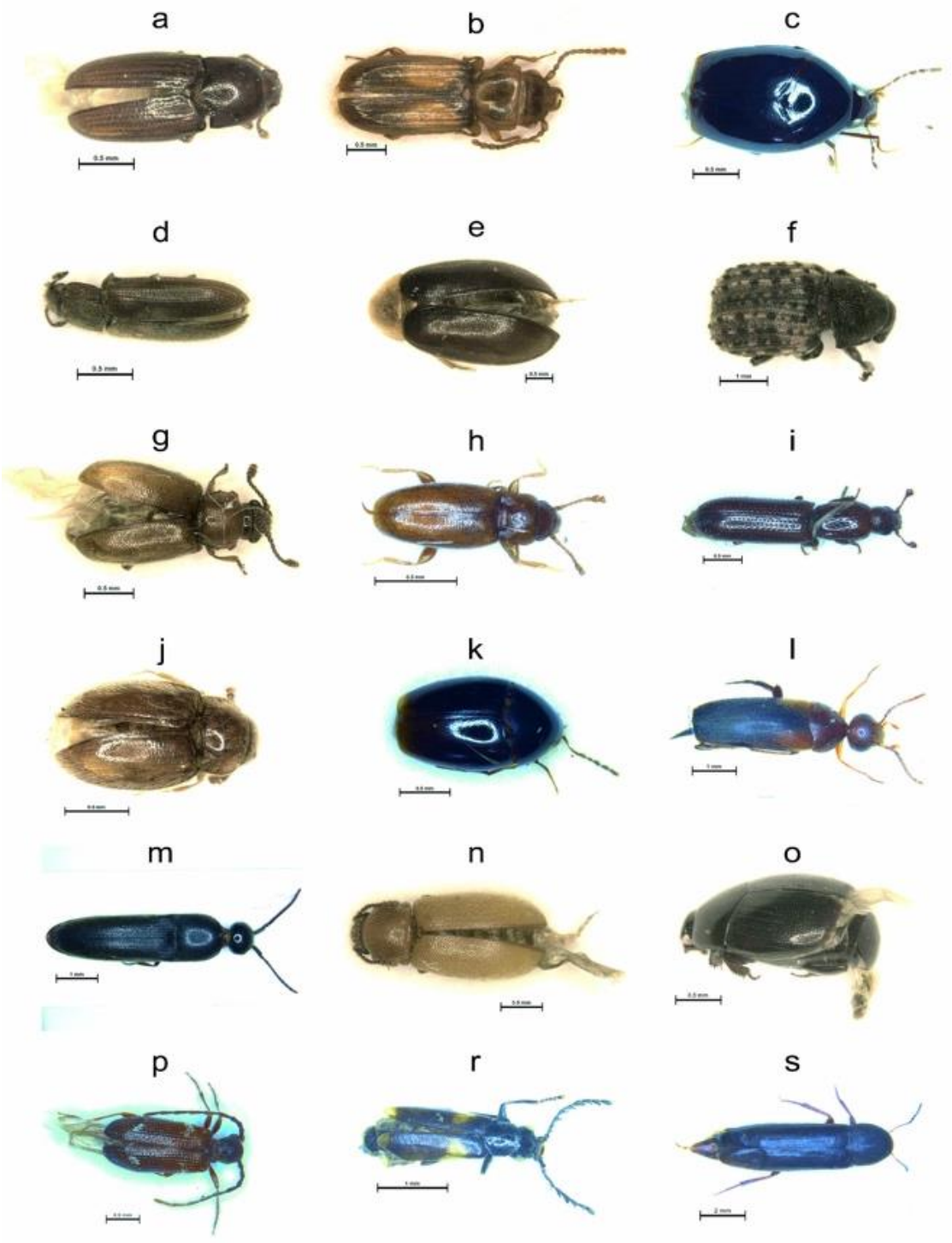

Figure 3. Examples of new species for the saproxylic insect fauna of Türkiye: a) *Cerylon histeroides*, b) *Laemophloeus monilis*, c) *Scaphisoma agaricinum*, d) *Diplocoelus fagi*, e) *Sacodes flavicollis*, f) *Anthribus nebulosus*, g) *Cryptophagus quercinus*, h) *Holoparamecus* (*Calyptobium*) *caularum*, i) *Oxylaemus cylindricus*, j) *Symbiotes gibberosus*, k) *Scaphisoma subalpinum*, l) *Mordellistena* (*Mordellistena*) *neuwaldeggiana*, m) *Clypeorhagus clypeatus*, n) *Atomaria* (*Atomaria*) *slavonica*, o) *Gnathoncus buyssoni*, p) *Ptinus* (*Gynopterus*) *diversipennis*, r) *Nepachys amaenus*, s) *Phloiotrya* (*Phloiotrya*) *rufipes*.

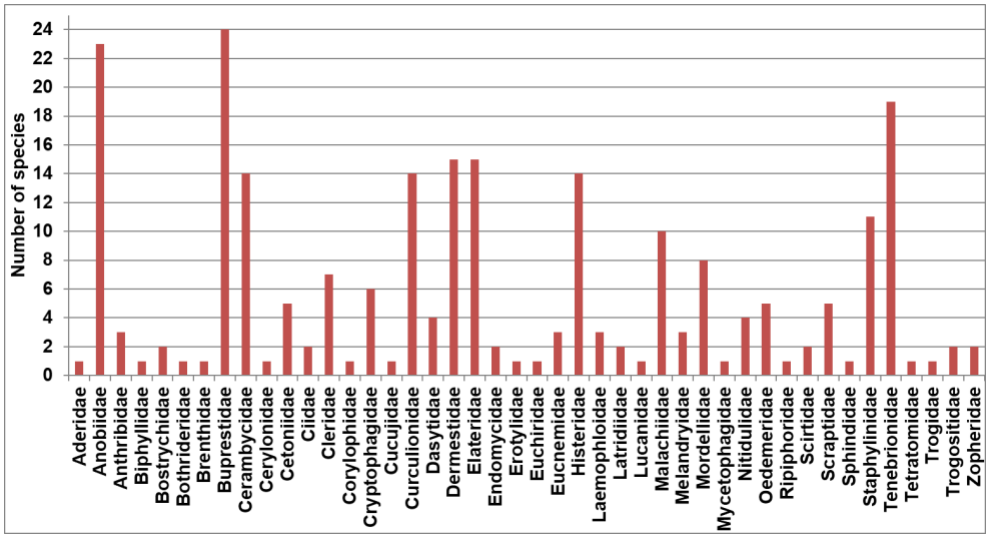

Figure 4. The number of species per family.

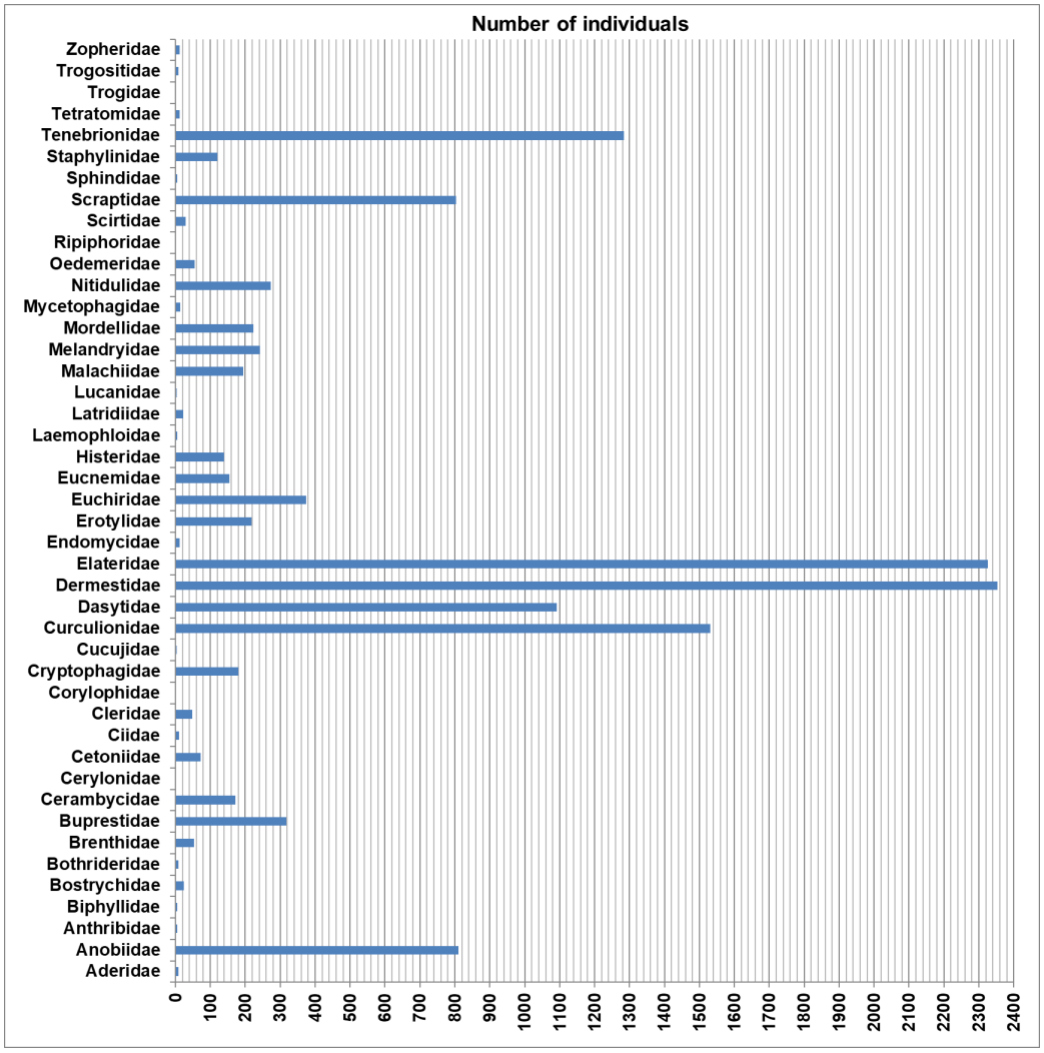

Figure 5. The number of individual beetles found per family.

A literature review conducted by Avgin et al. (2014) suggested that 151 saproxylic beetle species in eight families were present in Türkiye, showing that the families Cerambycidae, with 77 species, and Elateridae, with 46 species, were the most important families in terms of the number of species. In the present study, these two families were also amongst the most prevalent in terms of the number of species as the Cerambycidae family was represented by 14 species and the Elateridae family was represented by 15 species.

Laz (2015) found 2 323 individuals and 82 species in a study conducted in three different forests in the Kahramanmaraş/Andırın region. The results from the study's oak habitat have indicated that the highest numbers of species were in the Elateridae and Buprestidae families, a finding also matched in that of the present work.

Old oaks (*Quercus petraea* (Matt.) Liebl., *Q. frainetto* Ten. and *Q. frainetto* x *Q. petraea*) in the Kaz Dağları (Balıkesir-Edremit Bay) and Kapıdağ Peninsulas (Balıkesir-Erdek) were examined by Varlı et al. (2019). Anobiidae, Cerambycidae, Tenebrionidae and Scraptiidae were the richest families in terms of the number of species in the Kapıdağ Peninsula and Elateridae, Cerambycidae, Tenebrionidae and Anobiidae the richest families in terms of number of species in the Kaz Mountains. Again, these results were similar to those reported in the present study, with the exception of the Scraptiidae family.

The two most common species in this study were the curculionid *Xyleborus dryographus* species (Ratzeburg, 1837) from the Scolytinae subfamily and the elaterid *Cardiophorus* (*Cardiophorus*) *parvulus* species (Platia & Gudenzi, 2000) from the *Cardiophorinae* subfamily. For both species, 1 109 individuals were trapped. The next most abundant species was the dermestid *Ctesias maculifasciata* (Reitter, 1899) with 990 individuals. Previous studies have also shown that *X. dryographus* is a common species in old oak forests as larval development occurs under the bark of dead branches. The old trees in the present study area, therefore, provided a good habitat.

Sarıkaya (2013) obtained eight Scolytinae subfamily species in a study conducted with red sticky traps in the *Quercus cerris* forests of the Aksu province of Isparta province. The most collected species was *Xyleborinus saxesenii* (Ratzeburg, 1837), but only three individuals from the *Xyleborus dryographus* species were obtained. In further studies conducted in the oak (*Q. vulcanica)* forest in the Isparta Kasnak Oak Nature Reserve, eight species of the Scolytinae subfamily were recorded, with the *X. saxesenii* species being the most abundant (Sarıkaya & Sayın, 2016). While nine species from the Scolytinae subfamily were obtained in the present study, the *X. saxesenii* species was not detected.

From the 242 beetle species identified in this study, 22 species from four categories have been red listed by the IUCN. A total of 17 species were classified with eight species from the Cerambycidae family, two species from the Trogositidae family, and one species each from the Bostrichidae, Cetoniidae, Elateridae, Erotylidae, Eucnemidae, Lucanidae and Mycetophagidae families in at least the concern category (LC).

In total, two species found in the present study are listed on the red list, namely the *Megapenthes lugens* (L. Redtenbacher, 1842) (Coleoptera: Elateridae) and *Cerambyx cerdo* (L., 1758) (Coleoptera: Cerambycidae), and were assessed as near threatened (NT). *Protaetia mirifica* (Mulsant, 1842) (Coleoptera: Cetonidae) and *Propomacrus bimucronatus* (Pallas, 1781) (Coleoptera: Euchiridae) were classified in the Vulnerable (VU) category. The other identified species were classified as data deficient (DD) (the *Reitterelater dubius* species in the Elateridae family).

For trophic category classification, 228 of the 242 species were assessed and categorised. In some families, such as the Buprestidae, Cerambycidae and Mordellidae, the species had a uniform feeding pattern and were classified into the same single category (XY: Xylophagous), but the species in other families such as the Cryptophagidae (MY: Mycophagous, MB: Mycetophagous, SP: Saprophytophagous,

NI: feeds on dead insects and other animal parts in cavities), Laemophloeidae (MY, SX: Saproxylophagous) and Tenebrionidae (SX, MB, MY, PR: Predator) families had more mixed feeding patterns and were, therefore, placed in many different categories. Most of the species were classified into the trophic XY category, developing in fresh wood or bark but some also thrived on healthy trees.

This study confirmed that old oaks are a highly valuable, important habitat and support the status of Türkiye as a key biodiverse hot spot. At the same time, it is clear that further research is needed to expand knowledge of the biodiversity of old oaks in Türkiye. The records of many species that are rare and threatened in Europe show the high value of the oak habitat for biodiversity conservation as well as the importance for future scientific research. Earlier studies on birds showed that oak habitats in Türkiye supported a rich and diverse fauna, including several species of European conservation concern (Bergner et al., 2015). To preserve the important biological hot spots and structures in wood producing areas, it is crucial to implement an ecosystem-based management approach as well as an appropriate management protocol for these species in protected areas and across the entire forest landscape. Since many beetles are a key food source for woodpeckers and other insectivorous birds, conservation work is also important for the protection of insectivorous birds (Bergner et al., 2015; Kalay Göktepe et al., 2019).

Taking shoots and branches with foliage from trees for animal grazing fodder has been a traditional livelihood representing a cultural heritage practiced in Türkiye for at least two thousand years (Kaniewski et al., 2007), although similar techniques were used throughout Europe until a century ago. It has been suggested that pollarding is a valuable management strategy and an important driver for the creation of microhabitats needed by saproxylic organisms (Sebek et al., 2013; Quinto et al., 2014). The results presented in this study show that this tradition has created a rich habitat for wood-living beetles on the Taşeli plateau. It is, therefore, essential to maintain traditional management in some larger areas with pollarded (pruned) oaks, as in the studied area, of different climatic zones to preserve the unique biodiversity associated with oak trees in Türkiye. It is essential that natural and old oak forests in Türkiye are treated with methods most suitable to encourage biodiversity and that as great an area of these forests as possible is a priority for protection to preserve all the richness of the ecosystem for future generations.

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Appendix

Table 1. Species and individual numbers by family

Family / Species name	Number of individuals	Presence rate (%)	IUCN Category Europa (Mediterranean)	Trophic category
ADERIDAE				
Aderus populneus (Creutzer in Panzer, 1796)	**9**	**0.07**		**SX**
ANOBIIDAE				
Anobiinae				
Falsogastrallus unistriatus (Zoufal, 1897)	**8**	**0.06**		**SX**
Oligomerus ptilinoides (Wollaston, 1854)	**2**	**0.02**		**SX**
Dorcatominae				
Caenocara anatolica Zahradník, 1998	**1**	**0.01**		**SX**
Dorcatoma sp.1	**1**	**0.01**		**SX**
Dorcatoma sp.2	**1**	**0.01**		**SX**
Stagetus sp.	**1**	**0.01**		**SX**
Stagetus byrrhoides (Mulsant & Rey, 1861)	**449**	**3.40**		**SX**
Stagetus elongatus (Mulsant & Rey, 1861)	**72**	**0.54**		**SX**
Stagetus franzi Español, 1969	**98**	**0.74**		**SX**
Eucradinae				
Ptinomorphus imperialis (L., 1767)	**6**	**0.05**		
Ptininae				
Dignomus fridsvaldszkyi (Reitter, 1884)	**28**	**0.21**		**SX**
Dignomus kroliki Borowski, 2002	**10**	**0.08**		**SX**
**Ptinus* (*Gynopterus*) *diversipennis* Pic, 1907	**10**	**0.08**		**SX**
**Ptinus (Ptinus) hirsutus* Pic, 1895	**1**	**0.01**		**SX**
**Ptinus (Ptinus) gylippus* Reitter, 1906	**1**	**0.01**		**SX**
/*Ptinus (Bruchoptinus) libanicus* Pic, 1899	**2**	**0.02**		**SX**
Ptinus (Ptinus) phlomidis Boieldieu, 1854	**2**	**0.02**		**SX**
**Ptinus (Cyphoderes) schlerethi* (Reitter, 1884)	**10**	**0.08**		**SX**
Ptinus (Bruchoptinus) torretassoi Pic, 1934	**5**	**0.04**		**SX**
Ptinus (Gynopterus) variegatus Rossi, 1792	**71**	**0.54**		**SX**
Xyletininae				
Lasioderma sp.	**1**	**0.01**		**UN**
Xyletinus sp.	**1**	**0.01**		
Xyletinus (Xyletinus) laticollis (Duftschmid, 1825)	**29**	**0.22**		
ANTHRIBIDAE				
Anthribinae				
**Anthribus nebulosus* Forster, 1770	**4**	**0.03**		**XY (SX,MY)**
Anthribus scapularis Gebler, 1833	**1**	**0.01**		**XY (SX,MY)**
**Noxius variegatus* (Fahraeus, 1839)	**1**	**0.01**		**XY (SX,MY)**
BIPHYLLIDAE				
**Diplocoelus fagi* Guérin-Méneville, 1838	**5**	**0.04**		**SX (MY,PR)**
BOSTRICHIDAE				
Bostrichinae				
Scobicia pustulata (Fabricius, 1801)	**1**	**0.01**	LC (LC)	**XY**
Sinoxylon muricatum (L., 1767)	**24**	**0.18**		**XY**
BOTHRIDERIDAE				
Teredinae				
**Oxylaemus cylindricus* (Panzer, 1796)	**8**	**0.06**		**PR**
BRENTHIDAE				
Amorphocephala coronata (Germar, 1817)	**53**	**0.40**		**MM**
BUPRESTIDAE				
Agrilinae				
Agrilus biguttatus (Fabricius, 1776)	**2**	**0.02**		**XY**
Agrilus cyanescens Ratzeburg, 1837	**5**	**0.04**		**XY**
Agrilus relegatus subsp. *alexeevi* Bellamy, 1998	**72**	**0.54**		**XY**
Coraebus sp.	**1**	**0.01**		**XY**
Coraebus rubi (L., 1767)	**1**	**0.01**		**XY**
Buprestinae				
Anthaxia (*Anthaxia*) *bicolor* Falderman, 1835	**22**	**0.17**		**XY**
Anthaxia (*Cratomerus*) *diadema* (Fischer, 1824)	**8**	**0.06**		**XY**
Anthaxia (*Anthaxia*) *discicollis* Gory & Laporte, 1839	**4**	**0.03**		**XY**
Anthaxia (*Cratomerus*) *eugeniae* Ganglbauer, 1885	**27**	**0.20**		**XY**
Anthaxia (*Anthaxia*) *herbertschmidi* Novak, 1992	**1**	**0.01**		**XY**
Anthaxia (*Anthaxia*) *midas* Kiesenwetter, 1857	**1**	**0.01**		**XY**
Anthaxia (*Haplanthaxia*) *millefolii* (Fabricius, 1801)	**1**	**0.01**		**XY**
Anthaxia (*Haplanthaxia*) *mundula* Kiesenwetter, 1857	**27**	**0.20**		**XY**
Anthaxia myrmidon Abeille de Perrin, 1891	**15**	**0.11**		**XY**
Anthaxia (*Melanthaxia*) *nigrojubata* Roubal, 1913	**76**	**0.58**		**XY**
Anthaxia (*Anthaxia*) *plicata* Kiesenwetter, 1859	**1**	**0.01**		**XY**
Anthaxia (*Haplanthaxia*) *praeclara* Mannerheim, 1837	**1**	**0.01**		**XY**
Sphenoptera (*Tropeopeltis*) *tappesi* Marseul, 1865	**1**	**0.01**		**XY**

Table 1. Continued

Family / Species name	Number of individuals	Presence rate (%)	IUCN Category Europa (Mediterranean)	Trophic category
Chrysobothrinae				
Chrysobothris (*Chrysobothris*) *affinis* (Fabricius, 1794)	**2**	**0.02**		**XY**
Polycestinae				
Acmaeodera (*Acmaeotethya*) *degener* (Scopoli, 1763)	**1**	**0.01**		**XY**
Acmaeodera (*Acmaeodera*) *flavolineata* Laporte & Gory, 1835	**1**	**0.01**		**XY**
Acmaeodera (*Acmaeotethya*) *ottomana* (Frivaldszki, 1837)	**3**	**0.02**		**XY**
Acmaeodera (*Acmaeotethya*) *saxicola* Spinola, 1838	**44**	**0.33**		**XY**
Acmaeoderella (*Omphalothorax*) *longissima* (Abeille de Perrin, 1904)	**1**	**0.01**		**XY**
CERAMBYCIDAE				
Cerambycinae				
Axinopalpis gracilis (Krynicky, 1832)	**37**	**0.28**	LC	**XY**
Callimus angulatus (Schrank, 1789)	**1**	**0.01**	LC	**XY**
Cerambyx cerdo L., 1758	**16**	**0.12**	NT (LC)	**XY**
Chlorophorus nivipictus Halperin & Holzschuh, 1993	**3**	**0.02**	LC (LC)	**XY**
Delagrangeus (Delagrangeus) angustissimus Pic, 1892	**3**	**0.02**		
Phymatodes testaceus (L., 1758)	**5**	**0.04**	LC	**XY**
Phymatodes (Poecilium) wrzecionkoi (Rapuzzi et G. Sama, 2010)	**5**	**0.04**	LC	**XY**
Stenhomalus (Obriopsis) bicolor (Kraatz, 1852)	**2**	**0.02**	LC (LC)	**XY**
Stromatium unicolor (Olivier, 1795)	**2**	**0.02**	LC (LC)	**XY**
Trichoferus griseus (Fabricius, 1792)	**2**	**0.02**	LC (LC)	**XY**
Lepturinae				
Cortodera imrasanica Sama & Rapuzzi, 1999	**12**	**0.09**		**XY**
Grammoptera merkli Frivaldszky, 1884	**1**	**0.01**		**XY**
Rhagium syriacum Pic, 1892	**6**	**0.05**		**XY**
Spondylidinae				
Alocerus moesiacus (Frivaldsky, 1838)	**77**	**0.58**		
CERYLONIDAE				
Ceryloninae				
**Cerylon histeroides* (Fabricius, 1792)	**1**	**0.01**		**MY**
CETONIIDAE				
Cetoniinae				
Chromovalgus peyroni (Mulsant, 1852)	**1**	**0.01**	**(VU)**	**SX**
Protaetia (Netocia) angustata (Germar, 1817)	**18**	**0.14**	LC	**SX**
Protaetia (Eupotosia) mirifica (Mulsant, 1842)	**8**	**0.06**	VU (VU)	**SX**
Protaetia (Cetonischema) speciosa (Adams, 1817)	**14**	**0.11**		**SX**
Tropinota (Epicometis) hirta (Poda, 1761)	**32**	**0.24**		**UN**
CIIDAE				
Cis tauriensis Krolik, 2002	**7**	**0.05**		**MB**
Xylographus bostrichoides (Dufour, 1843)	**4**	**0.03**		**MB**
CLERIDAE				
Clerinae				
Clerus mutillarius Fabricius, 1775	**2**	**0.02**		**PR**
Opilo taeniatus (Klug, 1842)	**27**	**0.20**	**(DD)**	**PR**
Trichodes punctatus Fischer von Waldheim, 1829	**2**	**0.02**		
Korynetinae				
Korynetes caeruleus (De Geer, 1775)	**13**	**0.10**		**PR**
Korynetes coxalis Reitter, 1894	**2**	**0.02**	**(DD)**	**PR**
Tillinae				
Tilloidea unifasciata (Fabricius, 1787)	**1**	**0.01**		**PR**
CORYLOPHIDAE				
Corylophinae				
Clypastraea sp.	**1**	**0.01**		**MY**
CRYPTOPHAGIDAE				
Atomariinae				
**Atomaria (Atomaria) slavonica* Johnson, 1971	**8**	**0.06**		**MY**
Cryptophaginae				
Cryptophagus cylindrellus Johnson, 2007	**1**	**0.01**		**MB**
Cryptophagus jakowlewi Reitter, 1888	**18**	**0.14**		**MY**
Cryptophagus punctipennis C.N.F. Brisout de Barneville, 1863	**39**	**0.30**		**SP**
**Cryptophagus quercinus* Kraatz, 1852	**1**	**0.01**		**MY (MM)**
Cryptophagus uncinatus Stephens, 1830	**114**	**0.86**		**NI**
CUCUJIDAE				
Cryptolestes sp.	**3**	**0.02**		
CURCULIONIDAE				
Cossoninae				
Rhyncolus (Rhyncolus) ater (L., 1758)	**4**	**0.03**		**SX**
Cryptorhynchinae				
Camptorhinus statua (Rossi, 1790)	**10**	**0.08**		**SX**

Table 1. Continued

Family / Species name	Number of individuals	Presence rate (%)	IUCN Category Europa (Mediterranean)	Trophic category
Mesoptilinae				
Magdalis (Laemosaccidius) exarata (H. Brisout de Barneville, 1862)	**1**	**0.01**		**XY**
Magdalis (Magdalis) frontalis (Gyllenhal, 1827)	**11**	**0.08**		**XY**
Platypodinae				
Platypus simulans (Schedl, 1941)	**4**	**0.03**		
Scolytinae				
Carphoborus perrisi (Chapuis, 1869)	**8**	**0.06**		**XY**
Hylesinus crenatus (Fabricius, 1787)	**1**	**0.01**		**XY**
Hylesinus varius (Fabricius, 1775)	**9**	**0.07**		**XY**
Hypothenemus eruditus Westwood, 1836	**1**	**0.01**		**XY**
Scolytus rugulosus (Muller, 1818)	**37**	**0.28**		**XY**
Taphrorychus hirtellus (Eichhoff 1879)	**1**	**0.01**		**XY**
Taphrorychus ramicola (Reitter, 1894)	**206**	**1.56**		**XY**
Xyleborus monographus (Fabricius, 1792)	**129**	**0.98**		**MY**
Xyleborus dryographus (Ratzeburg, 1837)	**1109**	**8.39**		**MY**
DERMESTIDAE				
Attageninae				
Attagenus brunnescens Pic, 1904	**4**	**0.03**		**NI**
Attagenus quadrimaculatus Kraatz, 1858	**305**	**2.31**		**NI**
Attagenus pantherinus (Ahrens, 1814)	**3**	**0.02**		**NI**
Attagenus unicolor (Brahm, 1791)	**33**	**0.25**		**NI**
Dermestinae				
Dermestes (Dermestinus) undulatus Brahm, 1790	**32**	**0.24**		**NI**
Megatominae				
Anthrenus (Anthrenus) delicatus Kiesenwetter, 1851	**383**	**2.90**		**NI**
Anthrenus (Anthrenus) scrophulariae (L., 1758)	**70**	**0.53**		**NI**
Anthrenus (Florilinus) sordidulus Reitter, 1889	**7**	**0.05**		**NI**
Anthrenus (Florilinus) verbasci (L., 1767)	**21**	**0.16**		**NI**
Ctesias maculifasciata (Reitter, 1899)	**990**	**7.49**		**SX**
Globicornis (Globicornis) kafkai Háva, 2000	**27**	**0.20**		**SX**
Globicornis (Globicornis) picta (Kuester, 1851)	**437**	**3.31**		**SX**
Phradonoma (Phradonoma) nobile (Reitter, 1881)	**1**	**0.01**		**NI**
Trogoderma glabrum (Herbst, 1783)	**26**	**0.20**		**SX**
Orphilinae				
Orphilus niger (Rossi, 1790)	**15**	**0.11**		**SX**
DASYTIDAE				
Danaceinae				
Danacea (Danacea) marginata (Kuster, 1850)	**13**	**0.10**		**UN**
Danacea (Danacea) olivacea Baudi, 1873	**269**	**2.04**		**UN**
Rhadalinae				
Aplocnemus sp.	**808**	**6.11**		**PR**
Aplocnemus (Aplocnemus) rufipes Miller, 1862	**1**	**0.01**		**PR**
ELATERIDAE				
Agrypninae				
Lacon ladae (Mertlik & Dusanek, 2006)	**1**	**0.01**	**(LC)**	**PR**
Ampedini				
Nothodes parvulus (Panzer, 1799)	**28**	**0.21**		
Peripontius terminatus (Erichson, 1841)	**75**	**0.57**		
Reitterelater dubius Platia & Cate, 1990	**1**	**0.01**	**DD**	**PR**
Cardiophorinae				
Cardiophorus analis (Schwarz, 1892)	**1**	**0.01**		**PR**
Cardiophorus (Cardiophorus) anticus Erichson, 1840	**17**	**0.13**	**(LC)**	**PR**
Cardiophorus (Cardiophorus) discicollis (Herbst, 1806)	**14**	**0.11**		**PR**
Cardiophorus kindermanni Candeze, 1860	**50**	**0.38**	**(LC)**	**PR**
Cardiophorus (Cardiophorus) parvulus Platia & Gudenzi, 2000	**1109**	**8.39**		**PR**
Dicronychus merkli (Pic, 1910)	**166**	**1.26**		
Dendrometrinae				
Elathous nurayae Platia, 2011	**9**	**0.07**		
Elaterinae				
Melanotus (Melanotus) fusciceps (Gyllenhal, 1817)	**394**	**2.98**	**LC**	**PR**
Mulsanteus guillebeaui (Mulsant & Godart, 1853)	**458**	**3.47**		**PR**
Megapenthes lugens (L. Redtenbacher, 1842)	**1**	**0.01**	**NT**	**PR**
Lissominae				
Drapetes mordelloides (Host, 1789)	**1**	**0.01**		**UN**
ENDOMYCIDAE				
Anamorphinae				
**Symbiotes gibberosus* (Lucas, 1846)	**12**	**0.09**		**MB**
Holoparamecinae				
**Holoparamecus (Calyptobium) caularum* Aube, 1843	**1**	**0.01**		

Table 1. Continued

Family / Species name	Number of individuals	Presence rate (%)	IUCN Category Europa (Mediterranean)	Trophic category
EROTYLIDAE				
Tritominae				
Triplax russica (L., 1758)	**219**	**1.66**	**LC**	**MB**
EUCHIRIDAE				
Euchirinae				
Propomacrus bimucronatus (Pallas, 1781)	**374**	**2.83**	VU (VU)	**SX**
EUCNEMIDAE				
Melasinae				
**Clypeorhagus clypeatus* (Hampe, 1850)	**143**	**1.08**		**SX**
Farsus dubius (Piller & Mitterbacher, 1783)	**11**	**0.08**	(NT)	**SX**
Isoriphis melasoides (Laporte de Castelnau, 1835)	**1**	**0.01**	LC	**SX**
HISTERIDAE				
Abraeinae				
Pleuroleptus rothi (Rosenhauer, 1856)	**45**	**0.34**		**PR**
Dendrophilinae				
Cyclobacanius soliman (Marseul, 1862)	**1**	**0.01**		**PR**
Dendrophilus (Dendrophilus) punctatus (Herbst, 1792)	**14**	**0.11**		**PR**
Platylomalus gardineri (Scott, 1913)	**2**	**0.02**		**PR**
Paromalus (Paromalus) filum Reitter, 1884	**2**	**0.02**		**PR**
Histerinae				
Atholus corvinus (Germar, 1817)	**2**	**0.02**		**PR**
Cylister cornix (Marseul, 1861)	**1**	**0.01**		**PR**
Merohister ariasi (Marseul, 1864)	**24**	**0.18**		**PR**
Platysoma (Platysoma) compressum (Herbst, 1783)	**35**	**0.26**		**PR**
**Platysoma (Platysoma) deplanatum* (Gyllenhal, 1808)	**3**	**0.02**		**PR**
Platysoma (Platysoma) inexpectatum Lackner, 2004	**3**	**0.02**		**PR**
Saprininae				
**Gnathoncus buyssoni* Auzat, 1917	**2**	**0.02**		**PR**
Gnathoncus rotundatus (Kugelann, 1792)	**1**	**0.01**		**PR (NI)**
Tribalinae				
Epierus comptus Erichson, 1834	**4**	**0.03**		**PR**
LAEMOPHLOEIDAE				
Laemophloeinae				
**Cryptolestes* sp.	**4**	**0.03**		**MY**
Laemophloeus monilis (Fabricius, 1787)	**2**	**0.02**		**MY**
**Placonotus testaceus* (Fabricius, 1787)	**1**	**0.01**		**SX**
LATRIDIIDAE				
Corticariinae				
Corticaria elongata (Gyllenhal, 1827)	**7**	**0.05**		**MY**
Latridiinae				
Enicmus rugosus (Herbst, 1793)	**16**	**0.12**		**MY**
LUCANIDAE				
Lucaninae				
Dorcus parallelipipedus (L., 1785)	**4**	**0.03**	LC	**SX**
MALACHIIDAE				
Malachiinae				
**Acromalachius clavicornis* (Peyron, 1877)	**1**	**0.01**		**PR**
**Anthocomus semipolitus* Abeille de Perrin, 1882	**36**	**0.27**		**PR**
**Cephaloncus albozonatus* (Abeille de Perrin, 1883)	**8**	**0.06**		**PR**
**Cephaloncus rhinoceros* Marseul, 1868	**6**	**0.05**		**PR**
**Charopus thoracicus* Morawitz, 1861	**2**	**0.02**		**PR**
Ebaeus sp.	**1**	**0.01**		**PR**
Hypebaeus senaci (Abeille, 1890)	**17**	**0.13**		**PR**
Malachius fucatus Peyron, 1877	**119**	**0.90**		**PR**
**Nepachys amaenus* Peyron, 1877	**1**	**0.01**		**PR**
**Sphinginus coarctatus* (Erichson, 1840)	**4**	**0.03**		**PR**
MELANDRYIDAE				
Melandryinae				
Abdera (Abdera) quadrifasciata (Curtis, 1829)	**2**	**0.02**		**MY**
Orchesia (Orchesia) micans (Panzer, 1794)	**239**	**1.81**		**MY**
**Phloiotrya (Phloiotrya) rufipes* (Gyllenhal, 1810)	**1**	**0.01**		**MY**

Table 1. Continued

Family / Species name	Number of individuals	Presence rate (%)	IUCN Category Europa (Mediterranean)	Trophic category
MORDELLIDAE				
Mordellinae				
Mediimorda attalica Schilsky, 1895	**3**	**0.02**		
Mordellistena sp.1 (episternalis-group)	**25**	**0.19**		**SX**
Mordellistena sp.2 (pumila-group)	**2**	**0.02**		**SX**
Mordellistena sp.3 (pumila-group)	**1**	**0.01**		**SX**
Mordellistena sp. 4	**10**	**0.08**		**SX**
Mordellistena episternalis- group bis	**1**	**0.01**		**SX**
**Mordellistena (Mordellistena) neuwaldeggiana* (Panzer, 1796)	**155**	**1.17**		**SX**
cf. Mordellochroa humerosa bis	**27**	**0.20**		**SX**
MYCETOPHAGIDAE				
Mycetophaginae				
Mycetophagus (Mycetophagus) quadripustulatus (L., 1761)	**14**	**0.11**	LC	**MY**
NITIDULIDAE				
Carpophilinae				
Carpophilus bipustulatus (Heer, 1841)	**142**	**1.07**		**SF**
Cryptarchinae				
Cryptarcha bifasciata Baudi, 1870	**79**	**0.60**		**MY**
Epuraeinae				
Epuraea pallescens (Stephens, 1835)	**47**	**0.36**		**MY**
Nitidulinae				
Soronia oblonga C. Brisout de Barneville, 1863	**5**	**0.04**		**SF**
OEDEMERIDAE				
Oedemerinae				
Ischnomera auripennis (Reitter, 1903)	**1**	**0.01**		**SX**
Ischnomera caerulea (L., 1758)	**5**	**0.04**		**SX**
Ischnomera haemorrhoidalis (W. Schmidt, 1846)	**6**	**0.05**		**SX**
Ischnomera fuscipennis Švihla, 1988	**10**	**0.08**		**SX**
Oedemera (Oncomera) flavicans (Fairmaire, 1860)	**34**	**0.26**		**SX**
RIPIPHORIDAE				
Clinops spectabilis Schaufuss, 1872	**1**	**0.01**		
SCIRTIDAE				
Prionocyphon ornatus Abeille de Perrin, 1881	**27**	**0.20**		**SP**
**Sacodes flavicollis* (Kiesenwetter, 1859)	**3**	**0.02**		**UN**
SCRAPTIIDAE				
Anaspidinae				
Anaspis (Anaspis) sp.1	**208**	**1.57**		**SX**
Anaspis (Larisa) sp.2	**329**	**2.49**		**SX**
Anaspis (Larisa) sp.3	**30**	**0.23**		**SX**
Anaspis (Nassipa) melanostoma Costa, 1854	**22**	**0.17**		**SX**
Scraptiinae				
Scraptia sp.	**215**	**1.63**		**SX**
SPHINDIDAE				
Aspidiphorinae				
**Aspidiphorus orbiculatus* (Gyllenhal, 1808)	**6**	**0.05**		**MY**
STAPHYLINIDAE				
Aleocharinae				
Aleochara brevipennis Gravenhorst, 1806	**5**	**0.04**		**PR**
Aleochara intricata Mannerheim, 1830	**2**	**0.02**		**PR**
Gyrophaena sp.	**32**	**0.24**		
Haploglossa villosula (Stephens, 1832)	**63**	**0.48**		**PR**
Myllaena intermedia Erichson, 1837	**1**	**0.01**		**PR**
Oxytelinae				
Platystethus nitens (C. R. Sahlberg, 1832)	**6**	**0.05**		
Staphylininae				
Gyrohypnus angustatus Stephens, 1833	**1**	**0.01**		**PR**
Hypnogyra angularis (Ganglbauer, 1895)	**1**	**0.01**		**PR**
Scaphidiinae				
**Scaphisoma agaricinum* (L., 1758)	**1**	**0.01**		**MY**
**Scaphisoma subalpinum* Reitter, 1881	**6**	**0.05**		**MY**
Tachyporinae				
Tachyporus nitidulus (Fabricius, 1781)	**2**	**0.02**		**PR**

Table 1. Continued

Family / Species name	Number of individuals	Presence rate (%)	IUCN Category Europa (Mediterranean)	Trophic category
TENEBRIONIDAE				
Alleculinae				
Allecula oronthea Baudi di Selve, 1881	**102**	**0.77**		**SX**
Hymenalia atronitens Fairmaire, 1892	**644**	**4.87**		**SX**
Hymenalia graeca Seidlitz, 1896	**4**	**0.03**		**SX**
Mycetochara sp.1	**1**	**0.01**		**SX**
Mycetochara quadrimaculata (Latreille, 1804)	**408**	**3.09**		**SX**
Mycetocharina rufotestacea Reitter, 1898	**20**	**0.15**		**SX**
Prionychus cisteloides Seidlitz, 1896	**19**	**0.14**		**SX**
Prionychus nitidissimus Pic, 1905	**2**	**0.02**		**SX**
Pseudocistela ceramboides (L., 1761)	**1**	**0.01**		**SX**
Diaperinae				
*Alphitophagus bifasciatus (*Say, 1824)	**2**	**0.02**		**MY**
Corticeus turcicus Soldati, 2019	**6**	**0.05**		**CO (MY, PR)**
*Pentaphyllus testaceus (*Hellwig, 1792)	**1**	**0.01**		**MB**
Lagriinae				
Lagria atripes Mulsant & Guillebeau, 1855	**1**	**0.01**		
Palorinae				
*Palorus ratzeburgi (*Wissmann, 1848)	**22**	**0.17**		**SX**
Tenebrioninae				
*Lyphia tetraphylla (*Fairmaire, 1856)	**4**	**0.03**		**CO (MY)**
*Metaclisa azurea (*Waltl, 1838)	**8**	**0.06**		**SX**
Odocnemis evestigata Nabozhenko & Keskin, 2016	**8**	**0.06**		**SX**
Tenebrio obscurus Fabricius, 1792	**9**	**0.07**		**SX**
TETRATOMIDAE				
Eustrophus dermestoides (Hellwig, 1792)	**13**	**0.10**		**MY**
TROGIDAE				
Troginae				
Trox (Trox) scaber (L., 1767)	**1**	**0.01**		**NI**
TROGOSITIDAE				
Trogositinae				
Nemozoma elongatum (L., 1761)	**1**	**0.01**	LC	**PR**
Temnochila caerulea (Olivier, 1790)	**7**	**0.05**	LC (LC)	**PR**
ZOPHERIDAE				
Colydiinae				
Colobicus hirtus (Rossi, 1790)	**10**	**0.08**		**SX**
Colydium elongatum (Fabricius, 1787)	**3**	**0.02**		**PR**
TOTAL	**13,217**	**100**		

*New records for Türkiye

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Original article (Orijinal araştırma)

Resistance status in *Myzus persicae* (Sulzer, 1776) (Hemiptera: Aphididae) populations against single and mixture of neonicotinoid and synthetic pyrethroid insecticides[1]

Myzus persicae (Sulzer, 1776) (Hemiptera: Aphididae) popülasyonlarında neonikotinoid ve sentetik piretroid insektisitlerin tekli ve karışımlarına karşı direnç durumu

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Abstract

The peach potato aphid, *Myzus persicae (*Sulzer, 1776) (Hemiptera: Aphididae), a vector of many plant virus diseases, causes damage to its wide range of hosts by direct feeding. Chemical control has been the primary method to control this species, and the intensive use of insecticides has led to the development of resistance. In this study, conducted between the years 2017-2019, firstly resistance ratio of five *M. persicae* populations from Antalya, Türkiye were determined by leaf-dip bioassay method. The field populations showed significant resistance to thiamethoxam (between 201-332 fold) and lambda-cyhalothrin (between 50-103 fold) when compared to susceptible population. To identify whether resistance mediated by mutations in sodium channel and nicotinic acetylcholine receptor, DNA regions that encompass “mutation hot-spot” were sequenced. This revealed no population contained R81T mutation that has been previously linked with neonicotinoid resistance. As to synthetic pyrethroid resistance, the L1014F *kdr* mutation was fixed in all field populations. This study is the first description of *kdr* mutation in *M. persicae* populations from Türkiye. Bioassay results also indicated that the toxicity of thiamethoxam and lambda-cyhalothrin mixture was higher than that of lambda-cyhalothrin alone. Our findings can make significant contributions to *M. persicae* resistance management.

Keywords: Bioassay, insecticides, *kdr* mutation, *Myzus persicae*, resistance management

Öz

Birçok bitki virüs hastalığının vektörü olan yeşil şeftali yaprakbiti, *Myzus persicae* (Sulzer, 1776) (Hemiptera: Aphididae), doğrudan beslenerek çok sayıda konukçusuna zarar vermektedir. Kimyasal kontrol, bu tür ile mücadelede birincil yol olması ve yoğun kimyasal kullanımı insektisit direnci gelişimine yol açmaktadır. 2017-2019 yılları arasında yapılan bu çalışmada ilk olarak, Antalya, Türkiye'den beş *M. persicae* popülasyonunun direnç oranları yaprak daldırma biyoassay yöntemi ile belirlenmiştir. Örtüaltı popülasyonları, hassas popülasyona kıyasla, thiamethoxam için (201-332 kat aralığında) ve lambda-cyhalothrin için (50-103 kat aralığında) önemli direnç göstermiştir. Direncin sodyum kanalı ve nikotinik asetilkolin reseptöründeki mutasyonlardan kaynaklı olup olmadığını belirlemek için, “mutasyon sıcak nokta”larını kapsayan DNA bölgeleri dizilenmiştir. Popülasyonların neonikotinoid direncine neden olan R81T mutasyonunu içermediği belirlenmiştir. Sentetik piretroid direncinde, L1014F *kdr* mutasyonu tüm arazi popülasyonlarında tespit edilmiştir. Bu çalışma ile Türkiye'deki yeşil şeftali yaprakbiti popülasyonlarında *kdr* mutasyonu ilk defa gösterilmiştir. Biyoassay sonuçları ayrıca, thiamethoxam ve lambda-cyhalothrin karışım toksisitesinin, tek başına, lambda-cyhalothrin toksisitesinden daha yüksek olduğunu göstermiştir. Bulgularımız *M. persicae* direnç yönetimi için önemli katkılar sağlayabilir.

Anahtar sözcükler: Biyoassay, insektisitler, *kdr* mutasyonu, *Myzus persicae*, direnç yönetimi

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Introduction

The peach potato aphid, *Myzus persicae (*Sulzer, 1776) (Hemiptera: Aphididae), occurs worldwide in all vegetable-producing areas. *Myzus persicae* directly absorbs plant sap, causing discoloration and curling of young seedlings and leaves, so the plant growth slows down. In addition, *M. persicae* is the vector of over 100 plant virus diseases (Stevens & Lacomme, 2017). Since it is harmful all year long, especially in greenhouses, growers frequently apply insecticides to control this pest. As a result of this extensive use, resistance to several classes of insecticides including synthetic pyrethroids and neonicotinoids has been documented (IRAC, 2022).

Pyrethroids are neurotoxic insecticides widely used to control many insect pests due to their rapid action, high insecticidal activity and low toxicity on mammals (Rinkevich et al., 2013). The target of pyrethroids are the voltage-gated sodium channels and binding pyrethroids lead to incapacitation of the insect, known as 'knock-down' (Williamson et al., 1993). The resistance to pyrethroids is mainly caused by a point mutation in the S6 segment of domain II of the channel, termed knock-down resistance (*kdr*), resulting in a leucine to phenylalanine (L1014F) replacement. Secondary mutations such as M918L/T and L932F on the segment IIS5 have been associated with an enhanced form of resistance, termed super-*kdr*, in a range of insect species (Davies et al., 2007).

Neonicotinoid insecticides are used against numerous sucking and certain chewing insect pests because of their high efficacy. As selective agonists, neonicotinoids act by binding on insect nicotinic acetylcholine receptors (nAChR) (Jeschke et al., 2011). Previous studies have identified both metabolic and target-site mechanisms underlying resistance to neonicotinoids (Bass et al., 2015). For metabolism-based mechanism, a single P450 gene, *CYP6CY3*, plays a primary role in resistance by metabolizing the nicotine to fewer toxic metabolites (Puinean et al., 2010; Bass et al., 2013). Work on target-site mechanism in *M. persicae* has demonstrated the importance of the arginine to threonine substitution (R81T) in the loop D region of the β1 subunit (Bass et al., 2014).

Due to the failure in control as a result of resistance development, overdose and frequent spraying is carried out, which increases the cost of the product. It causes residues in freshly consumed vegetables, threatens human health and causes problems in exports. It also causes many problems such as environmental pollution and affecting pollinators. Therefore, insecticide resistance management (IRM) is needed for preventing or slowing the development of resistance to insecticides. Monitoring insecticide resistance status is a prerequisite for effective interventions. Distinguishing the underlying mechanism of resistance and cross-resistance spectrum are of key importance to determine effective insecticides for the management of the problem (Vontas & Mavridis, 2019). Application of the different IRM strategies such as rotations and mixtures of two or more insecticides having different modes of action could help to slow the rate of resistance evolution (Georghiou et al., 1986).

In a previous study (Velioğlu et al., 2008) revealing the insecticide susceptibility of *M. persicae* populations collected from vegetable growing fields and greenhouses in Ankara, Antalya and Mersin the concurrent occurrence of high levels of resistance to imidacloprid and thiamethoxam over 4,000 ppm have been recorded. However, the current resistance status and molecular basis of resistance to insecticides in Turkish *M. persicae* populations remains unknown.

In recent years, there has been an increase in the applications of plant protection products in mixtures from different groups in Türkiye. It is stated that the reason for making mixtures is the development of resistance in target pests and that mixture insecticides solve this problem. Therefore, there is a need to clarify the status of active ingredients such as thiamethoxam and lambda-cyhalothrin, which are known to develop resistance alone, in mixture insecticides.

In the present study, insecticide bioassays were conducted to measure the resistance level of *M. persicae* populations against neonicotinoid and synthetic pyrethroid insecticides. The mutations responsible for the resistance were screened across populations. We also tested the efficacy of binary mixtures of neonicotinoid and synthetic pyrethroid insecticides as a resistance management strategy. According to the results of this study, it will be possible to restrict the use of ineffective insecticides due to resistance. In this way, excessive and unnecessary use of insecticides will be prevented, protecting human and environmental health while contributing to the national economy.

Materials and Methods

Aphid populations

The *M. persicae* populations were collected from vegetable greenhouses in Antalya, Türkiye where insecticides were intensively applied in 2017 (Table 1). Susceptible population collected from the unsprayed home garden over years in Çubuk district located at Ankara province in June 2017. Bioassay studies were completed in 2017-2018 and molecular studies were conducted in 2019. Using a modified version of Velioğlu & Toros (2002) approach, the populations were reared in plexiglass cabinets in insect rearing rooms with 27±2°C temperature, 50-60% relative humidity, and 16 hours light and 8 hours dark conditions. In addition, eggplant (*Solanum melongena* L., Solanaceae) plants grown in a temperature-controlled greenhouse were used for maintaining the populations.

Table 1. Sample areas of *M. persicae* populations collected from greenhouses

Population name	Location	Date	Host
Kocaahmetler	Aksu	May 2017	Eggplant
Bahtılı	Konyaaltı	May 2017	Eggplant
Göçmenler	Konyaaltı	May 2017	Pepper
Topallı	Aksu	May 2017	Courgette
Kurşunlu	Kurşunlu	May 2017	Tomato

Insecticides and bioassay studies

Synthetic pyrethroid, lambda-cyhalothrin 50 g/l (EC) and neonicotinoid insecticides, thiamethoxam 240 g/l (SC) and their mixtures, thiamethoxam+lambda-cyhalothrin 141g/l+106 g/l (SC) were used in bioassay studies. Dose-response bioassays were based on the standard leaf-dip method (Cahill et al.,1995). Briefly, eggplant leaf discs (3.7 cm in diameter) were immersed in serial dilutions of insecticide for 20 seconds. Leaf discs were placed in bioassay containers poured with 1% agar and left to dry under a fume hood for 2 hours. 20 wingless adults *M. persicae* were then transferred onto the dried leaf discs. Control treatment consisted of only 0.02% Triton X-100. Mortality was assessed after 48 hours in a climate cabinet with 25 ±1°C temperature and 16 hours of light and 8 hours of darkness. Trials were carried out between 30-400 ppm and 3 replications for each insecticide.

Detection of target-site resistance mutations

Sanger sequencing were used to screen the populations for the *kdr* and *super-kdr* resistance (M918L/T, L932F, L1014F) for synthetic pyrethroid resistance; R81T for neonicotinoid resistance. DNA extraction was performed using “HP PCR product purification (Roche)” kit following the manufacturer’s instructions (Roche Diagnostics, Meylan, France). The quality and quantity of DNA samples were evaluated by spectrophotometer (Nanodrop). A partial gene fragments containing aforementioned mutations were amplified by PCR from 50 ng aliquots of DNA with the primers in Table 2.

Table 2**.** Primer sequences used in molecular studies

Primer name	Sequence 5′-3′	Fragment size	Reference
kdr-F1	TCGTGGCCCACACTGAATCT	578 bp	Cassanelli et al., 2005
kdr-R4	GTTCATGTAAGATACATGAATTC		
MpB1TMF	TAGTTCTAACTTATTGCCTGCAGCTAT	225 bp	Puinean et al., 2011
MpB1TMR	GCGGTCAGGAAGTCTAATACGTTA		

The PCR reactions consisted of 4 µl of 5xPCR Buffer Solution (FirePol, Solis BioDyne, Tartu, Estonia), 0.5 µl of each primer (10 mM), 1 µl gDNA, 14 µl distilled water in a final volume of 20 µl. Thermal cycling amplification consisted of an initial denaturation phase of 3 min at 95°C and then 30 cycles (95°C for 30 s, 60°C for 30 s, 72°C for 1 min) with a final extension at 72°C for 7 min. Electrophoresis on 1.2% agarose gel in 1X TBE buffer for 1 h at 100 V was performed to verify the size of the PCR products.

Data analysis

LC_{50} values and 95% confidence intervals (CI) were calculated by probit analysis using PoloPC (LeOra Software,1994 Company, Petaluma, USA). Resistance ratios were calculated as the LC_{50} value of field populations with respect to the LC_{50} calculated for the susceptible population. LC_{50} values were considered statistically significant if their respective 95% confidence limits (CL) did not overlap (Robertson et al., 2007).

The presence/absence of insecticide resistance mutations were determined by visual examination of sequencing chromatograms. Obtained chromatograms were analyzed using the Geneious 11.1.4 software (http: //www.geneious.com, Kearse et al., 2012) and compared with the sequences deposited in NCBI database (GenBank accession: AM711603 for sodium channel, GenBank accession: AJ251838 for nAChR).

Results

Toxicity of single insecticides

Results of the *M. persicae* bioassays with insecticides alone are shown in Table 3. Between 10-90% mortality has been observed among populations. A mortality rate below 10% was observed in the control group. The response of thiamethoxam and lambda-cyhalothrin were different from susceptible population (non-overlapping of 95% CI) in all field populations**.** The LC_{50} value of the susceptible population for thiamethoxam and lambda-cyhalothrin were found to be 0.172 and 0.971 ppm. The LC_{50} values of field populations were in the range of 34.578 to 57.256 pmm and 48.758 to 100.066 ppm for thiamethoxam and lambda-cyhalothrin, respectively. While Göçmenler recorded lowest resistance ratio of 201.03 and 50.214 fold, Kocaahmetler was showing highest resistance ratio of 332.88 and 103.05 fold to thiamethoxam and lambda-cyhalothrin, respectively in comparison to susceptible population.

Table 3. Toxicity of thiamethoxam and lambda-cyhalothrin alone to *M. persicae*

Insecticide	Populations	N	LC_{50} (CI 95%)		Slope+SE	X^2 (df)	*P* value	RR_{50}
Thiamethoxam	Susceptible	400	0.17 (0.028-0.295)	a[1]	1.02±0.29	2.11 (14)	0.99	-
	Bahtılı	400	46.43 (36.662-57.365)	bc	2.24±0.33	12.55 (15)	0.64	269.94
	Göçmenler	400	34.57 (26.358-43.500)	b	2.11±0.24	16.37 (15)	0.36	201.03
	Kocaahmetler	420	57.25 (45.573-70.432)	c	1.70±0.16	12.65 (16)	0.70	332.88
	Kurşunlu	400	49.86 (38.386-63.078)	bc	1.77±0.17	16.50 (15)	0.35	289.91
	Topallı	400	48.07 (38.103-58.618)	bc	2.19±0.30	9.46 (15)	0.85	279.48
Lambda-cyhalothrin	Susceptible	400	0.97 (0.761-1.039)	a[1]	1.78±0.22	8.41 (14)	0.86	-
	Bahtılı	420	94.81 (80.045-111.042)	d	2.18±0.20	12.62 (16)	0.70	97.64
	Göçmenler	400	48.75 (39.494-57.921)	b	2.35±0.25	12.30 (15)	0.66	50.21
	Kocaahmetler	420	100.06 (84.846-116.642)	d	2.34±0.22	9.02 (16)	0.91	103.05
	Kurşunlu	400	69.12 (59.279-79.607)	c	2.75±-0.25	6.34 (15)	0.97	71.18
	Topallı	400	93.26 (77.854-110.237)	cd	2.14±0.22	9.76 (15)	0.83	96.04

[1] Values followed by the same letter within a row are not statistically different.
N: number of individuals; *SE*: Standard Error; *df*: degrees of freedom; X^2: chi-squared test; *CI*: confidence interval; *RR*: resistance ratio.

Toxicity of binary mixture

Results of the *M. persicae* bioassays with insecticides mixture are shown in Table 4. The response of thiamethoxam and lambda-cyhalothrin binary mixture were different from susceptible population (non-overlapping of 95% CI) in all field populations (Robertson et al., 2007). The LC_{50} values were ranging from 30.758 to 76.114 ppm in field populations resulting in 39.841 to 98.593 fold resistance compared to susceptible population. Interestingly, the mixture of thiamethoxam and lambda-cyhalothrin exhibited significantly higher toxicity to Bahtılı, Kurşunlu and Topallı ($p < 0.05$, non-overlapping 95% CI) and marginally significant toxicity in Göçmenler and Kocaahmentler than the toxicity of lambda-cyhalothrin alone Table 3-4). Thiamethoxam and lambda-cyhalothrin together have been found to be slightly more effective than thiamethoxam taken alone.

Table 4. Toxicity of thiamethoxam and lambda-cyhalothrin in combination to *M. persicae*

Insecticide	Populations	N	LC_{50} (CI 95%)		Slope+SE	X^2 (df)	*P* value	RR
Thiamethoxam + Lambda-cyhalothrin	Susceptible	340	0.77 (0.131-1.261)	a[1]	1.76±0.21	5.44 (12)	0.94	-
	Bahtılı	340	57.88 (46.798-68.822)	c	2.54±0.29	4.55 (12)	0.97	74.97
	Göçmenler	340	30.75 (25.904-40.424)	b	3.50±0.54	3.56 (12)	0.99	39.84
	Kocaahmetler	340	76.11 (62.983-89.833)	c	2.45±0.26	6.67 (12)	0.88	98.59
	Kurşunlu	340	44.83 (36.639-54.390)	bc	2.59±0.33	5.66 (12)	0.93	58.07
	Topallı	340	55.36 (44.406-66.075)	bc	2.52±0.29	4.56 (12)	0.97	71.72

[1] Values followed by the same letter within a row are not statistically different.
N: number of individuals; *SE*: Standard Error; *df*: degrees of freedom; X^2: chi-squared test; *CI*: confidence interval; *RR*: resistance ratio.

Detection of target-site resistance mutations

The ~ 578 and 225 bp fragments of the partial (IIS4-IIS6) sodium channel and nAChR β1 subunit were PCR amplified and sequenced. The sequences were aligned against known sequences of *M. persicae* (GenBank accession: AM711603 for sodium channel, GenBank accession: AJ251838 for nAChR) and depicted in Figure 1.

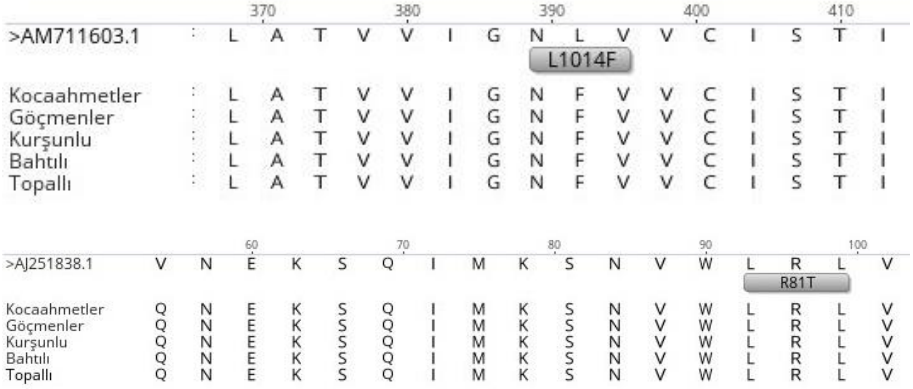

Figure 1. Detection of *kdr* (L1014F) mutation on sodium channel and R81T mutation in nAChR.

The comparisons revealed a *kdr* mutation (L1014F mutation, numbering based on the housefly sequence GenBank accession: X96668) was fixed in all field populations of *M. persicae*. The secondary mutations, M918T\L and L932F were absent. In the case of neonicotinoids, the R81T mutation could not be verified by direct sequencing (Figure 1). Thus, the involvement of R81T mutation in neonicotinoid resistance seems unlikely. Whether other target-site mechanisms and/or metabolic mechanisms play a role in the resistance warrants further investigation.

Discussion

Resistance to insecticides in *M. persicae* in previous studies is widespread worldwide. Insecticide resistance of *M. persicae* has been detected in Europe, Japan, North America and Australia (Soderlund, 2008). Velioğlu&Toros (2002) stated that more than 600-fold resistance was observed against pirimicarb in three different *M. persicae* populations collected from the province of İçel. Additionally, Velioğlu et al. (2008) investigated the resistance of *M. persicae* populations collected from vegetable growing fields and greenhouses in Ankara, Antalya and Mersin. They found the highest LC_{50} value for imidacloprid as 7,624 ppm and for thiamethoxam as 4,098 ppm for the populations collected from Antalya. In this study, different levels of LC_{50} values against insecticides from different groups were found in *M. persicae* populations. Different levels of resistance could be influenced by operational factors such as application frequency and insecticide coverage (Rosenheim & Tabashnik, 1990). According to the results there was a significant level of resistance to the insecticides tested in five different populations (Kocaahmetler, Topallı, Kurşunlu, Bahtılı, Göçmenler) collected from greenhouse vegetable fields in Antalya province. Overall, it has been confirmed that *M. persicae* has gained serious resistance to insecticides in and around Antalya province where pest control depends intensely on insecticide use.

To explore the molecular basis of lambda-cyhalothrin resistance, DNA regions of voltage-gated sodium channel that encompass a 'mutation hot-spot' was amplified and sequenced. This revealed a *kdr* mutation was fixed in all field populations of *M. persicae*. No populations were observed that carry either M918L/T and L932F. The current study is the first report of L1014F mutation in pyrethroid-resistant aphid populations from Türkiye. This mutation has been associated with pyrethroid resistance in over 20 different arthropod species including houseflies, cockroaches, mosquitoes, and aphids (Miyazaki et al., 1996; Martinez-Torres et al., 1997; Martinez-Torres et al., 1999). It was shown that the presence of kdr both reduced the action of insecticide and made the sodium channel less likely to open by changing the gating properties (Davies & Williamson, 2009; Du et al., 2010).

To investigate whether neonicotinoid resistance was mediated by R81T mutation in *M. persicae* we PCR amplified and sequenced the β1 subunit of the nAChR. The R81T mutation could not be verified by direct sequencing in this study. This mutation was first reported in a neonicotinoid-resistant population of *M. persicae* from France (Bass et al., 2011; Slater et al., 2011). Studies using enzyme inhibitors and microarray analysis revealed that P450-mediated detoxification, with the overexpression of *CYP6CY3*, may contribute to neonicotinoid resistance (Puinean et al., 2010; Bass et al., 2013). Indeed, further study confirmed that functionally expressed *CYP6CY3* is highly efficient at metabolizing nicotine to fewer toxic metabolites in vitro (Bass et al., 2013). Therefore, further investigations are needed to identify whether overexpression of *CYP6CY3* confer resistance to neonicotinoids in aphids populations from Türkiye.

The high selection pressure to *M. persicae* is seems to be the main cause about insecticide resistance in Antalya. To delay the onset of resistance development, it is essential to use IRM strategies which include use of insecticide in mixture. In theory, a binary mixture could ensure that insects that survive exposed to one compound will be killed by the other compound (Shi et al., 2012). In the current study, bioassay results indicated that the toxicity of thiamethoxam and lambda-cyhalothrin mixture was higher than that of lambda-cyhalothrin alone. Carbamates and organophosphates have been shown to synergize pyrethroids in *Helicoverpa armigera* (Hübner, 1808) (Lepidoptera: Noctuidae), *Anopheles gambiae* Giles, 1900 (Diptera: Culicidae), *Leptinotarsa decemlineata (*Say, 1824) (Coleoptera: Chrysomelidae) and *Frankliniella occidentalis* (Pergande, 1895) (Thysanoptera: Thripidae) (Guillet et al., 2001; Martin et al., 2003; Bielza et al., 2007; Jiang et al., 2011). Thus, the high rates of resistance to insecticides in insects could be reduced by the use of insecticide mixtures, making it a good option for an anti-resistance strategy. However, it is important to bear in mind that it should be used carefully since it may accelerate the development of multiple resistance (Sayyed et al., 2004).

In conclusion, our research findings suggest that over use of these insecticides to control *M. persicae* is likely to contribute to the development of resistance to thiamethoxam and lambda-cyhalothrin. In the study, all field populations were found to be resistant to thiamethoxam and lambda-cyhalothrin. This is supported by the presence of L1014F, a *kdr* mutation in the sodium channel. Further study is required to identify whether P450-based detoxification confer resistance to neonicotinoids in aphid populations from Türkiye. The present data show the application of thiamethoxam and lambda-cyhalothrin mixture could be considered to slow the development of resistance to lambda-cyhalothrin. Our findings may have considerable implications for *M. persicae* resistance management.

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Original article (Orijinal araştırma)

Lethal and sublethal effects of lambda-cyhalothrin on *Aphis fabae* (Scopoli, 1763), *Myzus persicae* (Sulzer, 1776) and *Acyrthosiphon pisum* (Harris, 1776) (Hemiptera: Aphididae)

Lambda-cyhalothrin'nin *Aphis fabae* (Scopli, 1763), *Myzus persicae* (Sulzer, 1776) ve *Acyrthosiphon pisum* (Harris, 1776) (Hemiptera: Aphididae) üzerindeki letal ve subletal etkileri

Ali KAYAHAN[1*]

Abstract

In this study, sublethal doses of lambda-cyhalothrin to the species *Aphis fabae* (Scopoli, 1763), *Myzus persicae* (Sulzer, 1776) and *Acyrthosiphon pisum* (Harris, 1776) (Hemiptera: Aphididae) were determined, and the effects of these doses on the life cycles of the species were revealed and evaluated. The lethal effects of different concentrations (0.3125, 0.625, 1.25, 2.5, 5, 10 and 20 µL L^{-1}) prepared by distillated water of lambda-cyhalothrin on the species were determined according to Abbott. Based on the results obtained, the effects of LC_{30} and LC_{40} concentrations of the insecticide on the life cycles of the species were determined. The insecticide caused different mortality rates in the species. The sublethal concentrations of the insecticide were found to be effective for the life cycles of the species. In all three species, intrinsic rate of increase (r_m), net reproduction rate (R_0) and gross reproduction rate (*GRR*) values were found to decrease when insecticide was applied. The results will provide guidance to researchers working in this specific field. However, it would be beneficial to replicate this study under field conditions to obtain clear information.

Keywords: Aphids, ecotoxicology, life table parameters, pyrethroid, toxicity

Öz

Bu çalışmada, lambda-cyhalothrin'in *Aphis fabae* (Scopoli, 1763), *Myzus persicae* (Sulzer, 1776) ve *Acyrthosiphon pisum* (Harris, 1776) (Hemiptera: Aphididae) üzerinde subletal dozları belirlenmiş ve bu dozların türlerin yaşam döngüleri üzerindeki etkileri ortaya konularak değerlendirilmiştir. Lambda-cyhalothrin'in saf su ile hazırlanan farklı konsantrasyonlarının (0.3125, 0.625, 1.25, 2.5, 5, 10 ve 20 µL L^{-1}) türler üzerindeki öldürücü etkileri Abbott'a göre belirlenmiştir. Elde edilen sonuçlara göre insektisitin LC_{30} ve LC_{40} konsantrasyonlarının türlerin yaşam döngüleri üzerindeki etkileri araştırılmıştır. Çalışmada kullanılan insektisitin türlerde farklı ölüm oranlarına neden olduğu gözlenmiştir. Ayrıca insektisitin subletal konsantrasyonlarının türlerin yaşam döngüleri üzerinde etkili olduğu bulunmuştur. Her üç türde de insektisit uygulandığında kalıtsal üreme yeteneği (r_m), net üreme gücü (R_0) ve toplam üreme oranı (*GRR*) değerlerinin düştüğü tespit edilmiştir. Elde edilen sonuçların bu konuda çalışan araştırmacılara yol gösterici olacağı düşünülmektedir. Ancak daha net sonuçlar elde etmek için bu çalışmayı saha koşullarında tekrarlamak faydalı olacaktır.

Anahtar sözcükler: Yaprak bitleri, ekotoksikoloji, yaşam çizelgesi parametreleri, piretroid, toksisite

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Introduction

Aphids cause growth disorders in plants and even the death of the plant in the case of a very dense population. Due to this negative situation in production, there is a loss of quality and yield of plants. In addition, aphids cover the plant surface with a substance they produce during their feeding, causing sooty mold formation on the plant. This causes secondary factors (fungi etc.) to multiply in the environment and indirectly cause damage to the plant. In addition, aphids cause indirect damage to plants because they secrete toxic substances and transmit phytopathogenic viruses (Lodos, 1986; Catherall et al., 1987; Kovalev et al., 1991; Elmalı & Toros, 1997; Will & Vilcinskas, 2015; Boissot et al., 2016; Kloth et al., 2017).

The black bean aphid, *Aphis fabae* (Scopoli, 1763) (Hemiptera: Aphididae) is a small and black-colored pest (Kennedy et al., 1962). The plants it feeds on include more than 200 wild plants, but also vegetables, sugar beets, broad beans, beans, potatoes, sunflowers and tomatoes (Völkl & Stechmann, 1998; Barnea et al., 2005; Fericean et al., 2012). *Acyrthosiphon pisum* (Harris, 1776) (Hemiptera: Aphididae), one of the most important pea pests was first detected on the alfalfa plant, *Medicago sativa* (L.) (Leguminosae) in Turkey (Düzgüneş & Tuatay, 1956). These pests cause deformations in the fruits and a reduction in grain weight by sucking up the sap in the young shoots of the plant (Bouchery, 1977; Cors & Depfort, 1993). Although this species is known to be one of the main causes of damage to wild plants, it also causes harm to beans, lentils, clover, sainfoin, vetch and some legumes (Stary, 1970; Ali & Habtewold, 1994). The green peach aphid *Myzus persicae* (Sulzer, 1776) (Hemiptera: Aphididae) is known as a species that causes damage to more than 400 plants. This species not only causes damage by absorbing plant sap, but also indirect damage by transmitting more than 100 viral diseases (Blackman & Eastop, 2007). Like other aphids, it reproduces parthenogenetically and can rapidly increase its population thanks to its short development time (Foster et al., 2000). The population of these pests can only be kept under control with chemical insecticides. This has led to the fact that this species has become resistant to various chemicals (Elbert et al., 1998; Bass et al., 2014; Gill & Garg, 2014; Sial et al., 2018; An et al., 2020).

Insecticides disrupt physiological functions of insects (fecundity, development, sex ratio, behavioral conditions, nutrition, egg laying, and orientation) and make them ineffective (Galvan et al., 2005; Desneux et al., 2007). Pyrethroids show flesh faster compared to other insecticides and are very effective in control of various pests such as aphids, moths and thrips. In addition, they are widely used in agricultural production areas thanks to their low prices (Liu et al., 2015; Zhang et al., 2015). Lambda-cyhalothrin is a non-systemic insecticide from the pyrethroid group with rapidly degradable properties. It is very effective against insects (aphids etc.) that cause damage in agricultural production. While lambda-cyhalothrin acts on insects, it acts on sodium channels of axon membrane and disrupts normal function. It prevents sodium channels that are important for nerve transmission from closing; sequential nerve stimulation occurs, resulting in the death of the insect (He et al., 2008).

Sublethal doses of insecticides can have different effects on the biology, physiology, and behavior of plant pests (Desneux et al., 2007; Liu et al., 2008). Moreover, these doses may stress the insects rather than kill them (Piiroinen et al., 2014; Wang et al., 2017). This event occurs due to environmental conditions that prevent an insect from continuing its normal biological cycle (Ghalambor et al., 2007). The resulting stress can have negative effects on insect development, offspring/egg production, feeding and mating behavior (Arn'o & Gabarra, 2011; Quan et al., 2016). These negative effects impact the insect population, and it is believed that these effects can be genetically transmitted to the offspring of generations exposed to sublethal doses (Stark & Banks, 2003; Guo et al., 2013). In addition, different effects occur between generations in insects exposed to different sublethal doses of different insecticides. For this reason, it is recommended that life tables data be obtained to evaluate the results of this study (Stark & Banks, 2003). From some studies, sublethal doses of pyrethroids and some insecticides are nonlethal to both aphids and some insects, but have different negative effects on agricultural pests (Kidd et al., 1996; Desnuex et al.,

2004, 2005; Quan et al., 2016; Xiao et al., 2016; Qu et al., 2020; Afza et al., 2021; Alfaro-Tapia et al., 2021; Garily-Moradi et al., 2021; Tan et al., 2021; Shi et al., 2022). In this study, sublethal doses of lambda-cyhalothrin to the species *A. fabae*, *M. persicae* and *A. pisum* were determined, and the effects of these doses on species life cycles were demonstrated and evaluated. Although there are studies on the effects of sublethal doses of insecticides on aphids, this study was conducted to address the deficiencies in the effects on different aphids.

Materials and Method

In this study, an insecticide containing the active ingredient lambda-cyhalothrin (Passat 50 g/L, Ferbis, Türkiye) was used to determine its effect on aphids.

Production of plants for aphids

In the experiments, the bell pepper plant (*Capsicum annuum* L. var. *grossum*), used for the production of *M. persicae*, and the faba bean plant (*Vicia faba* L. var. *major*), used for the production of *A. fabae,* and *A. pisum*, were grown in plastic containers (200 ml) with soil in a 1:1 ratio to peat. Production was carried out in a climate room with 25±1°C, 60±5% proportional humidity and 16:8 (light:dark) light conditions.

Mass production of aphids

The aphids in the last nymphal stage were transferred to bell pepper and field bean plants that had reached the length (15 cm) and number of leaves (6 pieces) intended for the experiments. They were propagated separately in different cages of 50x50x50 cm covered with tulle. The initial population of aphids infested on clean plants was obtained from ongoing mass production in the laboratory. Aphids, which were collected on pepper plants in Serik in Antalya and identified by Prof. Dr. İsmail Karaca in nature, were used for the experiments. To ensure continuity of mass production, old and decaying plants were replaced with new plants at weekly intervals. Aphid production was carried out in a climate room with 25±1°C, 60±5% proportional humidity, and 16:8 (light:dark) light conditions.

Lethal effect of lambda-cyhalothrin on the aphids

The lethal effects of different concentrations (0.3125, 0.625, 1.25, 2.5, 5, 10, and 20 µL L^{-1}) of the insecticide (It was prepared as 7 concentrations by diluting 50% from the highest dose on the label of the insecticide) used in the first phase of the study were determined on *A. fabae*, *M. persicae* and *A. pisum*. Petri dishes with filter paper of 9 cm diameter were used for the experiments. The prepared concentrations were sucked into the filter paper at 1 ml in each Petri dish. One-day-old aphids were transferred to these papers using a thin sable brush, ensuring contact with the dose on the paper (tarsal, ventral and labial contact). Subsequently, the plant leaves were left in the Petri dish for the aphids to feed on. Twenty-four hours after the start of the experiments, the live and dead individuals were recorded and the effect of the insecticide was determined. At this time, 10 Petri dishes were used for each dose and 10 aphids were used for each Petri dish. Pure water was used for the control application. The experiments were conducted in a climatic room with 25±1°C, 60±5% proportional humidity, and 16: 8 (light: dark) light conditions. This phase of the experiment was repeated separately for each aphid species.

Abbott's formula was used to determine mortality rates over living and dead individuals and the percentage of mortality rates was calculated (Abbott, 1925). Analysis of variance (ANOVA) was applied to the obtained results. If the difference between the means was statistically significant, groups were compared using Tukey's HSD. The level of this significance was determined according to the TUKEY multiple comparison test. Lethal concentrations of the insecticide on aphids (LC_{30}, LC_{40}, LC_{50}) were determined using the mortality rates obtained at this stage of the study. PROBIT analysis was used to determine these concentrations.

$$\text{Percent effect} = \left(\frac{\text{Number of live individuals in control-Number of live individuals in application}}{\text{Number of live individuals in control}}\right) \text{X } 100$$ (Abbott, 1925).

Sublethal effect of lambda-cyhalothrin on the aphids

The effects of LC_{30} and LC_{40} concentrations of the insecticide applied in this phase of the experiment on *A. fabae*, *A. pisum* and *M. persicae* were determined. The prepared doses were absorbed by the filter papers in the Petri dishes, and the one-day-old individuals transferred to the Petri dish using a sable brush were exposed to the dose. Cotton was left on the bottom of the filter paper to prevent the leaves from fading, and it was moistened, and plant leaves were placed as food for the aphids. Then, the daily development of individuals was monitored, and the newborns were recorded and removed from the environment. The counts were continued until the aphids died. This part of the experiments was performed with 60 replicates for each dose. Standard size Petri dishes were opened to allow air circulation in the Petri dish and covered with tulle to prevent escape. This procedure was performed separately for each aphid species. Experiments were performed in a climate room with 25±1°C, 60±5% proportional humidity and 16: 8 (light: dark) light conditions.

The data were recorded to determine the development of age-related life tables for each dose used. The parameters of the aphid life tables were calculated using RmStat-3 software (Özgökçe & Karaca, 2010) according to the Euler-Lotka equation (Birch, 1948) and analyzed separately. Tukey multiple comparison test was used for comparison of the periods with Minitab (ver. 16) at a significant difference level, $p<0.05$. Several equations were used to calculate the parameters, which are:

Intrinsic Rate of Increase (r_m), $\sum e^{(-r_m.x)} l_x.m_x = 1$ (Birch, 1948);

Net reproduction Rate (R_0), $R_0 = \sum l_x.m_x$ (Birch, 1948);

Mean Generation Time (T_0), $T_o = \frac{\ln R_0}{r_m}$ (Birch, 1948);

Gross Reproduction Rate (*GRR*), $GRR = \sum m_x$ (Birch, 1948);

Daily maximum reproductive value (λ), $\lambda = e^{r_m}$ (Birch, 1948);

Doubling time (T_2), $T_2 = \frac{\ln 2}{r_m}$ (Kairo & Murphy, 1995).

Results

The results of the study have shown that high doses of insecticide caused a high mortality rate in the applied aphids. When the insecticide was applied to *A. fabae*, it was found that 5 µl L^{-1} and subsequent high concentrations caused more than 90% mortality and the resulting mortality was statistically different from low concentrations ($P<0.05$). Although similar situations were observed in the other two aphids (*M. persicae* and *A. pisum*), it was found that the mortality rate of 90% was at concentrations of 10 and 20 µl L^{-1}, in contrast to *A. fabae*. The mortality rates obtained were also statistically different from the low concentrations ($p<0.05$). Examining the data obtained, the lowest mortality rate (32.97%) was observed at the lowest dose (0.3125 µl L^{-1}) applied to *A. pisum* (Figure 1).

Lethal concentrations calculated by probit analysis were determined based on the mortality rates obtained. Accordingly, the lowest LC_{30}, LC_{40}, and LC_{50} values were found for *A. fabae* (0.156, 0.274 and 0.462 µl L^{-1}, respectively) and the highest values were found for *A. pisum* (0.238, 0.474 and 0.806 µl L^{-1}, respectively). All lethal concentrations resulting from the study are listed in Table 1.

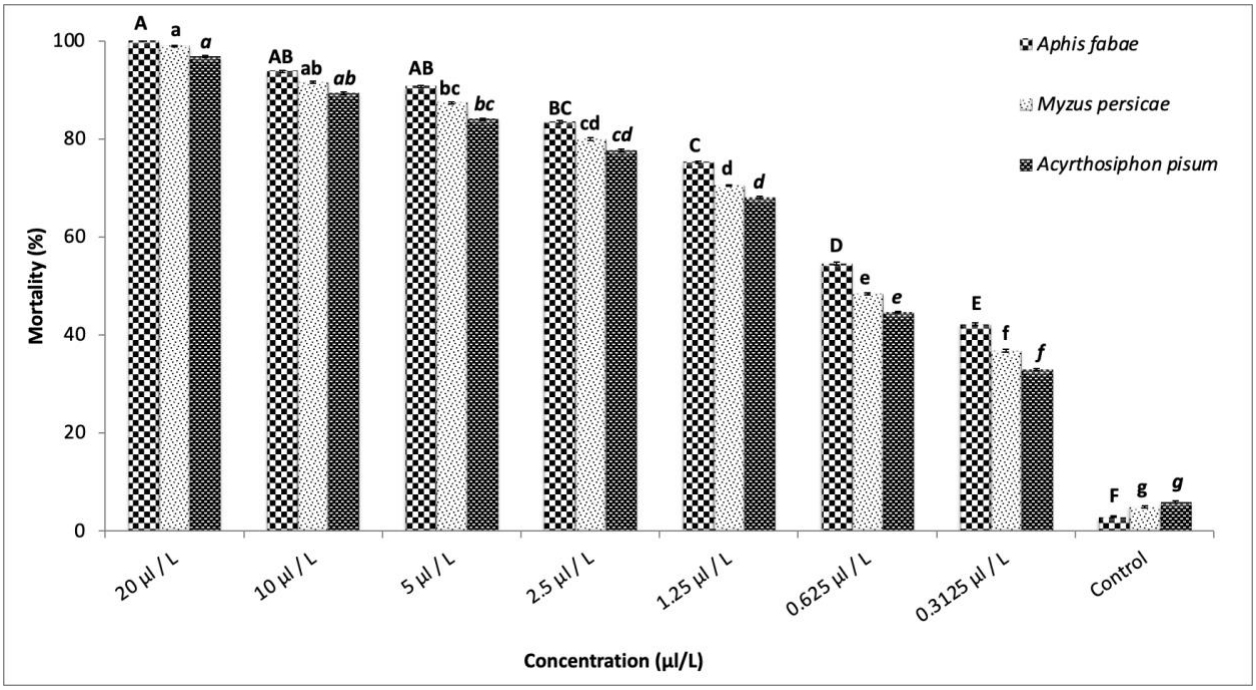

Figure 1. Mortality percentage of aphids (*Aphis fabae*, *Myzus persicae* and *Acyrthosiphon pisum*) exposed to different concentrations of lambda-cyhalothrin for 24 h. Means represented by different letters for each aphid species were significantly different according to Tukey ($F_{A.fabae}$: 187.95; $df_{A.fabae}$: 7, 79; $P_{A.fabae}$: 0.001 / $F_{M.persicae}$: 203.77; $df_{M.persicae}$: 7, 79; $P_{M.persicae}$: 0.001 / $F_{A.pisum}$: 196.93; $df_{A.pisum}$: 7, 79; $P_{A.pisum}$: 0.001).

Table 1. Toxicity of lambda-cyhalothrin on *Aphis fabae*, *Myzus persicae* and *Acyrthosiphon pisum* after 24 h

Aphids	N	Slope±SE[a]	LC_{30} µl L^{-1} (95% Cl)[b]	LC_{40} µl L^{-1} (95% Cl)[b]	LC_{50} µl L^{-1} (95% Cl)[b]	x^2 (*df*)[c]	*P* value
Aphis fabae	800	1.117±0.159	0.156 (0.076-0.320)	0.274 (0.134-0.561)	0.462 (0.226-0.948)	0.999 (5)	0.002
Myzus persicae	800	1.043±0.162	0.202 (0.097-0.421)	0.369 (0.177-0.767)	0.646 (0.310-1.344)	0.995 (5)	0.001
Acyrthosiphon pisum	800	0.991±0.167	0.238 (0.112-0.504)	0.474 (0.211-0.947)	0.806 (0.380-1.709)	0.979 (5)	0.001

[a] Standard error

[b] 95% confidence intervals

[c] Chi-square value (x^2) and degrees of freedom (*df*)

In the second phase of the study, the effects of the lethal doses (LC_{30} and LC_{40}) on the aphids were investigated. It was found that the doses administered caused a prolongation of the preadult period of the aphids. When these results were evaluated for *A. fabae*, it was found that both 0.156 µl L^{-1} (LC_{30}) and 0.274 µl L^{-1} dose (LC_{40}) of the insecticide prolonged the total developmental period ($p<0.05$). When evaluating *M. persicae*, it was found that both doses (0.202 and 0.369 µl L^{-1}) caused an increase in nymphal stages and total development time ($p<0.05$). When the effects of lethal doses (0.238 and 0.474 µl L^{-1}) on *A. pisum* were examined, it was found that the LC_{40} dose in particular increased preadult developmental times ($p<0.05$). When total development times were examined, it was found that time increased and both doses and control treatments were statistically different from each other ($p<0.05$) (Table 2).

It was found that the doses applied in the experiments (LC_{30} and LC_{40}) also had effects on the development time of aphids after adulthood and the number of offspring sired by them. When considering oviposition time, adult longevity and total longevity, it was found that the doses applied were not effective in the periods of *A. fabae* and *A. pisum*. Therefore, oviposition time, adult longevity and total longevity are in the same statistical group at both doses for all the aphid species (P>0.05). When the oviposition time and adult longevity of *M. persicae* were evaluated, it was found that the times obtained with the LC_{40} dose were different from those of the control (p<0.05). While the daily number of offspring and the total number of offspring at all doses (LC_{30} and LC_{40}) were different compared with the control (P<0.05), statistical similarity was observed between the cats of the doses on *M. persicae* and *A. fabae* (P>0.05). In addition to these data, aphid biological parameters and differences between dosages are shown in Table 3.

Table 2. Effects of lambda-cyhalothrin on immature stages of *Aphis fabae*, *Myzus persicae* and *Acyrthosiphon pisum*

***Aphis fabae* / Lambda-cyhalothrin**									
Biological parameters	Control			LC_{30}			LC_{40}		
	N	Days (Mean±SE)		N	Days (Mean±SE)		N	Days (Mean±SE)	
First instar (N1)	60	1.517±0.065	b	50	1.660±0.073	ab	44	1.773±0.072	a
Second instar (N2)	58	1.328±0.062	c	48	1.458±0.079	b	44	1.523±0.083	a
Third instar (N3)	56	1.339±0.069	c	44	1.455±0.083	b	38	1.579±0.090	a
Forth instar (N4)	54	1.759±0.070	c	40	1.925±0.075	b	37	2.054±0.054	a
Total development times	54	6.019±0.120	c	40	6.625±0.159	b	37	7.000±0.155	a
***Myzus persicae* / Lambda-cyhalothrin**									
Biological parameters	Control			LC_{30}			LC_{40}		
	N	Days (Mean±SE)		N	Days (Mean±SE)		N	Days (Mean±SE)	
First instar (N1)	60	1.633±0.063	b	47	1.957±0.074	ab	40	2.000±0.080	a
Second instar (N2)	58	2.690±0.111	b	43	3.070±0.107	ab	39	3.179±0.103	a
Third instar (N3)	56	2.429±0.067	c	41	2.610±0.085	b	36	2.639±0.090	a
Forth instar (N4)	54	2.537±0.129	c	39	2.897±0.121	b	36	3.000±0.120	a
Total development times	54	9.370±0.243	c	39	10.564±0.229	b	36	10.917±0.201	a
***Acyrthosiphon pisum* / Lambda-cyhalothrin**									
Biological parameters	Control			LC_{30}			LC_{40}		
	N	Days (Mean±SE)		N	Days (Mean±SE)		N	Days (Mean±SE)	
First instar (N1)	60	1.767±0.055	c	54	2.019±0.062	b	47	2.213±0.080	a
Second instar (N2)	58	1.259±0.058	c	52	1.462±0.070	b	46	1.544±0.092	a
Third instar (N3)	56	1.446±0.072	c	51	1.686±0.091	b	46	1.848±0.108	a
Forth instar (N4)	54	1.926±0.058	c	50	2.060±0.078	b	42	2.214±0.080	a
Total development times	54	6.500±0.102	c	50	7.260±0.124	b	42	7.881±0.181	a

* Different letters in the same line were significantly different according to Tukey (p<0.05).

It was found that the doses applied in the experiments (LC_{30} and LC_{40}) were effective on the aphid life plates. When the data obtained were examined, it was found that the mean generation time (T_0) and doubling time (T_2) for all aphids increased with the increase in dose. Using these data, it was determined that there was a statistical difference between the times (p<0.05). When the intrinsic rate of increase (r_m) was examined, it was found that the results were different for all three aphids compared to the control. Although the results were close for the doses administered, there was a statistical difference (p<0.05). When the results were examined in detail, the same situation was observed for the values of net reproduction rate (R_0), gross reproduction rate (*GRR*), and finite rate of increase (λ). It was found that there was a statistical difference between the results obtained for all three-aphid species (p<0.05) (Table 4).

Table 3. Effects of lambda-cyhalothrin on biological parameters of *Aphis fabae*, *Myzus persicae* and *Acyrthosiphon pisum*

Aphis fabae									
Biological parameters	Control			LC_{30}			LC_{40}		
	N	Mean±SE		N	Mean±SE		N	Mean±SE	
Oviposition times (Days)	54	18.907±0.563	a	40	20.425±1.513	a	37	20.270±1.374	a
Adult longevity (Days)	54	20.426±0.554	a	40	22.150±1.542	a	37	22.027±1.391	a
Total longevity (Days)	60	24.200±0.996	a	52	22.942±1.924	a	46	24.065±1.885	a
Daily number of offspring	54	2.205±0.041	a	40	1.398±0.030	b	37	1.315±0.025	b
Total number of offspring	54	45.481±1.516	a	40	31.850±2.390	b	37	29.784±2.105	b
Myzus persicae									
Biological parameters	Control			LC_{30}			LC_{40}		
	N	Mean±SE		N	Mean±SE		N	Mean±SE	
Oviposition times (Days)	54	28.037±0.729	a	39	24.872±1.290	ab	40	24.333±1.307	b
Adult longevity (Days)	54	30.963±0.743	a	39	27.564±1.339	ab	39	26.417±1.323	b
Total longevity (Days)	60	36.950±1.467	a	49	31.347±2.211	a	36	34.250±1.925	a
Daily number of offspring	54	1.879±0.029	a	39	1.407±0.025	b	36	1.427±0.030	b
Total number of offspring	54	58.685±1.729	a	39	39.179±2.085	b	36	38.667±2.328	b
Acyrthosiphon pisum									
Biological parameters	Control			LC_{30}			LC_{40}		
	N	Mean±SE		N	Mean±SE		N	Mean±SE	
Oviposition times (Days)	54	18.907±0.563	a	50	18.500±0.528	a	42	18.548±0.573	a
Adult longevity (Days)	54	20.426±0.554	a	50	20.020±0.523	a	42	20.286±0.559	a
Total longevity (Days)	60	24.633±1.019	a	54	25.556±0.984	a	47	25.787±1.142	a
Daily number of offspring	54	2.205±0.041	a	50	1.760±0.022	b	42	1.616±0.029	c
Total number of offspring	54	45.481±1.516	a	50	35.560±1.148	b	42	33.071±1.210	b

* Different letters in the same line were significantly different according to Tukey ($p<0.05$).

Table 4. Effects of lambda-cyhalothrin on life table parameters (Mean±SE) of *Aphis fabae*, *Myzus persicae* and *Acyrthosiphon pisum*

Aphis fabae													
Treatments	N	Intrinsic rate of increase, r_m		Net reproduction rate, R_0		Mean generation time, T_0		Gross reproduction rate, ***GRR***		Doubling time, T_2		Finite rate of increase, λ	
Control	60	0.378±0.0002	a	45.289±0.041	a	10.095±0.003	c	60.343±0.030	a	1.835±0.0007	c	1.459±0.0002	a
LC_{30}	52	0.243±0.0003	b	25.810±0.055	b	13.395±0.014	b	51.390±0.022	b	2.856±0.0038	b	1.275±0.0004	b
LC_{40}	46	0.238±0.0007	c	25.581±0.066	c	13.598±0.033	a	48.952±0.041	c	2.908±0.0085	a	1.269±0.0008	c
Myzus persicae													
Treatments	N	Intrinsic rate of increase, r_m		Net reproduction rate, R_0		Mean generation time, T_0		Gross reproduction rate, ***GRR***		Doubling time, T_2		Finite rate of increase, λ	
Control	60	0.296±0.0002	a	59.922±0.053	a	13.847±0.009	c	74.788±0.031	a	2.345±0.002	c	1.344±0.0003	a
LC_{30}	49	0.202±0.0003	b	36.481±0.080	b	17.531±0.026	b	53.877±0.033	b	3.440±0.006	b	1.223±0.0004	b
LC_{40}	40	0.184±0.0002	c	34.196±0.067	c	19.588±0.015	a	53.278±0.037	c	3.775±0.004	a	1.202±0.0002	c
Acyrthosiphon pisum													
Treatments	N	Intrinsic rate of increase, r_m		Net reproduction rate, R_0		Mean generation time, T_0		Gross reproduction rate, ***GRR***		Doubling time, T_2		Finite rate of increase, λ	
Control	60	0.352±0.0012	a	44.531±0.066	a	10.806±0.040	c	60.240±0.065	a	1.973±0.008	c	1.421±0.002	a
LC_{30}	54	0.248±0.0001	b	33.767±0.032	b	14.219±0.003	b	45.160±0.033	b	2.800±0.001	b	1.281±0.001	b
LC_{40}	47	0.235±0.0001	c	31.049±0.042	c	14.620±0.006	a	41.845±0.027	c	2.950±0.002	a	1.265±0.001	c

* Different letters in the same parameters and columns were significantly different according to Tukey ($p<0.05$).

In this study, survival rate (l_x), fecundity (m_x, l_x*m_x), reproductive value (V_x) and expected lifetime (E_x), which are the most important parameters of life tables, were also calculated. These values were calculated and evaluated separately for each species (*A. fabae*, *M. persicae* and *A. pisum*) and for each concentration (Control, LC_{30} and LC_{40}). According to the data obtained, it was determined that the survival rates (l_x) of *A. fabae* and *M. persicae* at sublethal concentrations were longer than the control. However, the opposite was observed in *A. pisum*. According to the results, while the survival rate (l_x) of *A. fabae* showed a high decrease as of the 25th day in the control group, it was determined that this decrease started on the 5th day in sublethal concentrations of lambda-cyhalothrin (LC_{30} and LC_{40}) and decreased continuously in the following days (Figure 2). Survival rate (l_x) showed a high decrease in the control group of *M. persicae* starting on the 35th day, while this decrease at sublethal concentrations of lambda-cyhalothrin (LC_{30} and LC_{40}) started onthe 3rd day, which was yet followed by an increase in the following days (Figure 3). The same value (l_x) showed a high decrease as of the 25th day in the control group of *A. pisum*. However, this decreases at sublethal concentrations of lambda-cyhalothrin (LC_{30} and LC_{40}) started on day 5 and continued thereafter (Figure 4). It was determined that expected lifetime increased for both species at sublethal concentrations, and was shortened for *A. pisum* at these concentrations. It was determined that the fecundity (m_x, l_x*m_x) and reproductive values (V_x) of the applied concentrations (LC_{30} and LC_{40}) decreased compared to the control. It was observed that the data obtained are close when the concentrations were evaluated among themselves (Figures 2, 3, 4).

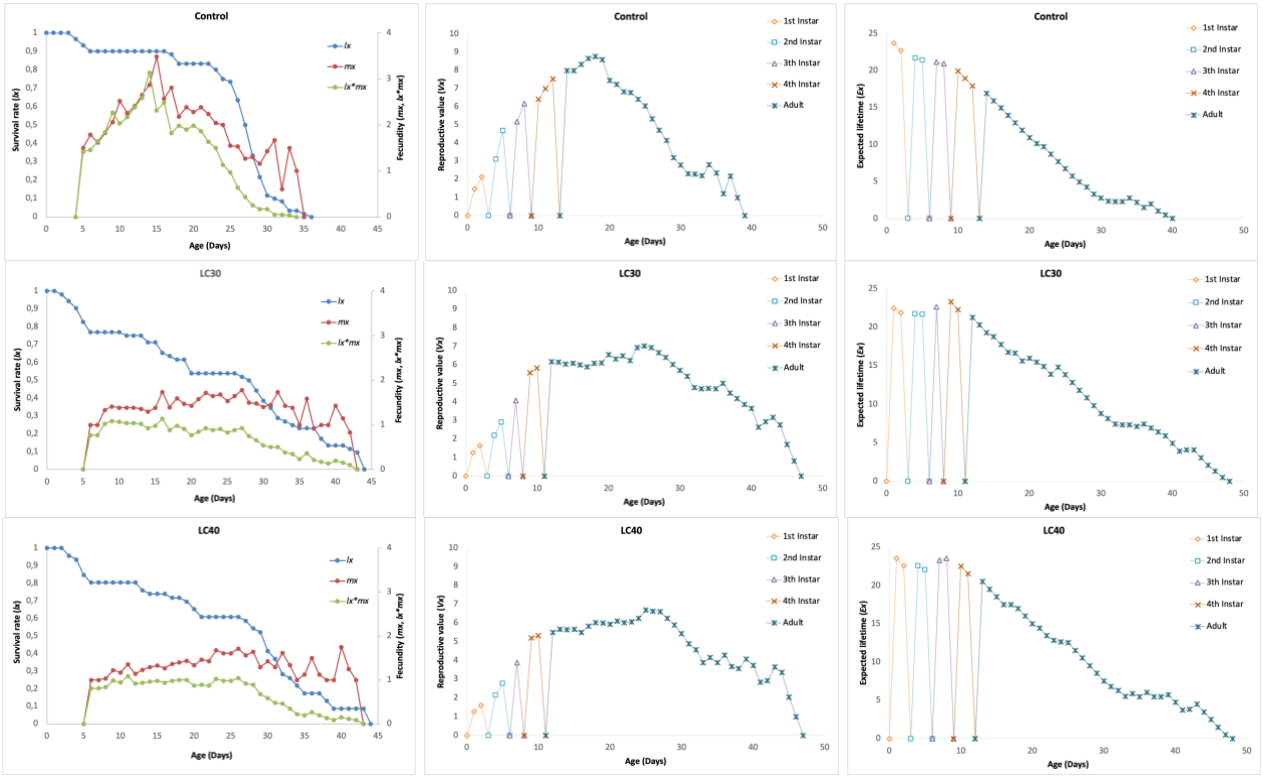

Figure 2. Survival rate (l_x), Fecundity (m_x, l_x*m_x), Reproductive value (V_x) and Expected lifetime (E_x) of *Aphis fabae* exposed to different concentrations (0.156 µl L^{-1} and 0.274 µl L^{-1}) of lambda-cyhalothrin.

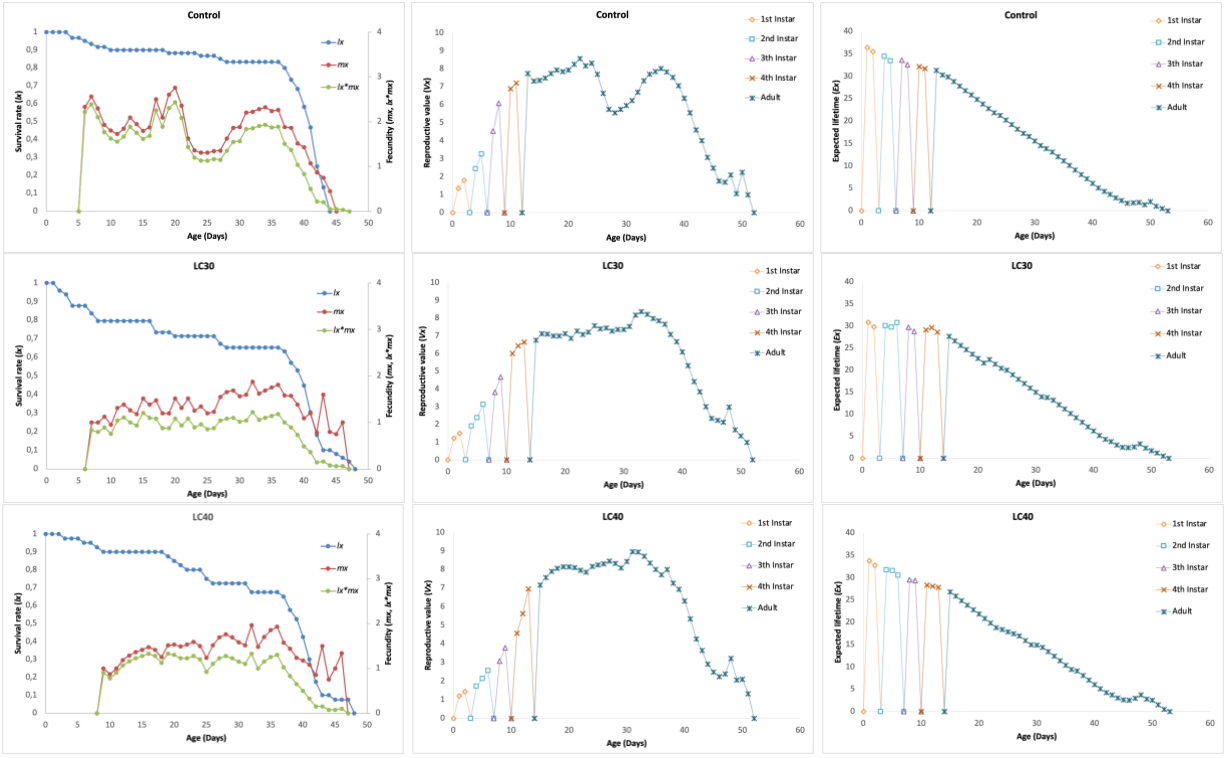

Figure 3. Survival rate (l_x), Fecundity (m_x, l_x*m_x), Reproductive value (V_x) and Expected lifetime (E_x) of *Myzus persicae* exposed to different concentrations (0.202 µl L^{-1} and 0.369 µl L^{-1}) of lambda-cyhalothrin.

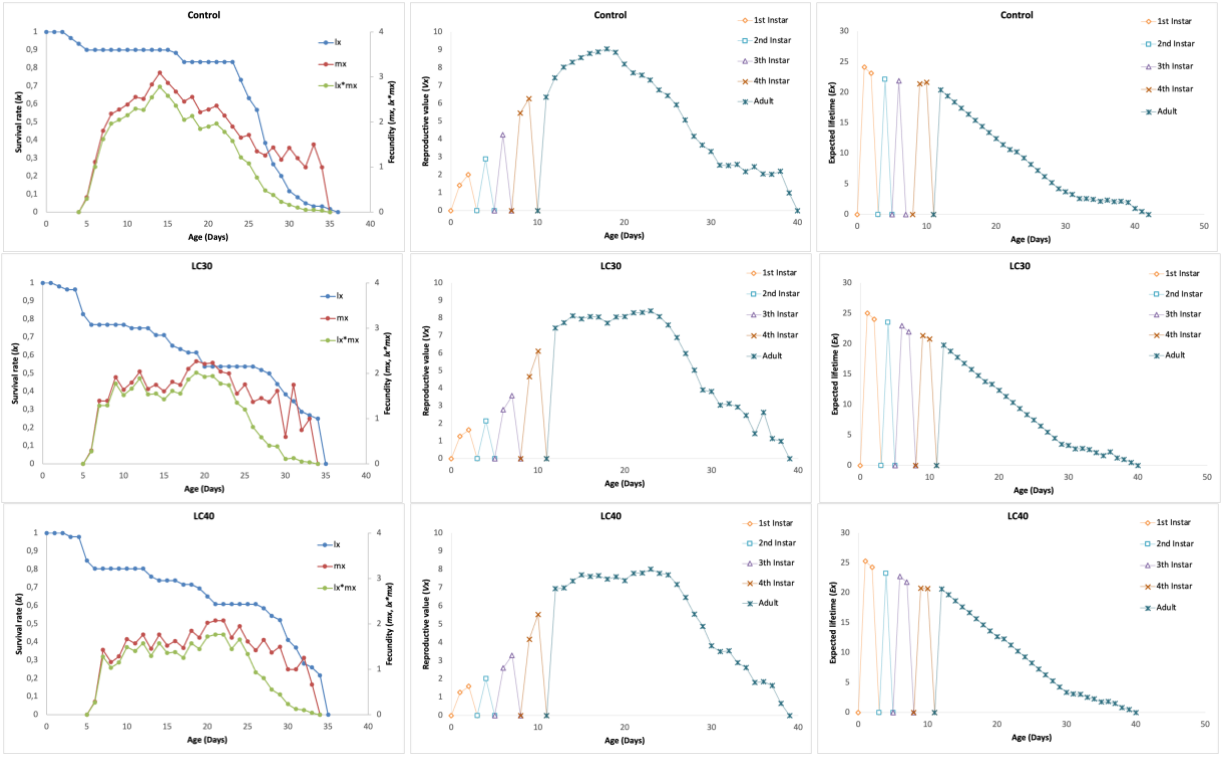

Figure 4. Survival rate (l_x), Fecundity (m_x, l_x*m_x), Reproductive value (V_x) and Expected lifetime (E_x) of *Acyrthosiphon pisum* exposed to different concentrations (0.238 µl L^{-1} and 0.474 µl L^{-1}) of lambda-cyhalothrin.

Discussion

In this study, reductions in pre- and post-emergence development times were observed in three different aphids exposed to two low-to-medium doses of lambda-cyhalothrin (LC_{30} and LC_{40}). Compared to the control group, it was found that the number of aphid progeny and related life table parameters were reduced. The full evaluation of the obtained data suggests that the application of doses below the mean dose may have effects on the physiological characteristics of aphids.

One of the most important factors in stimulating insect reproduction is the pesticides used in control. This excitation occurs when insects are exposed to these chemicals (Catae et al., 2017). This also occurs as a result of long-term control processes with the same chemical (Wang et al., 2017). The data analysis in our study revealed that all three aphid species (*A. fabae*, *M. persicae* and *A. pisum*) were affected by the chemical used and the reproduction rate decreased. For this reason, it is reasonable to use insecticides against these pests in crop rotation to prevent the species from developing resistance (Ayyanath et al., 2013; Wang et al., 2017). Some studies have shown that pyrethroid-based compounds affect reproduction of various insects and arthropods and the number of eggs/offspring laid decreases (Kidd et al., 1996; Kerns & Stewart, 2000; Fujiwara et al., 2002; Wang et al., 2008; Quan et al., 2016; Zuo et al., 2016). Ayyanath et al. (2013) reported that reproductive stimulation in insects can occur in different populations and different generations. In contrast, in our study, a decrease in reproduction was observed in all three aphids. To better understand the situation described by the researchers in *A. fabae*, *M. persicae* and *A. pisum*, it would be beneficial to conduct a similar study both in different generations and under field conditions. Ayyanath et al. (2013) and Wang et al. (2017) reported in their studies that low doses of an insecticide can prolong nymph/larval development and adult life span, thus reducing reproduction. They reported that this situation was caused by the introduction of insecticide into the embryo. In our study, these conditions were observed when low concentrations of lambda-cyhalothrin were applied. Therefore, it is reasonable to conduct molecular studies to transfer the adverse effects that occur when low doses of lambda-cyhalothrin are applied to aphids (*A. fabae*, *M. persicae* and *A. pisum*).

Low doses of lambda-cyhalothrin (LC_{30} and LC_{40}) used in our study, as with other insecticides, affected both the biological and physiological characteristics of the insects in our experiments. In our study, the application of lambda-cyhalothrin to aphids significantly decreased the intrinsic rate of increase (r_m), net reproduction rate (R_0), and gross reproduction rate (*GRR*) of all three aphids compared to the control. For this reason, it is suspected that the chemical used could have a negative impact on the next generations. However, as mentioned earlier, it would be beneficial to repeat a similar study both under field conditions and on different populations. There are several studies on the effects of insecticide trials on insect life cycle parameters. When examining the studies on pyrethroid-based insecticides, similar results were obtained (Kerns & Stewards, 2000; Whalen et al., 2012; Song et al., 2013; Zuo et al., 2016; Mahmoodi et al., 2020; Qu et al., 2020; Tan et al., 2021; O'Hara et al., 2022). As can be seen from the results obtained, when lambda-cyhalothrin is applied to *A. fabae*, *M. persicae* and *A. pisum* in the laboratory, development is delayed and the number of progeny decreases. This suppresses subsequent population growth in all three species. However, some researchers have reported that low doses of pyrethroid-based insecticides can have a beneficial effect on insect populations under field/greenhouse conditions (Kidd et al., 1996; Wang et al., 2008; Piiroinen et al., 2014). For this reason, it is advantageous to apply lambda-cyhalothrin, which has been tested in the laboratory on three different aphids, under field conditions.

It is unclear whether lambda-cyhalothrin has a typical effect in some aphid populations (Valmorbida et al., 2020). Similar results were obtained on different aphid species. It has been stated that the effects on the longevity and fecundity of aphids, especially for the first generations, vary according to the species and insecticide combination. For example, the adult longevity and fecundity of *M. persicae*, which was exposed to LC_{25} dose of flupyradifurone, decreased significantly (Heidel-Fischer & Vogel, 2015). In addition, there was no difference in lifespan and fertility when *A. gossypii* was exposed to LC_{25} of flupyradifurone (Liang et al., 2018) and sulfoxaflor (Chen et al., 2016). It has been reported that there was no difference in longevity

in *A. gossypii* exposed to LC_{10} and LC_{50} doses of Nitenpyram, while fecundity was greatly reduced (Wang et al., 2017). In our study, there was no difference in adult and total longevity of *A. fabae* and *A. pisum* exposed to different concentrations of lambda-cyhalothrin (LC_{30} and LC_{40}) compared to the control. However, this situation differed in *M. persicae*. It was determined that this species had a lower adult and total longevity compared to the control at these doses. The effects of sublethal concentrations of lambda-cyhalothrin used differed. In addition, it was determined that fecundity and reproductive value decreased at sublethal concentrations.

The results show that the application of low concentrations of lambda-cyhalothrin to aphids (*A. fabae*, *M. persicae* and *A. pisum*) prolongs growth times and reduces fecundity. In addition, the use of this chemical on agricultural commodities is thought to have an effect not only at lethal concentrations but also at lower concentrations (Ayyanath et al., 2013; Wang et al., 2017). The emergence of insecticide-induced resistance in agricultural pests can cause distressing situations. Therefore, the emergence of resistance means that pests increase their populations in the environment and become even more harmful (Guedes et al., 2017). At the same time, it is seen that the sensitivity of pests exposed to low concentrations (sublethal doses) of insecticides decreases (Gressel, 2011). In addition, it has been stated that insects can tolerate different stress conditions (resistant host plant) indirectly in case of exposure to sublethal concentrations (Brevik et al., 2018). Valmorbida et al. (2020) reported that *A. glycines*, which are exposed to low concentrations of lambda-cyhalothrin and fed under stress conditions, may have an advantage over individuals not under stress conditions. In our study, it was determined that low concentrations of lambda-cyhalothrin had negative effects (especially on their reproductive potential) on three different aphids. Based on the data obtained here, it would be beneficial to replicate the study under different environmental and stress conditions. As is well known, resistance problems arise from intensive use of chemicals for insect control. For example, the active ingredient that we consider effective in this study may become ineffective in the future due to resistance. For this reason, it is beneficial to use different pyrethroids or different insecticides in rotation at different times, especially under field conditions. This study was conducted to determine the effects of lambda-cyhalothrin on *A. fabae*, *M. persicae* and *A. pisum* and will benefit future studies.

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Original article (Orijinal araştırma)

Isolation and identification of a fungal pathogen, *Beauveria bassiana* (Bals.-Criv.) Vuill. (Ascomycota: Hypocreales) from the Hatay yellow strain of silkworm, *Bombyx mori* L., 1758 (Lepidoptera: Bombycidae) in Türkiye[1]

Türkiye'den Hatay sarısı ipekböceği ırkı, *Bombyx mori* L., 1758 (Lepidoptera: Bombycidae)'den bir fungal patojeni olan *Beauveria bassiana* (Bals.-Criv.) Vuill. (Ascomycota: Hypocreales)'nın izolasyonu ve tanımlanması

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Abstract

The Hatay yellow strain of silkworm, *Bombyx mori* L., 1758 (Lepidoptera: Bombycidae) which produces cocoons with an extraordinary yellow color range, is one of the most important endemic and endangered cultural assets in Türkiye. In this study, intense fungal infection and many deaths were detected in the breeding trays in the Hatay yellow breed production facility. An entomopathogenic fungus was isolated from Hatay yellow strain cadavers collected in 2020. According to the morphological and molecular analysis results of the isolate, which was brought into pure culture, it was identified as *Beauveria bassiana* (Bals.-Criv.) Vuill. (Ascomycota: Hypocreales), the isolate HS1. Phylogenetic analysis results showed that the HS1 strain was very similar (99 %) to the isolates of *B. bassiana* KJ6 (Iran) and ARSEF 300 (Europe). The concentration-response test using $1 \times 10^{4-8}$ conidia/ml concentrations produced LC_{50} values of the new strain of 1.2×10^3 and 0.6×10^6 conidia/ml within 7 days against the larvae of Hatay yellow strain and hybrid strain of silkworm, respectively. The results indicated that the virulence of the *B. bassiana* HS1 strain to the Hatay yellow strain was much more severe and that the Hatay yellow strain had to fight it to survive.

Keywords: *Beauveria bassiana*, *Bombyx mori*, entomopathogenic fungi, Hatay yellow strain, silkworm

Öz

Olağanüstü sarı renk dağılımına sahip kozalar üreten Hatay sarı ipekböceği ırkı olan *Bombyx mori* L., 1758 (Lepidoptera: Bombycidae), Türkiye'nin en önemli endemik ve tehlike altındaki kültür varlıklarından biridir. Bu çalışmada, Hatay sarı ırkı üretim tesisinde, yetiştirme tepsilerinde yoğun fungal enfeksiyonu ve birçok ölüm tespit edilmiştir. Entomopatojen bir fungus 2020 yılında toplanan Hatay sarısı ırkı kadavralarından izole edilmiştir. Saf kültür haline getirilen izolat morfolojik ve moleküler analiz sonuçlarına göre *Beauveria bassiana* (Bals.-Criv.) Vuill. (Ascomycota: Hypocreales) türüne ait HS1 izolatı olarak tanımlanmıştır. Filogenetik analiz sonuçları, HS1 izolatının *B. bassiana* KJ6 (İran) ve ARSEF 300 (Avrupa) izolatlarına çok benzer (%99) olduğunu göstermiştir. Konsantrasyon-doz testi $1 \times 10^{4-8}$ konidia/ml konsantrasyonları kullanılarak yapılmış ve yeni izolatın 7 günlük LC_{50} değerleri Hatay sarısı ırkı ve hibrid ırk ipekböceği larvalarına karşı sırasıyla 1.2×10^3 ve 0.6×10^6 konidia/ml olarak belirlenmiştir. Sonuçlar, *B. bassiana* HS1 izolatının Hatay sarı ırkına verdiği hasarın çok daha şiddetli olduğunu ve Hatay sarı ırkının hayatta kalabilmek için onunla mücadele etmek zorunda kaldığını göstermiştir.

Anahtar sözcükler: *Beauveria bassiana*, *Bombyx mori*, entomopatojen fungus, Hatay sarı ırkı, ipekböceği

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Introduction

Fungi are unicellular or multicellular eukaryotic organisms. They are found in almost all habitats, but most of them live on land, especially on soil, plant and animal material, rather than in water. A group called decomposers grow in soil or on dead biological material, where they play an important role in the cycling of carbon and other elements. Some of them are plant pathogens because they cause diseases such as mold, rust, scab, or thrush, which cause significant economic losses in agricultural production. Some of them cause significant diseases and parasitic infestations in animals, including humans. The group known as entomopathogens is one of the most important groups of arthropod infectious agents (Kocacevik et al., 2015; Sönmez et al., 2016, 2017; Biryol et al., 2020). These entomopathogenic fungi, which play a beneficial role in the control of agricultural and forestry pests (Biryol et al., 2021), are a major problem for beneficial insects. Although they have a beneficial role within populations, they cause severe epidemics leading to a sudden and rapid collapse of colonies (Wada et al., 2011; Vojvodic et al., 2012).

Bombyx mori L., 1758 (Lepidoptera: Bombycidae), the silkworm, is one of the best-known beneficial insects. It is an economically important insect as it is a major producer of silk. Silkworm breeding began in China at least 5000 years ago and spread to India, Korea, Nepal, Japan, and the West (Arunkumar et al., 2006). The domestic silk moth used in raw silk production was domesticated from the wild silk moth Bombyx mandarina, which was distributed from northern India to northern China, Korea, Japan, and even the far eastern parts of Russia (Maekawa et al., 1988; Adams & Barber, 1996). The domesticated silk moth originated in China, not Japan or Korea. As a result of thousands of years of selective breeding, it is entirely dependent on humans for its reproduction. Silk fiber is one of the indispensable textile fibers with high added value and its value and importance is increasing today as it has throughout the history. Silk fiber production is growing in parallel to meet the demand. Moreover, farmers face many problems due to the contamination of silkworms with various microbial diseases (Mishra, 2017; Sharma et al., 2020; Chopade et al., 2021). One of the most virulent microorganisms affecting *B. mori* is entomopathogenic fungi, which spread rapidly among individuals in the population and cause mass mortality (Saad et al., 2019). Various fungal diseases called muskardin, with types such as white, green and yellow, have been reported in silkworm (Mishra, 2017). Wada et al. (2010) revealed that some silkworm strains are extremely sensitive to various *Beauveria* spp. Vuill. (Ascomycota: Hypocreales) and strains. A study of commercial silkworm hybrids showed that a strain of *Metarhizium anisopliae* (Metschn.) Sorokīn (Ascomycota: Hypocreales) is an important pathogen of the silkworm (Ribeiro et al., 2017). Although governments and different organizations organize different programs and provide support to inform farmers and control these diseases, crop losses are not yet controlled as expected. The Hatay yellow strain, a privilege of Türkiye and one of the three native breeds in our country was domesticated as an insect about 5000 years ago (Ulaşlı et al., 2021). In sericulture, the only hybrid strain that weaves white cocoons has been cultivated in our country for many years. While the hybrid breed that produces only white cocoons has been widely used in our country for many years, the local breed of Hatay, the Hatay yellow strain, has not been produced for almost 50 years (İleri, 2019). Studies on the strain, which has a very high value as a biocultural heritage in our country, focus on the production of fibers naturally occurring in different shades of yellow. These fibers are in high economic demand worldwide, but research on them is limited. In a study, Ulaşlı et al. (2021) investigated some morphological and biological characteristics of the Hatay yellow strain, which is in danger of extinction. There is no study in the literature on fungal pathogens of the Hatay yellow strain, which is exceptionally susceptible compared to hybrids. The identification of fungal pathogens is important for survival of the Hatay yellow strain, which is our cultural heritage and is in danger of extinction. In this study, the entomopathogenic fungi of the Hatay yellow strain were investigated for the first time. The fungi isolated from the cadavers were identified, and their lethal effects on the Hatay yellow strain and hybrid strain were determined.

Materials and Method

Silkworm strains

Both silkworm strains were obtained from Defne Apollon İpekçilik (Harbiye Mah. Atatürk Cad. No:17 Defne, Hatay, Türkiye), a silkworm breeder operating as a family business in Hatay, Türkiye, in 2020. The Hatay yellow strain was collected as cadavers from the production tables and taken to the laboratory in sterile tubes. Cadavers showing signs of fungal infection were placed in a humid chamber. The contaminated cadavers were used for fungal isolation. Healthy silkworms of the Hatay yellow strain and hybrid strain were used for the insecticidal activity study.

Isolation of fungal strain

Fungi were isolated from the infected larvae of the Hatay yellow strain and transferred to artificial media (Sabouraud CAF agar, Liofilchem s.r.l., Italy) containing 40 µg/ml chloramphenicol to prevent bacterial contamination. Cultures were incubated at 26-28°C for 1-2 weeks to promote growth and sporulation. Pure cultures were maintained on CAF agar media and subcultured monthly. Strains were stored at -20°C in glycerol for long-term storage. Only one of the fungal strains obtained from the cadavers of mycosis larvae was cultured as an entomopathogen, named HS1, and used for bioassay studies after its identification.

Morphological and molecular identification

The appearance of fungal infection of fungal isolates on larvae and adults, colony morphology, spore size, and spore shape on CAF agar were used for initial identification. Fungal spores and mycelial structures were measured using a phase contrast microscope (Nicon, Exlipse Ni) and morphological identification was done according to Humber (2012).

Partial sequencing of the ITS1-5.8S-ITS2 gene region between the 18 S and 28 S rRNA subunits was performed to confirm the identity of the isolates. The partial sequence of the ITS1-5.8S-ITS2 gene region of the new strain was amplified by polymerase chain reaction (PCR) using primers ITS5 (5'-GGA AGTAAAAGTCGTAACAAGG-3)' and ITS4 (5'-TCCTCCGCTTATTGATATGC-3') (White et al., 1990). PCR reactions and gel imaging were performed as described in Gencer (2023). Sequencing of the amplicons was performed by MACROGEN sequencing service, Amsterdam. Sequences were compared with sequences in the NCBI (Anonymous, 2023). The sequences were assembled and edited with BioEdit version 7.0.5.3 (Hall, 1999). Multiple sequence alignment was performed, and phylogenetic trees for the ITS1-5.8S-ITS2 genes were constructed using MEGA software, version 7.0.26 (Tamura et al., 2013; Kumar et al., 2018) and phylogenetic analysis was performed to compare them to similar species (Benson et al., 2013). Finally, the sequences were compared to representative sequences described by Rehner & Buckley (2005) to determine the taxonomic position of the new strain within *Beauveria*.

Insecticidal activity tests

Preparation of conidiospore

The conidial suspension of the new strain was harvested by adding 10 ml of sterile water amended with 0.1% Tween 80 to the 4-week-old culture. The conidiospore-containing mixture was filtered through three layers of cheesecloth into sterile 15 ml Falcon tubes and vortexed, and the spores were counted using a hemocytometer. The final concentration of conidial suspensions was calculated and serially diluted in 0.01% Tween 80 from 1×10^9 to 1×10^3 conidia/ml. Fungal spores with viability greater than 95% of conidia were used for the bioassay, which was determined after 24 hours of incubation on CAF agar medium at 25°C.

Concentration-response test

Concentration-response assays were performed with the *Beauveria bassiana (*Bals.-Criv.) Vuill. (Ascomycota: Hypocreales) HS1 strain on both silkworm strains under laboratory conditions. Thirty 3rd instar larvae of both silkworm strains in each concentration and control group were used for the bioassay. The larva was placed in plastic boxes with mulberry leaves, and the conidial suspension was applied separately for each dilution using a mini hand sprayer. The control groups were sprayed water containing 0.01% Tween 80 only. Boxes were then incubated for 7 days in a climate chamber at 28°C, 60% RH, and a photoperiod of 12:12 (L:D). Boxes in the bioassay setup were checked daily and dead larvae were collected. Collected dead larvae were placed in a humid chamber to induce fungal sporulation. Mortality data were corrected for individuals with mycosis using the Schneider-Orelli formula (Püntener, 1981). The LC_{50} of the new strain was determined using probit analysis on MS Excel (Finney, 1971). The bioassay was repeated three times and the experiment was repeated three times on different days.

Results

Fungi were isolated from the larval cadavers of the Hatay yellow strain brought to the laboratory. Based on the morphological images of the isolated fungal isolates on agar plates, one of the strains (HS1) was determined to be a new entomopathogenic fungal isolate. The isolate was morphologically defined according to conidia shape, conidia size, colony morphology and color, and symptoms in cadavers (Figure 1).

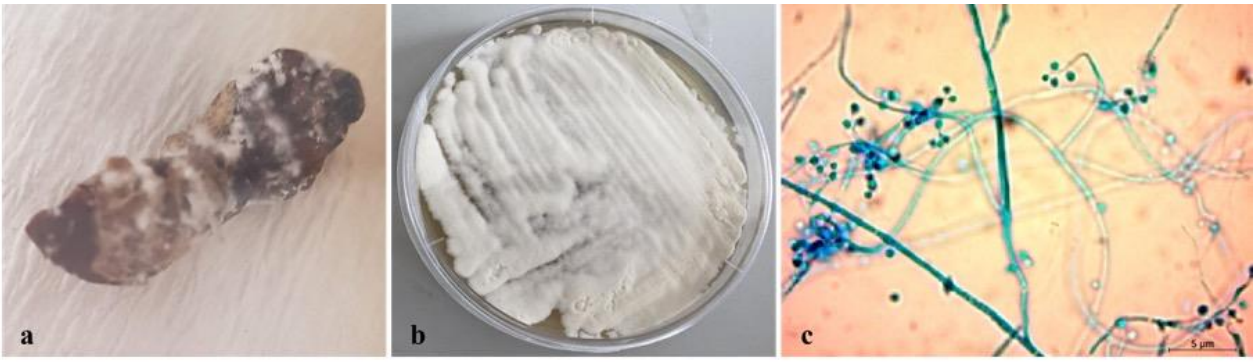

Figure 1. Macroscopy and microscopy of *Beauveria bassiana* HS1 strain. a) A larval cadaver showing mycosis naturally infected by the new strain. b) Macroscopic image of the HS1 strain on CAF. c) Mycelial and spore structures of the HS1 strain.

The new strain was morphologically identified as *B. bassiana*. Genomic analysis of the ITS1-5.8S-ITS2 gene region also revealed that the new strain was identical to *B. bassiana* (Figure 2). The HS1 strain was found to be very similar to *B. bassiana* strains KJ6 (Iran) and ARSEF 300 (Europe) in the Genbank database.

The new strain was found to be highly pathogenic on the larvae of the Hatay yellow strain (Figure 3). The mortality rates of the HS1 strain differed from those of the control groups within 7 days of application. The lowest concentration (1×10^4 conidia/ml) killed 60% of the Hatay yellow strain larvae used in the study. It was found that as the concentration increased, the mortality of the Hatay yellow strain larvae increased, and a concentration of $1 \times 10^{6-8}$ conidia/ml resulted in 100% death of the insect.

After the dose-response test, the LC_{50} value of the new fungal strain was calculated to be 1.2×10^3 conidia/ml within 7 days against the larvae of the Hatay yellow strain under laboratory conditions (Table 1). The presence of mycosis in all cadavers indicated that the deaths were due to fungal infection (Figure 4).

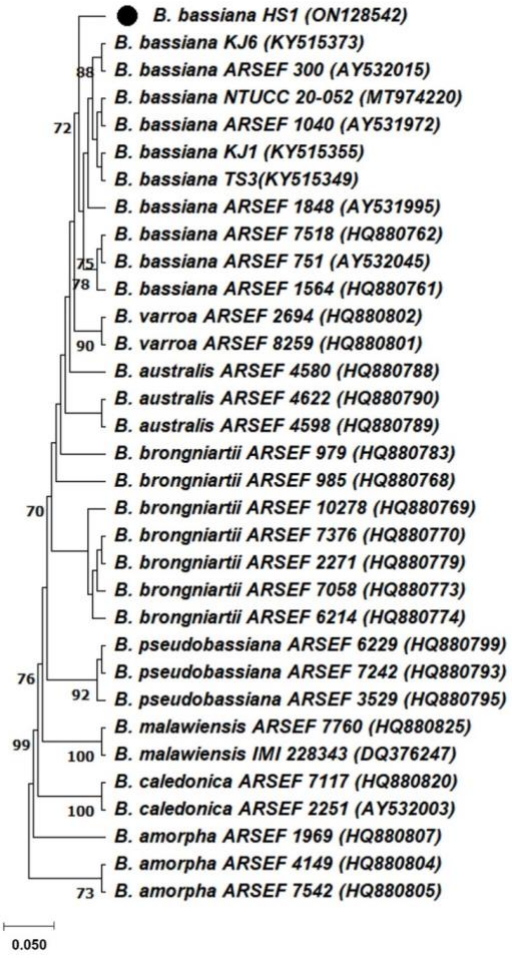

Figure 2. Neighbour-joining tree of the *Beauveria bassiana* HS1 strain and closely related fungal species based on the sequence of ITS1-5.8S-ITS2 gene region. The numbers at the nodes are bootstrap percentages based on 1000 replicates.

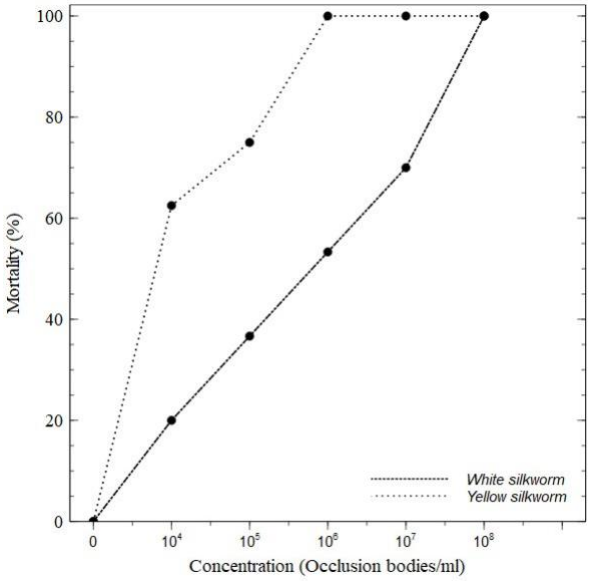

Figure 3. The insecticidal effect of the HS1 strain on *B.mori* Hatay yellow silkworm and *B.mori (*white silkworm) larvae within 7 days.

Table 1. Median lethal concentration (LC_{50}) of *B. mori* larvae exposed to five concentrations of *Beauveria bassiana* HS1 strain

Isolate HS1	N	LC_{50} (conidia/ml)	Intercept	Slope±SE	df	χ^2	95% Cl	
							Lower bound	Upper bound
B. mori (Hatay yellow silkworm)	90	1.2×10^3	3.895	0.356±0.616	3	0.548	0.07	20.4
B. mori (white silkworm)	90	0.6×10^6	2.369	0.452±0.421	3	0.764	0.09	4.3

N: number of individuals; SE: Standard Error; df: degrees of freedom; χ^2: chi-squared test; Cl: confidential limits.

Figure 4. Mycoses cadavers (Hatay yellow silkworm) at the end of the infection.

The experiments with the insecticidal activity of the white strain indicated that it was more tolerant than the Hatay yellow strain. While the lowest concentration (1×10^4 conidia/ml) caused 20% mortality, the concentrations of 1×10^5, 10^6, and 10^7 conidia/ml caused 39%, 53.33%, and 70% mortality, respectively, on the white strain. The concentration of 1×10^8 conidia/ml caused 100% mortality of the *B. bassiana* HS1 strain on the white strain of silkworm. After the concentration-response test, the LC_{50} value of the new strain with 0.6×10^6 conidia/ml was calculated within 7 days against the larvae of the white silkworm strain under laboratory conditions (Table 1).

Discussion

Fungi are one of the most important pathogens infecting insects and cause their death (Lacey et al., 2001). The strain of *B. bassiana* is obtained from cadavers that form a dense white coating on the exoskeleton, grow on agar as a white mould. The conidiogenous cells of *B. bassiana* are short and ovoid and terminate in a narrow apical process called a rachis (Figure 2). Conidiogenous cells with swollen bases and tooth-shaped, apically extending rachis with a conidium formed sequentially on each tooth are *B. bassiana*-specific morphological images under the microscope. All symptoms appeared to be highly compatible with *B. bassiana* infection in the cadavers of the Hatay yellow strain. The symptoms on cadavers, the growth characteristics on agar, and the microscopic images indicated that the pathogen was a new isolate of *B. bassiana*. This is the first detection of white muscardine in the Hatay yellow strain. White muscardine is one of the most common and best-studied fungal diseases of hybrid strain that weaves white cocoon. It causes significant problems and death of many insects during the larval and pupal periods, especially in cold and rainy seasons (Kumar et al., 1990; Lu, 1991). Symptoms such as larval inactivation, cessation of feeding, vomiting, and loss of larval elasticity were also observed in larvae of the Hatay yellow strain, which is the material of the present study. To confirm the morphological identification of the new strain, a fragment of the ITS1-5.8S-ITS gene region was sequenced. This sequence was compared with

representative sequences from the study by Rehner & Buckley (2005) and Rehner et al. (2011). Based on the dendrogram generated using ITS, the new strain was phylogenetically very close to *Beauveria* species and was included in the *Beauveria* cluster (Figure 2). In addition to recent interest in the genetic diversity and molecular ecology of *Beauveria* in relation to its role as a pathogen of insects in natural and agricultural environments, the genus has been under critical taxonomic review for several decades (Rehner et al., 2011). Specifically, *B. bassiana* includes an as yet undetermined number of cryptic lineages, many of which have an intercontinental distribution and occur as multispecies assemblages in both natural and agricultural habitats (Rehner et al., 2006; Meyling et al., 2009). In addition to the morphological data, molecular analyses and phylogenetic studies have shown, in great agreement with the literature, that the new fungus is a new strain of *B. bassiana*.

Entomopathogenic fungi are known to cause very severe infections in beneficial insects. One of the most important examples is the fungal diseases in bees caused by entomopathogenic fungi (Campano et al., 1999). One of the most striking examples of fungal infections in beneficial insects is silkworms. The first fungal infection in insects and even arthropods was white muscarine disease in silkworms caused by *B. bassiana*, described by Agostino Bassi (Bassi, 1835; Ainsworth, 1956). In a study examining the effects of *B. bassiana* on larval development and cocoon production of *Bombyx mori*, Seema et al. (2019) found significant reductions in mature larval weight, cocoon weight, shell weight, shell ratio, filament length, and unbreakable filament length as a result of fungal infection. In the current study, the new strain (HS1) isolated from the Hatay yellow strain and identified as *B. bassiana* also had a highly lethal effect on its host, which is very consistent with all the literature. All this shows that *B. bassiana* strains are a very important fungal species for many pests from different orders as well as for Lepidoptera.

The morphological appearance of the new strain of *B. bassiana* from the Hatay yellow strain that we discovered in the current study and its effect on larvae and cadavers are in excellent agreement with the isolates determined in other studies. Although there are many studies investigating the effects of *B. bassiana* strains isolated from white silkworms on their hosts and ways to control this pathogen, the fungal pathogens of the Hatay yellow strain have been neglected to date. Thus, the present study is the only one that demonstrates the virulence of a pathogenic fungus isolated from the Hatay yellow strain on its host. Virulence studies on larvae of the Hatay yellow strain showed that the fungal pathogen is highly effective on its host. Pathogenicity studies conducted with the white strain under the same conditions and concentration showed that the white strain was more resistant than the Hatay yellow strain. In one study, Lee et al. (1989) showed that the Chinese commercial silkworm strain was more resistant to *B. bassiana* than the Japanese commercial strain. A study comparing the susceptibility of three Indian commercial silkworm strains to *B. bassiana* showed significant differences in the LC_{50} value of the fungus on the insect (Lakshmi et al., 2005). In another study, Wada et al. (2011) investigated the susceptibility of twenty-two silkworm strains to *Beauveria brongniartii* (Saccc.) Petch (Ascomycota: Hypocreales), an isolate of an entomopathogenic fungus. They showed that the difference in susceptibility was in varying degrees between resistance and sensitivity. In the present study, the LC_{50} of a *B. bassiana* strain (HS1) isolated from the Hatay yellow strain was determined to be 1.2×10^3 spores/ml and 0.6×10^6 spores/ml on the third instar larvae of the Hatay yellow strain and the hybrid strain, respectively. This represents a very large difference in susceptibility of 500-fold between the two strains. Comparing this value with the susceptibility level between different silkworm breeds, it is clear that this value is extremely high. This is because in previous studies this value was found to be 20-fold (Lee et al., 1989) and 7-fold (Lakshmi et al., 2005). All these studies show that entomopathogenic fungi are important pathogens of silkworm commercial hybrids. The present study revealed that similar fungi are also very important pathogens of our cultural heritage the Hatay yellow strain. This is an extremely critical situation for the Hatay yellow strain.

In conclusion, to preserve the Hatay yellow strain, which is one of the most important cultural assets and biodiversity of Türkiye, very urgent and serious action plans must be prepared and implemented as soon as possible. Otherwise, tomorrow may be too late and the culture may be irrevocably lost. Fungal pathogens have the great advantage of spreading rapidly between individuals in populations. The climatic conditions in Hatay also favor the spread of fungal pathogens. Considering that the effects of climate change are having a negative impact on this situation, it becomes clear that the problem is much more serious.

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Original article (Orijinal araştırma)

Soft scale (Hemiptera: Coccomorpha: Coccidae) species on fruit orchards of Diyarbakır and Elazığ provinces in Türkiye[1]

Diyarbakır ve Elazığ illeri meyve bahçelerindeki Koşnil (Hemiptera:Coccomorpha: Coccidae) türleri

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Abstract

This study was carried out in order to determine the soft scale insects (Hemiptera: Coccomorpha: Coccidae) species, their host plants, distribution areas, densities and infestation rates on fruit production areas of Diyarbakır and Elazığ Provinces in 2017-2018. As a result of the study, 7 soft scale species were determined on 12 fruit plants. The most common species and the widest host plant range was *Parthenolecanium corni* (Bouché, 1844) in the study areas. *Sphaerolecanium prunastri* (Boyer de Fonscolombe, 1834) and *Didesmococcus unifasciatus* (Archangelskaya, 1923) were determined to be the most important species in the region. *Didesmococcus unifasciatus* caused heavy infestation on almond, *Prunus dulcis* (Mill.) (Rosales: Rosaceae) trees (Scale 4) at rates of 3.1% and 0,9% in Çınar district of Diyarbakır and Sivrice district of Elazığ, respectively. *Sphaerolecanium prunastri* caused heavy infestation on apricots, *Prunus armeniaca* L. (Rosales: Rosaceae) at rates of 3.8% and 4.6% in Elazığ's Central and Baskil districts, which have significant apricot production areas.

Keywords: Density, host plant, infestation rate, soft scale insect, Türkiye

Öz

Bu çalışma 2017-2018 yılları arasında, Diyarbakır ve Elazığ illeri meyve üretim alanlarındaki Koşnil (Hemiptera: Coccomorpha: Coccidae) türleri ile bu türlerin konukçuları, yayılış alanları, yoğunlukları ve bulaşma oranlarının belirlenmesi amacıyla yürütülmüştür. Çalışma sonucunda, 12 konukçu bitki üzerinde 7 Coccidae türü tespit edilmiştir. Sürvey alanlarında görülen en yaygın ve konukçu dizisi en geniş türün *Parthenolecanium corni* (Bouché, 1844) olduğu belirlenmiştir. *Sphaerolecanium prunastri* (Boyer de Fonscolombe, 1834) ve *Didesmococcus unifasciatus* (Archangelskaya, 1923)'un bölgedeki en önemli türler oldukları belirlenmiştir. *Didesmococcus unifasciatus*'un Diyarbakır'ın Çınar ilçesinde bademde, *Prunus dulcis* (Mill.) (Rosales: Rosaceae) %3,1 oranında Elazığ'ın Sivrice ilçesinde ise %0,9 oranında aşırı bulaşma oranına (Skala 4) sebep olduğu, *S. prunastri*'nin ise Elazığ'ın Merkez ve Baskil ilçelerinde kayısıda, *Prunus armeniaca* L. (Rosales: Rosaceae) sırasıyla %3,8 ve %4,6 oranında aşırı bulaşma oranına sebep olduğu tespit edilmiştir.

Anahtar sözcükler: Yoğunluk, konukçu bitki, bulaşma oranı, koşnil, Türkiye

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Introduction

Soft scale insects (Hemiptera: Coccomorpha: Coccidae) constitute the third most species-rich group in the infraorder Coccomorpha with 1221 species belonging to 173 genera (García Morales et al., 2016). The habitats, colors, shapes and forms differ among species of Coccidae (Ben-Dov, 1997; Kondo, 2022). They are plant-feeding insects which develop mostly on perennial, but rarely on annual plants (Hodgson, 1994). Coccids feed on nearly any part of the host plant, including the roots, even though many species develop on the leaves or twigs or the trunk. Actual take-up of nutrients is from the phloem and thus all species of Coccidae produce honeydew (Ben-Dov, 1997). Species of Coccidae include noxious insects, causing direct injury by depleting the host plant of nutrients and damaging tissues, and indirectly through honeydew secretion which accumulates on crops. The consequent cover of sticky honeydew and the development of black sooty mold on crops decreases significantly their market value (Öncüer, 1974; Hodgson, 1994; Ben-Dov, 1997). In order to prevent their damages, it is important to study the species of Coccidae which are found on crops (Kosztarab & Kozár, 1988).

In studies carried out in cultivated and non-cultivated areas in Türkiye, 72 Coccid species belonging to 30 genera have been identified so far (Ülgentürk et al., 2022). In addition, the studies conducted in fruit production areas in different geographical regions of Türkiye have revealed that many of Coccid species belonging to genera such as *Anapulvinaria* Borchsenius, 1952, *Ceroplastes* Gray, 1828, *Coccus* L., 1785, *Didesmococcus* Borchsenius,1953, *Eulecanium* Cockerell, 1893, *Filippia* Targioni Tozzetti, 1868, *Palaeolecanium* Šulc, 1908, *Parthenolecanium* Šulc, 1908, *Saissetia* Deplanche, 1859, *Sphaerolecanium* Šulc, 1908 (Hemiptera: Coccidae) cause damage on different fruit tree species and, they can cause economic losses if they are not kept under control (Altay et al., 1972; Öncüer, 1974; Soylu, 1976; İren, 1977; Kozar et al., 1979; Ecevit et al., 1987; Kılıç & Aykaç, 1989; Erol & Yaşar, 1996; Özgökçe et al., 1999; Bolu & Uygun, 2003; Kumral & Kovancı, 2004; Zeki et al., 2004; Özgen & Bolu, 2009; Ülgentürk et al., 2009; Bolu, 2012; Kaçar et al., 2012; Akşit & Apak, 2013; Yiğit, 2013; Ayaz et al., 2015; Kaplan & Turanlı, 2016; Şimşek & Bolu, 2017; Gülmez et al., 2022).

Along with the improvement in irrigation opportunities, fruit plantations have increased in Southeastern Anatolia and Eastern Anatolia Regions. For example, the apricot orchards area in Elazığ have increased from 3535 ha to 10211.2 ha between 2004 and 2021. Similarly, the almond production area in Diyarbakir province increased from 661 ha to 1984.9 ha between 2004 and 2021 (TURKSTAT, 2022). Since the increase in fruit diversity and production areas may cause proliferation of many harmful insect species, including coccids, it is important to conduct surveys of harmful insect species at regular intervals and to reveal their damage levels.

This paper deals with Coccidae species, their host plants, distributions, densities and infestation rates on fruit orchards of Diyarbakır and Elazığ provinces in Türkiye.

Materials and Methods

The study was carried out in the fruit orchards of Diyarbakır (Çermik, Çüngüş, Eğil, Ergani, Çınar) and Elazığ (Center district, Baskil, Keban, Sivrice) in April-September, in 2017-2018 (Figure 1). The fruit trees were examined every two weeks and the number of trees examined in the sampled orchards was determined according to Lazarov & Grigorov (1961). Specimens were slide mounted for light microscopy by using the methodology of Kosztarab & Kozár (1988). Dry and mounted material were deposited at the Plant Protection Research institution, Diyarbakır, Türkiye. In order to determine the densities of Coccidae species, individuals on 10 cm shoots or branches of the host plant were counted and evaluated according to the density scale determined by Kozár & Viktorin (1978) (Table 1), infestation rates of the species were calculated according to the formula “number of infested trees/total number of trees examined x100” for each host plant in the survey district.

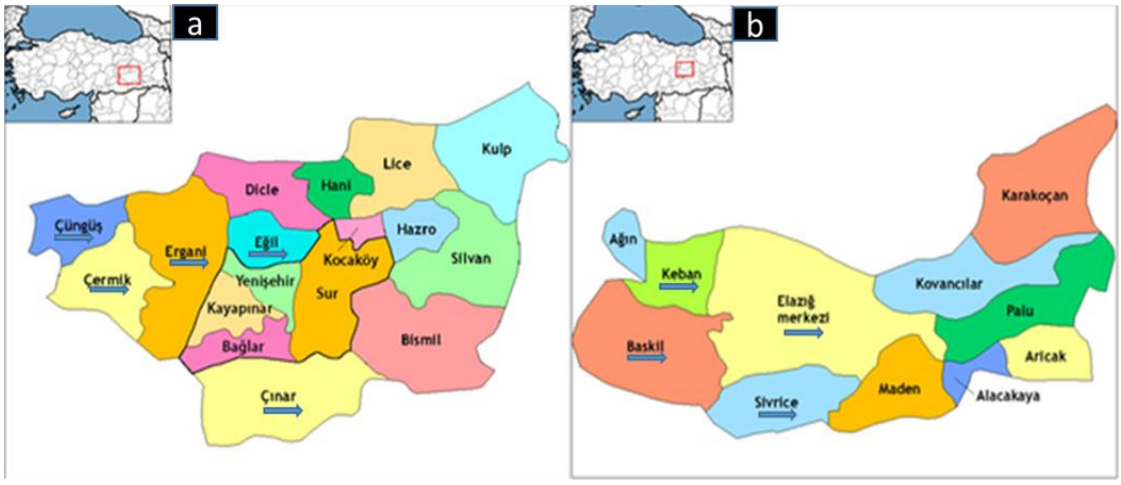

Figure 1. Survey districts of a) Diyarbakır and b) Elazığ.

Table 1. Density scales of scale insects on fruit trees

Scale	Individual number/10'cm shoot or branch	Evaluation
0	0	Clean
1	1-5	Low infestation
2	6-20	Small colony
3	21-50	Large colony
4	More than 50	Heavy infestation

Results and Discussion

Soft scale species on fruit plants in Diyarbakır Elazığ Provinces

As a result of the study carried out, it was determined that 26 of the 97 orchards surveyed in Diyarbakır were clean and 71 were infested with soft scale insects. In Elazığ, 47 out of 126 orchards were found to be clean and 79 infested. 7 Coccidae species belonging to 7 genera were determined on 12 host plants, and these species and their hosts are given in Table 2.

Table 2. Coccidae species and their hosts in the orchards of Diyarbakır and Elazığ provinces in 2017-2018

Coccidae species	Host plant
Anapulvinaria pistaciae (Bodenheimer, 1926)	*Pistacia vera* L. (Sapindales: Anacardiaceae)
Didesmococcus unifasciatus (Archangelskaya, 1923)	*Prunus dulcis* (Mill.), *Prunus persica* (L.) (Rosales: Rosaceae)
Eulecanium tiliae (L., 1758)	*Pistacia vera*, *Cydonia oblonga* Mill., *Malus domestica* Baumg, *Prunus domestica* A.Sav., *Prunus armeniaca* Blanco (Rosales: Rosaceae)
Parthenolecanium corni (Bouché, 1844)	*Cydonia oblonga, M. domestica, P. vera, P. dulcis, P. persica, P. domestica*, *P. armeniaca*, *Prunus avium* (L.), *Prunus cerasus* Scop., (Rosales: Rosaceae), *Diospyros kaki* Thunb., (Ericales: Ebenaceae), *Juglans regia* L. (Fagales: Juglandaceae), *Morus alba* Bureau (Rosales: Moraceae)
Palaeolecanium bituberculatum (Signoret, 1873)	*Malus domestica*
Pulvinaria vitis (L., 1758)	*Juglans regia*
Sphaerolecanium prunastri (Boyer de Fonscolombe, 1834)	*Prunus domestica*, *P. armeniaca*

***Anapulvinaria pistaciae* (Bodenheimer, 1926)**

Adult female approximately broadly circular with raised short oval central area and transverse wrinkles. Length 2.8-3.4 mm. Color light to dark brown, ovisac "cottony" white, length 4-5 mm width 3-4 mm (Figure 2a). The egg is oval and light green in color, crawlers and first-instar nymphs are also green, but second instar nymphs turn to red brown (Santas, 1985).

Material examined. Diyarbakır: Çınar, Aktepe, 37°43'15.6"N 40°34'10.6"E, 13.vii.2017, 2♀♀, *P. vera*; Çınar, Ekinveren, 37°41'10.6"N 40°25'51.5"E, 10.vii.2018, 3♀♀, *P. vera*.

Distribution in Türkiye. the pest has been detected in Central Anatolia, Eastern Anatolia and Southeastern Anatolia regions (Ülgentürk et al., 2022).

Distribution in the world. Afghanistan, Armenia, China, Iran, Lebanon, Mongolia, Pakistan, Tajikistan, Türkiye, Turkmenistan, Uzbekistan (García Morales et al., 2016).

***Didesmococcus unifasciatus* (Archangelskaya, 1923)**

The body of young females is elliptical, oval in shape. Body color is reddish brown, with an orange-yellowish band in the middle of the body (Figure 2b). Egg-laying adult females swell and take on a spherical shape (Hodgson, 1994).

Material examined. Diyarbakır: Çüngüş, Karşıyaka, 38°12'57.7"N 39°17'39.5"E, 16.VIII.2017, 4♀♀, *P. persica*; Çınar, Altınakar, 37°44'51.7"N 40°24'57.1"E, 20.IX.2017, 3♀♀, *P. dulcis*; Çüngüş, Keleşevler, 38°12'20.2"N 39°19'11.1"E, 10.V.2018, 3♀♀, *P. dulcis*; Çınar, Bağacık, 37°40'13.2"N 40°26'29.4"E, 16.VIII.2018, 5♀♀, *P. dulcis*; Elazığ: Sivrice, Günay, 38°29'14.2"N 39°20'01.1"E, 16.IV.2018, 4♀♀, *P. dulcis*; Sivrice, Cevizdere, 38°29'50.6"N 39°22'27.1"E 24.IV.2018, 4♀♀, *P. dulcis*; Sivrice, Şirinyazı, 38°27'17.6"N 39°17'44.4"E, 22.V.2018, 5♀♀, *P. dulcis*.

Distribution in Türkiye. It has been reported that the pest has been detected in Eastern Anatolia and Southeastern Anatolia regions (Kaydan & Kozár, 2010; Bolu, 2012; Çiftçi & Bolu, 2021).

Distribution in the world. Afghanistan, Armenia, China, Iran, Lebanon, Mongolia, Pakistan, Tajikistan, Türkiye, Turkmenistan, Uzbekistan (García Morales et al., 2016).

***Eulecanium tiliae* (L., 1758)**

The body of the young female is oval in shape and there are red and white colored bands extending from length to end on the dorsal (Figure 2c). The length of the young females is 3313±15.6 (2440-4200) µm, and the width is 3163±17.7 (2120-4320) µm. After oviposition periods, these colored bands disappear and color of body turns into a uniform yellowish light brown or brown. The bodies of adult females become swollen during ovulation and take the shape of a sphere (Ülgentürk, 1998). The body of first-instar nymphs is oval, brownish orange in color, and the young individuals of them are 0.43-0.59 µm length and 0.27-0.30 µm width; before molting, they are 800-960 µm length and 420-590 µm width. In Second instar nymphs, the body is elongated oval, greenish yellow. There are 10 reddish orange thin lines extending from the head to the anal lobe. the males of it are 1.2-2.05 mm length, 0.56-1.08 mm width and females are 1.02-1.08 mm tall, 0.6-0.8 mm wide (Đurović, 2015).

Material examined. Diyarbakır: Çınar, Aktepe, 37°43'57.4"N 40°33'49.7"E, 13.VII.2018, 2♀♀, *P. vera*; Çınar, Aktepe, 37°43'15.6"N 40°34'10.6"E, 13.VII.2018, 5♀♀, *P. vera*; Çınar, Yaprakbaşı, 37°38'37.3"N 40°33'43.0"E, 13.VII.2018, 2♀♀, *P. vera*; Çınar, Ekinveren, 37°41'09.7"N 40°25'51.8"E, 12.IV.2018, 4♀♀, *P. vera*; Çınar, Öncülü, 37°42'48.6"N 40°31'55.2"E, 12.IV.2018, 4♀♀, *P. vera*; Çınar, Öncülü, 37°42'45.3"N 40°32'21.6"E, 12.IV.2018, 2♀♀, *P. vera*; Eğil, Bahşilar, 38°14'00.2"N 40°03'51.7"E, 24.IV.2018, 3♀♀, *P. vera*; Eğil, Ilgın, 38°16'07.4"N 40°05'44.0"E, 12.VII.2018, 2♀♀, *P. vera*; Çınar, Bilmece, 37°33'21.5"N

40°13'08.9"E, 27.viii.2018, 5♀♀, *P. vera*; Elazığ: Baskil, Alangören, 38°27'41.4"N 38°37'31.1"E, 20.VII.2017, 2♀♀, *P. armeniaca*; Baskil, Hacımehmetli, 38°30'00.8"N 38°32'06.5"E, 20.VII.2017, 4♀♀, *P. domestica*; Center district, Veran, 38°40'59.3"N 39°08'35.1"E, 09.IV.2018, 2♀♀, *M. domestica*, 2♀♀, *C. oblonga*; Central district, Sarıcubuk, 38°40'59.3"N 39°06'18.0"E, 03.v.2018, 4♀♀, *P. domestica*.

Distribution in Türkiye. The pest spreads in the Mediterranean, Eastern Anatolia, Black Sea, Marmara and Central Anatolia Regions (Ülgentürk et al., 2022).

Distribution in the world. Armenia, Austria, Bulgaria, Canada, Canary Corsica, Croatia, Czechia, Denmark, England, France, Georgia, Germany, Greece, Hungary, Iran, Iraq, Ireland, Islands, Israel, Italy, Latvia, Luxembourg, Malta, Moldova, Netherlands, Northern Ireland, Pakistan, Poland, Portugal, Romania, Russia, Sardinia, Scotland, Slovenia, Spain, Sweden, Switzerland, Türkiye, Turkmenistan, Ukraine, United States,Yugoslavia (García Morales et al., 2016).

***Parthenolecanium corni* (Bouché, 1844)**

The body shape and color of the pest may vary depending on the age of the individual, species of host plant and the region. Young females are usually grayish yellow, light brown colors, and are 3550±20.2 (2200-5320) µm length, 2862±22.3 (1240-4600) µm width. there are longitudinal 2 dark-colored apparent stripes and latitudinal vague lines on dorsum (Figure 2e). Adult females which have completed ovulation are dark brown and varies from slightly swollen or spherical. On dorsum, there is a distinct longitudinal carina on mid-dorsum of the body (Ülgentürk, 1988).

Material examined. Diyarbakır: Çınar, Aktepe, 37°43'57.4"N 40°33'49.7"E, 13.VII.2017, 2♀♀, *P. vera*; Eğil, Dere, 38°13'45.5"N 40°05'16.6"E, 19.VII.2017, 4♀♀, Eğil, Dere, 38°14'13.1"N 40°04'14.1"E, 19.VII.2017, 3♀♀, *P. dulcis*;*P. dulcis*; Çermik, Karamusa, 38°08'52.3"N 39°29'25.8"E, 01.VIII.2017, 3♀♀, *P. armeniaca*, 2♀♀, *P. domestica*; Çermik, Tepe, 38°07'57.3"N 39°28'41.5"E, 01.VIII.2017, 3♀♀, *P. domestica*; Çermik, Sinek, 38°10'04.3"N 39°28'00.1"E, 01. VIII.2017, 4♀♀, *P. persica*, 2♀♀, *M. domestica*; Çermik, Haburman, 38°08'17.5"N 39°28'19.4"E, 01.VIII.2017, 3♀♀, *P. dulcis*; Ergani, Sanayi, 38°14'21.9"N 39°47'13.7"E, 03.VIII.2017, 5♀♀, *P. armeniaca*; Ergani, Şirinevler, 38°15'37.8"N, 39°44'27.1"E, 03.VIII.2017, 3♀♀, *M. domestica*, 3♀♀, *P. avium*; Çüngüş, Değirmensuyu, 38°13'07.7"N 39°15'05.9"E, 16.VIII.2017, 2♀♀, *P. domestica*; Çüngüş, Hindibaba, 38°13'27.3"N 39°13'14.9"E, 16.VIII.2017, 2♀♀, *M. domestica*, 3♀♀, *P. persica*; Çınar, Altınakar, 37°46'02.4"N 40°24'48.8"E 20.IX.2017, 3♀♀, *P. dulcis*; Ergani, Yukarı karbuzlu, 38°11'48.4"N 39°50'33.8"E, 21.IX.2017, 2♀♀, *P. dulcis*; Ergani, Kömürtaş, 38°13'28.3"N 39°48'08.4"E, 21.IX.2017, 3♀♀, *M. domestica*, 3♀♀, *P. domestica*; Ergani, Bahçekaşı, 38°15'31.8"N 39°43'59.8"E, 21.IX.2017, 3♀♀, *P. armeniaca*, 2♀♀, *M. domestica*; Çermik, Karakaya, 38°03'51.5"N 39°18'43.0"E 05.IV.2018, 5♀♀, *M. domestica*, 2♀♀, *P. domestica*; Çermik, Kırmatepe, 05.IV.2018, 3♀♀, *J. regia*, 5♀♀, *P. armeniaca*, ♀, *P. domestica*, 38°03'46.2"N 39°19'37.3"E; Çermik, Göktepe, 38°04'53.8"N 39°20'39.8"E, 05.IV.2018, 3♀♀, *P. armeniaca*, 3♀♀, *P. persica*; Çınar, Beşpınar köyü, 12.IV.2018, 5♀♀, *P. dulcis*, 3♀♀, *P. armeniaca*, 2♀♀, *M. domestica*, 6♀♀, *P. domestica*, 2♀♀, *J. regia*, 4♀♀, *M. alba*, 37°46'38.5"N 40°21'51.8"E; Çınar, Beşpınar, 12.IV.2018, 2♀♀, *M. domestica*, 37°46'05.4"N 40°22'23.5"E; Çınar, Beşpınar, 12.IV.2018, 4♀♀, *M. alba*, 2♀♀, *M. domestica*, 2♀♀, *P. armeniaca*, 37°46'05.4"N 40°22'23.5"E; Çınar, Öncülü, 12.IV.2018, 2♀♀, *P. vera*, 37°42'48.6"N 40°31'55.2"E; Ergani, Tevekli, 17.IV.2018, 4♀♀, *P. armeniaca*, 3♀♀, *P. domestica*, 38°11'37.2"N 39°50'39.6"E; Ergani, Kavurmaköprü, 17.IV.2018, 5♀♀, *P. domestica*, 38°17'34.2"N 39°44'04.5"E; Ergani, Kavurmaköprü, 17.IV.2018, 4♀♀, *M. domestica*, 38°18'02.1"N 39°43'20.4"E; Ergani, Ortaağaç, 17.IV.2018, 2♀♀, *P. armeniaca* 38°18'09.9"N 39°42'55.4"E; Çüngüş, Oyuklu, 18.IV.2018, 2♀♀, *P. armeniaca* 38°11'36.2"N 39°22'33.5"E; Çüngüş, Oyuklu, 18.IV.2018, 2♀♀, *P. armeniaca*, 38°11'45.4"N 39°21'17.2"E; Çüngüş, Keleşevleri, 18.IV.2018, 3♀♀, *P. avium*, 38°11'47.8"N 39°20'58.4"E; Çüngüş, Keleşevleri, 38°11'48.4"N 39°21'01.3"E, 18.IV.2018, 2♀♀, *P. domestica*, 3♀♀, *J. regia*; Eğil, Kırkkuyu, 38°13'06.6"N 40°03'28.9"E, 24.IV.2018, 2♀♀, *P. dulcis*; Eğil, Kırkkuyu, 38°13'12.9"N 40°03'39.0"E, 24.IV.2018, 3♀♀, *P. dulcis*; Eğil, Kırkkuyu, 38°13'39.3"N 40°03'51.7"E, 24.IV.2018, 2♀♀, *P.*

dulcis; Ergani, Kavurmaköprü, 38°19'58.7"N 39°43'47.8"E, 17.V.2018, 4♀♀, *J. regia*, 3♀♀, *P. armeniaca*; Ergani, Ortaağaç, 38°18'26.0"N 39°42'45.3"E, 17.V.2018, 3♀♀, *P. armeniaca*; Ergani, Bahçekaşı, 38°17'01.0"N 39°43'59.0"E, 17.V.2018, 4♀♀, *M. domestica*, 3♀♀, *P. armeniaca*; Çermik, Sinek, 38°10'04.3"N 39°28'00.1"E, 21.V.2018, 4♀♀, *C. oblonga*; Çüngüş, Handere, 38°14'02.5"N 39°10'37.9"E, 21.V.2018, 3♀♀, *J. regia*; Çüngüş, Karşıyaka, 38°12'49.1"N 39°17'37.8"E, 21.V.2018, 4♀♀, *M. domestica*; Çınar, Höyükdibi, 37°42'51.4"N 40°15'34.8"E, 23.V.2018, 2♀♀, *P. domestica*; Çınar, Kazıktepe, 37°48'38.0"N 40°15'36.7, 30.V.2018, 3♀♀, *P. persica*; Çınar, Şeyhçoban, 37°41'13.6"N 40°14'18.6"E, 30.V.2018, 3♀♀, *P. dulcis*; Çınar, Karababa, 37°39'53.5"N 40°13'42.2"E, 30.V.2018, 4♀♀, *P. domestica*, 3♀♀, *M. domestica*; Çınar, Öncülü, 37°43'02.5"N 40°31'55.5"E, 30.V.2018, 4♀♀, *M. domestica*; Eğil, Ilgın, 38°16'07.4"N 40°05'46.0"E, 12.VII.2018, 2♀♀, *P. dulcis*; Çüngüş, Geçitköy, 38°11'00.8"N 39°15'59.3"E, 03.VIII.2018, 3♀♀, *P. dulcis*; Çüngüş, ibikkaya, 38°07'24.4"N 39°16'21.2"E, 03.VIII.2018, 4♀♀, *P. vera*; Çüngüş, Karşıyaka, 38°12'47.7"N 39°17'42.3"E, 03.VIII.2018, 6♀♀, *M. domestica*; Çermik, Sinek, 38°09'59.2"N 39°27'36.6"E, 09.VIII.2018, 6♀♀, *M. domestica*; Çermik, Çukur, 38°08'05.2"N 39°27'05.6"E, 09.VIII.2018, 2♀♀, *P. armeniaca* 2♀♀, *P. domestica*; Çermik, Tepe, 38°08'21.3"N 39°27'24.2"E, 09.VIII.2018, 2♀♀, *P. armeniaca*, ♀, *P. dulcis*; Çınar, Yıllarca, 37°44'19.9"N 40°08'27.3"E, 16.VIII.2018, 4♀♀, *P. domestica*; Çınar, Akçomak, 37°39'29.7"N 40°27'44.9"E, 16.VIII.2018, 4♀♀, *J. regia*, 3♀♀, *P. armeniaca*; Çınar, Bağacık, 37°40'13.2"N 40°26'29.4"E, 16.VIII.2018, 3♀♀, *P. dulcis*; Çınar, Tilver, 37°37'19.6"N 40°14'27.8"E, 27.VIII.2018, 6♀♀, *M. domestica*; 2♀♀, *P. domestica*; Elazığ: Central districts, Öküzuşağı, 38°28'02.1"N 39°03'05.3"E, 26.VII.2018, 4♀♀, *J. regia*; Central district, Sinan, 38°37'17.4"N 39°02'11.0"E, 14.VII.2017, 5♀♀, *P. armeniaca*; Central district, Sinan, 38°37'13.8"N 39°02'11.1"E, 14.VII.2017, 2♀♀, *M. domestica*; Sivrice, Hazar, 14.VII.2017, 3♀♀, *M. domestica*, 4♀♀, *P. domestica* 38°27'35.3"N 39°17'11.4"E; Baskil, Hacımehmetli, 38°30'00.8"N 38°32'06.5"E, 20.VII.2017, 3♀♀, *P. domestica*; Keban, Nimri, 38°49'43.5"N 38°41'26.5"E, 02.viii.2017, 4♀♀, *P. dulcis*; Keban, Nimri, 38°48'18.8"N 38°41'04.6"E, 02.08.2017, 4♀♀, *M. domestica*; Central district, Yenicami, 38°33'02.3"N 39°02'04.9"E 15.VIII.2017, 2♀♀, *J. regia*; Sivrice, Üçgöz, 15.VIII.2017, ♀, *P. persica*, 38°27'22.5"N 39°17'38.1"E; Baskil, Canbeyler, 38°33'47.8"N 38°48'47.9"E, 18.IX.2017, 3♀♀, *P. armeniaca*: Baskil, Şahaplı, 38°32'27.7"N, 38°47'46.4"E, 18.IX.2017, 4♀♀, *J. regia*; Baskil, Şahaplı, 38°32'01.0"N 38°47'36.7"E, 18.IX.2017, 3♀♀, *P. armeniaca*; Keban, Sağdıclar, 38°47'02.6"N 38°49'56.0"E, 19.IX.2017, 4♀♀, *P. armeniaca*, 3♀♀, *M. domestica*, 4♀♀, *D. kaki*; Baskil, Odabaşı, 38°34'36.8"N 38°48'13.8"E, 04.IV.2018, 3♀♀, *P. armeniaca*; Central district, Veran, 09.IV.2018, 4♀♀, *M. domestica*, 2♀♀, *P. cerasus*, 3♀♀, *C. oblonga*, 4♀♀, *J. regia*, 38°40'59.3"N 39°08'35.1"E; Centtal district, Cipköy, 09.IV.2018, 2♀♀, *J. regia*, 38°41'19.3"N 39°03'41.8"E; Central district, Sarıcubuk, 09.IV.2018, 2♀♀, *P. avium*, 38°40'59.3"N 39°06'18.0"E; Central district, Poyraz, 09.IV.2018, 3♀♀, *M. domestica*, 3♀♀, *J. regia*, 38°41'15.0"N 39°02'38.8"E; Sivrice, Kürk, 16.IV.2018, 3♀♀, *P. avium*, 38°25'24.2"N 39°13'57.0"E; Baskil, Habibuşağı, 38°26'18.6"N 38°46'45.9"E, 26.IV.2018, 2♀♀, *P. armeniaca*; Baskil, Habibuşağı, 38°26'16.5"N 38°46'16.0"E, 26.IV.2018, 3♀♀, *P. armeniaca*; Baskil, Pınarlı, 38°26'28.6"N 38°44'48.9"E, 26.IV.2018, 3♀♀, *P. armeniaca*; Central district, Dilek, 38°34'46.7"N 39°02'11.1"E, 02.V.2018, 3♀♀, *P. armeniaca*; Central district, Dilek, 38°34'46.7"N 39°02'11.1"E, 02.v.2018, 4♀♀, *M. domestica*; Central district, Dilek, 38°34'33.2"N 39°03'13.6"E, 02.V.2018, 2♀♀, *P. armeniaca*; Central district, Durupınar, 38°33'28.3"N 39°02'58.7"E, 02.V.2018, 3♀♀, *P. armeniaca*; Central district, Işıkyolu, 38°31'53.9"N 39°02'08.2"E, 02.V.2018, 3♀♀, *P. armeniaca*; Central district, Yolçatı, 38°32'06.8"N 39°03'12.9"E, 02.V.2018, 3♀♀, *J. regia*; Sivrice, Şirinyazı, 38°27'17.6"N 39°17'44.4"E, 02.V.2018, 3♀♀, *M. domestica*; Baskil, Pınarlı, 38°26'16.4"N 38°45'39.1"E, 16.V.2018, 3♀♀, *P. armeniaca*; Baskil, Gedebük, 38°26'29.7"N 38°43'01.0"E, 16.V.2018, 3♀♀, *P. armeniaca*; Baskil, Kuşsarayı, 38°26'45.3"N 38°40'01.6"E, 16.V.2018, 2♀♀, *P. armeniaca*; Central district, Öküzuşağı, 38°28'01.7"N 39°03'01.5"E, 31.V.2018, 2♀♀, *M. domestica*: Central district, Öküzuşağı, 38°28'38.9"N 39°03'03.6"E, 31.V.2018, 2♀♀, *J. regia*; Central district, Bölüklü, 38°28'17.2"N 39°02'11.5"E, 31.V.2018, 2♀♀, *J. regia*; Central district, Sakabaşı, 38°28'21.0"N 39°01'30.5"E, 31.V.2018, 3♀♀, *J. regia*; Keban, Ulupınar, 38°44'30.9"N 38°51'10.7"E, 06.VI.2018, 3♀♀, *J. regia*; Keban, Ulupınar, 38°44'49.4"N 38°51'18.3"E, 06.VI.2018, 3♀♀, *P. armeniaca*;

Keban, Sağdıçlar, 38°47'19.6"N 38°49'53.4"E, 06.VI.2018, 2♀♀, *J. regia*; Baskil, Alangören, 38°27'21.2"N 38°38'48.5"E, 08.VI.2018, 3♀♀, *P. armeniaca*; Central district, Öksüzuşağı, 38°27'41.8"N 39°03'05.2"E, 26.VII.2018, 4♀♀, *M. domestica*: Central district, Küllük, 38°28'01.2"N 39°01'32.2"E, 26.VII.2018, 3♀♀, *P. armeniaca*; Central district, Çaydaçıra, 38°41'12.2"N 39°10'09.0"E, 02.VIII.2018, 3♀♀, *P. armeniaca*; Central district, Bölüklü, 38°28'49.1"N 39°02'30.3"E, 02.VIII.2018, 3♀♀, *J. regia*; Baskil, Karaali, 38°27'46.5"N 38°53'32.9"E, 10.VIII.2018, 2♀♀, *P. armeniaca*; Central district, Yalındamlar, 38°28'33.5"N 38°58'41.2"E, 10.VIII.2018, 3♀♀, *P. armeniaca*; Baskil, Kuşsarayı, 38°27'07.3"N 38°40'05.0"E, 17.VIII.2018, 3♀♀, *P. armeniaca*; Baskil,Alangören, 38°26'57.4"N 38°39'27.6"E, 17.VIII.2018, 2♀♀, *P. armeniaca*; Baskil, Gemici, 38°29'18.1"N 38°33'34.5"E, 17.VIII.2018, 4♀♀, *P. armeniaca*; Baskil, Çiğdemlik, 38°30'01.1"N 38°30'21.5"E, 17.VIII.2018, 5♀♀, *P. armeniaca*; Central district, Temürköy, 38°29'46.1"N 39°03'04.5"E, 31.VIII.2018, 2♀♀, *J. regia*; Central district, Aşağıdemirtaş, 38°36'52.7"N 39°05'55.1"E, 31.VIII.2018, 3♀♀, *J. regia*; Central district, Sarıcubuk, 38°40'22.6"N 39°07'05.8"E, 06.IX.2018, 3♀♀, *J. regia*; Central district, Sarıcubuk, 38°40'08.0"N 39°07'26.0"E, 06.IX.2018, 2♀♀, *J. regia*.

Distribution in Türkiye. The pest was reported in the Mediterranean, Eastern Anatolia, Southeastern Anatolia, Aegean, Black Sea, Marmara and Central Anatolia regions (Ülgentürk et al., 2022).

Distribution in the world. Afghanistan, Albania, Algeria, Argentina, Armenia, Australia, Austria, Azerbaijan, Balearic Islands, Belarus, Belgium, Brazil, Bulgaria, Canary Islands, Chile, China, Corsica, Croatia, Cyprus, Czechia, Denmark, Egypt, Ethiopia, Finland, France, Georgia, Germany, Hungary, India, Iran, Ireland, Israel, Italy, Japan, Kazakhstan, Kyrgyzstan, Latvia, Lebanon, Libya, Lithuania, Luxembourg, Malta, Mexico, Moldova, Mongolia, Netherlands, New Zealand, North Korea, Norway, Pakistan, Peru, Poland, Portugal, Romania, Russia, Serbia, Slovakia, Slovenia, South Korea, Spain, Sweden, Switzerland, Syria, Tajikistan, Türkiye, Turkmenistan, Ukraine, United Kingdom, USA, Uzbekistan, Yugoslavia (García Morales et al., 2016).

***Palaeolecanium bituberculatum* (Signoret, 1873)**

The body of the young adult females on the leaves is quite thin, flat and oval, and the color is greenish yellow. On the dorsal, brown bands that expand transversely and towards the body edge are seen. The body color turns brown after settle on branches in autumn. Young females are 3140±12.9 (2863-3390) µm long, 2559±11.9 (2331-2797) µm wide. As it gets older, the body becomes swollen, and after ovulation the color becomes darker and sclerotized. Indistinct bands formed by white spots in the middle part of the body can also be seen. There are two pairs of knoblike tubercles on dorsum (Figure 2e). The pair of these anterior tubercles is larger than posterior ones (Ülgentürk, 1998).

Material examined. Elazığ: Sivrice, Gözeli, 38°25'43.3"N 39°04'54.1"E, 22.V.2018, 9♀♀, *M. domestica*; Central district, Öksüzuşağı, 38°27'41.8"N 39°03'05.2"E, 26.VII.2018, 2♀♀, *M. domestica*; Sivrice, Şirinyazı, 38°27'17.6"N 39°17'44.4"E, 26.VII.2018, 2♀♀, *M. domestica*.

Distribution in Türkiye. It has been reported that *Palaeolecanium bituberculatum* is distributed in the Mediterranean, Eastern Anatolia, Southeastern Anatolia, Aegean, Black Sea, Marmara and Central Anatolia regions (Ülgentürk et al., 2022).

Distribution in the world. Afghanistan, Armenia, Azerbaijan, Bulgaria, Croatia, Cyprus, Czechia, Denmark, France, Georgia, Germany, Hungary, Iran, Iraq, Israel, Italy, Kazakhstan, Kyrgyzstan, Luxembourg, Moldova, Netherlands, Poland, Romania, Russia, Sardinia, Slovenia, Spain, Sweden, Switzerland, Tajikistan, Türkiye, Turkmenistan, UK, Ukraine, USA, Uzbekistan, Yugoslavia (García Morales et al., 2016).

***Pulvinaria vitis* (L., 1758)**

The body of young adult females is yellowish-brown, the body shape is elongated, elliptical and oval (Figure 2f). Body shape can vary depending on the host and the shape of the place where it is settled (Ülgentürk, 1988).

Material examined. Diyarbakır: Ergani, Kavurmaköprü, 38°19'58.7"N 39°43'47.8"E, 17.V.2018, 2♀♀, *J. regia*; Elazığ: Central district, Yolçatı, 38°32'06.8"N 39°03'12.9"E, 02.V.2018, 3♀♀, *J. regia*; Central district, Bölüklü, 38°28'17.2"N 39°02'11.5"E, 31.V.2018, 2♀♀, *J. regia*; Keban, Ulupınar, 38°28'17.2"N 39°02'11.5"E, 06.VI.2018, ♀, *J. regia*; Central district, Bölüklü, 38°28'49.1"N 39°02'30.3"E, 02.VIII.2018, ♀, *J. regia*; Central district, Sarıcubuk, 38°40'57.7"N 39°07'15.5"E, 06.IX.2018, 2♀♀, *J. regia*; Central district, Sarıcubuk, 38°40'08.0"N 39°07'26.0"E, 06.IX.2018, 2♀♀, *J. regia*.

Distribution in Türkiye. *Pulvinaria vitis* has been detected in the Mediterranean, Eastern Anatolia, Southeastern Anatolia, Aegean, Black Sea, Marmara and Central Anatolia regions (Ülgentürk et al., 2022).

Distribution in the world. Algeria, Argentina, Armenia, Austria, Brazil, Bulgaria, Canada, China, Corsica, Crete island, Croatia, Czechia, Denmark, Finland, France, Georgia, Germany,GreeceHungary, Iran, Ireland, Israel, Italy, Japan, Jordan, Kazakhstan, Latvia, Lithuania, Luxembourg, Malta, Moldova, Mongolia, Morocco, Netherlands, New Zealand, Norway, Poland, Portugal, Romania, Russia, Sardinia, Slovenia, Spain, Sweden, Switzerland, Tajikistan, Türkiye, Turkmenistan, UK, Ukraine, USA, Uzbekistan, Yugoslavia (García Morales et al., 2016).

***Sphaerolecanium prunastri* (Boyer de Fonscolombe, 1834)**

The postovipositon females are round, almost spherical, dark brownish black in color (Figure 2g). It is smaller than similar species. It is 2297±16 (1680-3400) µm length and 2077±20 (1320-3200) µm width (Ülgentürk, 1998).

Material examined. Diyarbakır: Ergani, Bahçekaşı, 38°17'01.0"N 39°43'59.0"E, 17.V.2018, 4♀♀, *P. domestica*; Elazığ: Baskil, Gemici, 38°28'27.1"N 38°34'20.5"E, 20.VII.2017, 2♀♀, *P. armeniaca*; Baskil, Alangören, 38°27'41.4"N 38°37'31.1"E, 20.VII.2017, 2♀♀, *P. armeniaca*; Baskil, Cumhuriyet, 38°34'22.3"N 38°49'50.9"E, 20.VII.2017, 4♀♀, *P. armeniaca*; Central district, Sarıcubuk, 38°40'59.3"N 39°06'18.0"E, 09.IV.2018, 4♀♀, *P. domestica*; Baskil, Pınarlı, 38°26'28.6"N 38°44'48.9"E, 26.IV.2018, 2♀♀, *P. armeniaca*; Baskil, Pınarlı Köyü, 38°26'12.1"N 38°44'11.2"E, 26.IV.2018, 3♀♀, *P. armeniaca*; Baskil, Gedebük, 38°26'28.2"N 38°43'35.2"E, 26.IV.2018, 3♀♀, *P. armeniaca*; Baskil, Karaali, 38°27'41.5"N 38°52'52.3"E, 02.V.2018, *P. armeniaca*, 3♀♀; Baskil,Pınarlı, 38°26'16.4"N 38°45'39.1"E, 16.V.2018, 4♀♀, *P.* armeniaca; Cental district, Sürsürü, 38°39'53.9"N 39°12'36.7"E, 16.V.2018, 4♀♀, *P. armeniaca*; Baskil, Gedebük, 38°26'29.7"N 38°43'01.0"E, 16.V.2018, 3♀♀, *P. armeniaca*; Baskil, Kuşsarayı, 38°26'45.3"N 38°40'01.6"E, 16.V.2018, 3♀♀, *P. armeniaca*; Baskil, Kuşsarayı, 38°27'11.5"N 38°40'38.1"E, 16.V.2018, 2♀♀, *P. armeniaca*; Baskil, Deliktaş, 38°27'47.2"N 38°36'15.7"E, 08.VI.2018, 4♀♀, *P. armeniaca*; Baskil, Alangören, 38°27'21.2"N 38°38'48.5"E, 08.VI.2018, 4♀♀, *P. armeniaca*; Baskil, Alangören, 38°27'29.4"N 38°38'15.0"E, 08.VI.2018, 3♀♀, *P. armeniaca*; Baskil, Sinan, 38°28'49.5"N 38°34'40.8"E, 20.VII.2018, 3♀♀, *P. armeniaca*; Baskil, Alibaba, 38°29'01.2"N 38°33'25.1"E, 20.VII.2018, 3♀♀, *P. armeniaca*; Baskil, Konacık, 38°29'32.9"N 38°33'00.9"E 20.VII.2018, 3♀♀, *P. armeniaca*; Central district, Ortaçalı, 38°28'04.9"N 39°01'27.8"E, 26.07.2018, 3♀♀, *P. armeniaca*; Baskil, Karaali, 38°27'44.1"N 38°53'09.4"E, 02.VIII.2018, 3♀♀, *P. armeniaca*; Central district, Yalındamlar, 38°28'33.5"N 38°58'41.2"E, 10.VIII.2018, 5♀♀, *P. armeniaca*; Central district, Kuşsarayı, 38°27'07.3"N 38°40'05.0"E, 17.VIII.2018, 2♀♀, *P. armeniaca*; Baskil, Güllüce, 38°26'57.4"N 38°39'27.6"E, 17.VIII.2018, 4♀♀, *P. armeniaca*; Baskil, Alibaba, 38°29'18.1"N 38°33'34.5"E, 17.VIII.2018, 3♀♀, *P. armeniaca*; Baskil, Çiğdemlik, 38°30'01.1"N 38°30'21.5"E, 17.VIII.2018, 3♀♀, *P. armeniaca*; Baskil, Şahinkaya, 38°40'56.1"N 39°07'38.4"E, 06.IX.2018, 3♀♀, *P. armeniaca*.

Distribution in Türkiye. *Sphaerolecanium prunastri* have distributed in the Mediterranean, Eastern Anatolia, Southeastern Anatolia, Aegean, Black Sea, Marmara and Central Anatolia regions (Ülgentürk et al., 2022).

Distribution in the world. Armenia, Austria, Azerbaijan, Balaer Islands, Belgium, Bulgaria, China, Crete island, Croatia Iran, Czechia, France, Georgia, Germany, Greece, Hungary, Israel, Italy, Moldova, Poland, Romania, Russia, Sardinia, Slovenia, South Korea, Spain, Switzerland, Syria, Türkiye, Turkmenistan, Ukraine, USA, Uzbekistan, Yugoslavia (García Morales et al., 2016).

Figure 2. Coccidae species determined in Diyarbakır and Elazığ; a) *Anapulvinaria pistaciae*; b) *Didesmococcus unifasciatus*; c) *Palaeolecanium bituberculatum*; d) *Eulecanium tiliae*; e) *Parthenolecanium corni*; f) *Pulvinaria vitis*; g) *Sphaerolecanium prunastri*.

The density levels and infestation rates of the Coccidae species in the Diyarbakır and Elazığ fruit orchards

In Çınar district of Diyarbakır, *A. pistaciae, P. corni, D. unifasciatus* and *E. tiliae* were identified. *Anapulvinaria pistaciae* was detected only in pistachio trees, and it was determined that 1.3% of the 377 examined trees were infested at scale 1 density level. *Eulecanium tiliae* was also detected only in pistachio

and 9% of pistachio trees were infested at scale 1, and 6.4% of them were infested at scale 2 and 2.1% of them were infested at scale 3. *Didesmococcus unifasciatus* was detected only on almond trees and it was found that 4.4% of the 318 examined almond trees were infested at scale 1, 14.7% at scale 2, 15.6% at scale 3 and 3.8% at scale 4 density. In addition, this species caused drying in the shoots and branches of almond trees, where it was heavily infested. *Parthenolecanium corni* was found to be the most common Coccidae species with the widest host range in Çınar district. Although *P. corni* was able to form large colonies (scale 3) at limited infestation rates on apple, mulberry and plum trees, it was found in low population densities such as scale 1 and scale 2 on walnut, pistachio, apricot, almond and peach trees in Çınar (Table 3).

Table 3. Density levels and infestation rates of the Coccidae species in Çınar

Host plant	Number of trees sampled	Infestation rate (%)																
		Anapulvinari pistaciae					*Didesmococcus unifasciatus*				*Eulecanium tiliae*				*Parthenolecanium corni*			
		0	1	2	3	4	1	2	3	4	1	2	3	4	1	2	3	4
J. regia	51	86.3	0	0	0	0	0	0	0	0	0	0	0	0	5.9	7.8	0	0
M. domestica	68	50.0	0	0	0	0	0	0	0	0	0	0	0	0	14.7	17.7	17.7	0
M. alba	25	92.0	0	0	0	0	0	0	0	0	0	0	0	0	0	4	4	0
P. vera	377	75.3	1.3	0	0	0	0	0	0	0	9	6.4	2.1	0	5.8	0	0	0
P. armeniaca	56	62.5	0	0	0	0	0	0	0	0	0	0	0	0	26.8	10.7	0	0
P. avium	33	100.0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
P. domestica	75	53.3	0	0	0	0	0	0	0	0	0	0	0	0	20	24	2.7	0
P. dulcis	318	55.3	0	0	0	0	4.4	14.7	15.6	3.8	0	0	0	0	4.1	2.2	0	0
P. persica	27	92.6	0	0	0	0	0	0	0	0	0	0	0	0	7.4	0	0	0

0: Clean, 1: Low infestation, 2: Small colony, 3: Large colony, 4: Heavy infestation.

In the orchards of Çermik district of Diyarbakır, only the *P. corni* was detected on almond, apricot, apple, peach plum, quince and walnut trees. *Parthenolecanium corni* could infest at a scale 3 density level besides scale 1 and scale 2 on apple and walnut trees in Çermik. The pest was commonly seen in the orchards of Çermik district, it could form dense populations and therefore it is a potential pest that could cause economic damage in future.

Table 4. Density levels and infestation rates of the Coccidae species in Çermik

Host plant	Number of trees sampled	Infestation rate (%)				
		Parthenolecanium corni				
		0	1	2	3	4
C. oblonga	27	77.8	7.4	14.8	0	0
J. regia	34	82.4	0	11.8	5.9	0
M. domestica	64	64.1	17.2	12.5	6.3	0
P. vera	108	100.0	0	0	0	0
P. armeniaca	65	61.5	9.2	29.2	0	0
P. avium	41	100.0	0	0	0	0
P. domestica	35	68.6	8.6	22.9	0	0
P. dulcis	81	91.4	8.6	0	0	0
P. persica	22	81.8	18.2	0	0	0

0: Clean, 1: Low infestation, 2: Small colony, 3: Large colony, 4: Heavy infestation.

In Çüngüş district of Diyarbakır, *D. unifasciatus* and *P. corni* were detected. it was found that 2.4% of the examined almond trees were infested at scale 1, 1.2% at scale 2, 2.4% at scale 3 density and 3.6% of the peach trees were infested at scale 2 with *D. unifasciatus*. *Parthenolecanium corni* was found at different infestation rates and density levels (Scale1,2,3) on its host plants (Table 5). Consequently, both of these soft scale insects did not have heavy population density which can cause serious damage on their hosts.

Table 5. Density levels and infestation rates of the Coccidae species in Çüngüş

Host plant	Number of trees sampled	Infestation rate (%)								
		Didesmococcus unifascitus					*Parthenolecanium corni*			
		0	1	2	3	4	1	2	3	4
C. oblonga	24	100.0	0	0	0	0	0	0	0	0
J. regia	41	82.9	0	0	0	0	7.3	9.8	0	0
M. domestica	81	74.1	0	0	0	0	3.7	13.6	8.6	0
P. vera	22	90.9	0	0	0	0	9.1	0	0	0
P. armeniaca	48	89.6	0	0	0	0	10.4	0	0	0
P. avium	168	98.8	0	0	0	0	1.2	0	0	0
P. domestica	44	68.2	0	0	0	0	13.6	18.2	0	0
P. dulcis	84	83.3	2.4	1.2	2.4	0	8.3	2.4	0	0
P. persica	55	90.9	0	3.6	0	0	5.5	0	0	0

0: Clean, 1: Low infestation, 2: Small colony, 3: Large colony, 4: Heavy infestation.

Eulecanium tiliae and *P. corni* were identified as a result of the surveys carried out in Eğil district of Diyarbakır. It was determined that 3.6% of the examined almond trees were infested at scale 1 and 1.4% at scale 2 density with *P. corni* and 2.3% of pistachios were also infested at scale 1 density with *E. tiliae* (Table 6). Infestation rates and population densities of both pests were low in Eğil district.

Table 6. Density levels and infestation rates of the Coccidae species in Eğil

Host plant	Number of trees sampled	Infestation rate (%)								
		Eulecanium tiliae					*Parthenolecanium corni*			
		0	1	2	3	4	1	2	3	4
P. vera	85	97.7	2.3	0	0	0	0	0	0	0
P. domestica	83	100.0	0	0	0	0	0	0	0	0
P. dulcis	560	95.0	0	0	0	0	3.6	1.4	0	0

0: Clean, 1: Low infestation, 2: Small colony, 3: Large colony, 4: Heavy infestation.

Parthenolecanium corni, *P. vitis* and *S. prunastri* were determined in the Ergani district of Diyarbakır. *Parthenolecanium corni* could create scale 3 population density besides scale 1 and scale 2 on apple and walnut trees, it was also found at low population densities such as scale 1 and scale 2 on almond, apricot, cherry, peach and plum trees in Ergani. Moreover, it was determined that 5.1% of the examined plum trees were infested at scale 1 and 2.6% at scale 2 density with *S. prunastri* and 1.3% of walnut trees were also infested at scale 1 density with *P. vitis* (Table 7). *Pulvinaria vitis* was found only on walnut in Ergani, in study areas.

Table 7. Density levels and infestation rates of the Coccidae species in Ergani

Host plant	Number of trees sampled	Infestation rate (%)												
		Parthenolecanium corni					*Pulvinaria vitis*				*Sphaerolecanium prunastri*			
		0	1	2	3	4	1	2	3	4	1	2	3	4
C. oblonga	21	100.0	0	0	0	0	0	0	0	0	0	0	0	0
J. regia	77	74.0	14.3	7.8	2.6	0	1.3	0	0	0	0	0	0	0
M. domestica	151	83.4	8.0	6.0	2.7	0	0	0	0	0	0	0	0	0
P. armeniaca	143	64.3	25.2	10.5	0	0	0	0	0	0	0	0	0	0
P. avium	97	96.9	3.1	0	0	0	0	0	0	0	0	0	0	0
P. cerasus	14	100.0	0	0	0	0	0	0	0	0	0	0	0	0
P. domestica	39	61.5	15.4	15.4	0	0	0	0	0	0	5.1	2.6	0	0
P. dulcis	137	93.4	4.4	2.2	0	0	0	0	0	0	0	0	0	0
P. persica	42	100.0	0	0	0	0	0	0	0	0	0	0	0	0

0: Clean, 1: Low infestation, 2: Small colony, 3: Large colony, 4: Heavy infestation.

Eulecanium tiliae, *P. corni*, and *S. prunastri* were determined in Baskil district of Elazığ. It was detected that 6.2% of plum trees were infested at scale 1, 3.6% at scale 2 and 0.4% of the apricot trees were infested at scale 1 with *E. tiliae*. It did not reach population densities that could cause damage on its hosts. As in other districts, *P. corni* was the most common species and have the largest host range in Baskil, but it could

not create dense populations on its hosts. The density of *S. prunastri* was scale 1 in 14.2%, scale 2 in 11.4%, scale 3 in 4.6% and scale 4 in 3.3% of the examined apricot trees. it was also scale 1 in 6.2%, scale 2 in 10,6%, scale 3 in 1.8%, scale 4 in 0.9% of the plum trees (Table 8).

Table 8. Density levels and infestation rates of the Coccidae species in Baskil

Host plant	Number of trees sampled	Infestation rate (%)												
		Eulecanium tiliae					*Parthenolecanium corni*				*Sphaerolecanium prunastri*			
		0	1	2	3	4	1	2	3	4	1	2	3	4
J. regia	33	84.9	0	0	0	0	9.1	6.1	0	0	0	0	0	0
M. domestica	38	81.6	0	0	0	0	10.5	5.3	2.6	0	0	0	0	0
P. armeniaca	1297	60.7	0.4	0	0	0	3.7	1.7	0	0	14.2	11.4	4.6	3.3
P. domestica	113	53.1	6.2	3.5	0	0	8.0	9.7	0	0	6.2	10.6	1.8	0.9
P. persica	39	100.0	0	0	0	0	0	0	0	0	0	0	0	0

0: Clean, 1: Low infestation, 2: Small colony, 3: Large colony, 4: Heavy infestation.

In the Keban district of Elazığ, *P. corni* and *P. vitis* species were detected. *Pulvinaria vitis* was found only on walnut trees and it was determined that 0.9% of the 114 trees examined were infested with the pest at scale 1 density. *Parthenolecanium corni* was generally detected at scale 1 and scale 2 density levels on almond, apricot, apple, cherry, persimmon and walnut trees (Table 9). Both pests could not create dense populations, and thus did not cause any economic damage in Keban.

Table 9. Density levels and infestation rates of the Coccidae species in Keban

Host plant	Number of trees sampled	Infestation rate (%)								
		Parthenolecanium corni					*Pulvinaria vitis*			
		0	1	2	3	4	1	2	3	4
D. kaki	11	81.8	18.2	0	0	0	0	0	0	0
J. regia	114	86.8	10.5	1.8	0	0	0.9	0	0	0
M. domestica	88	88.6	9.1	2.3	0	0	0	0	0	0
P. armeniaca	189	96.3	3.7	0	0	0	0	0	0	0
P. avium	23	100.0	0	0	0	0	0	0	0	0
P. dulcis	212	98.6	1.4	0	0	0	0	0	0	0

0: Clean, 1: Low infestation, 2: Small colony, 3: Large colony, 4: Heavy infestation.

In the studies carried out in the Sivrice district of Elazığ, *D. unifasciatus*, *P. bituberculatum* and *P. corni* species were found. The density of *D. unifasciatus* was scale 1 in 7.7%, scale 2 in 5.8%, scale 3 in 3.9% and scale 4 in 1% of the examined almond trees. *Didesmococcus unifasciatus* was observed to cause drying in the branches and shoots of the trees, which are heavily infested. *P. bituberculatum* was determined only in apple trees and its density was scale 1 in 3.6%, scale 2 in 2.8%, scale 3 in 2% of the examined apple trees. *Parthenolecanium corni* was able to form scale 3 population density besides scale 1 and scale 2 on apple, it was also detected at low population densities such as scale 1 and scale 2 on the almond, apricot, cherry, peach, plum and sour cherry trees (Table 10).

Table 10. Density levels and infestation rates of the Coccidae species in Sivrice

Host plant	Number of trees sampled	Infestation rate (%)												
		Parthenolecanium corni					*Palaeolecanium bituberculatum*				*Didesmococcus unifasciatus*			
		0	1	2	3	4	1	2	3	4	1	2	3	4
M. domestica	252	84.5	5.6	1.2	0.4	0	3.6	2.8	2.0	0	0	0	0	0
P. avium	168	91.1	5.4	3.6	0	0	0	0	0	0	0	0	0	0
P. dulcis	104	73.1	5.8	2.9	0	0	0	0	0	0	7.7	5.8	3.9	1.0
J. regia	78	100.0	0	0	0	0	0	0	0	0	0	0	0	0
P. armeniaca	64	100.0	0	0	0	0	0	0	0	0	0	0	0	0
P. domestica	59	74.6	18.6	6.8	0	0	0	0	0	0	0	0	0	0
P. cerasus	48	100.0	0	0	0	0	0	0	0	0	0	0	0	0
P. persica	38	97.4	2.6	0	0	0	0	0	0	0	0	0	0	0

0: Clean, 1: Low infestation, 2: Small colony, 3: Large colony, 4: Heavy infestation.

Eulecanium tiliae, *P. bituberculatum*, *P. corni*, *P. vitis* and *S. prunastri* were determined in the studies carried out in the central district of Elazığ (Table 11). It was determined that 0.5% of the examined apple trees were infested with *E. tiliae* at a scale 1 density, 4.26% of plum trees at a scale 2 density, and 3.85% of quince trees at a scale 1 density. *Palaeolecanium bituberculatum* was detected only in apple trees and the density of it was scale 1 in 2.1%, scale 2 in 1% of the examined apricot trees. *Parthenolecanium corni* was generally found at low infestation rates and density levels (Scale1 and 2) on the apple, apricot, cherry, peach, plum, walnut trees. 3.9% and 0.8% of the walnut trees were infested with *P. vitis* at scale 1 and scale 2, respectively. The density of *S. prunastri* was scale 1 in 6%, scale 2 in 8%, scale 3 in 4.4% and scale 4 in 3.8% of the examined apricot trees (Table 10). The highest number of Coccidae species were found in Elazığ Central district. Considering the population densities in the orchards and the drying of the branches and shoots, as well as the damages such as sooty moulds, which are caused by the honeydew they secrete, *S. prunastri* was the most destructive species in the region.

Table 11. The density levels and infestation rates of the Coccidae species in Central district of Elazığ

Host plant	Number of trees sampled	Infestation rate (%)																			
		Eulecanium tiliae					*Palaeolecanium bituberculatum*				*Parthenolecanium corni*				*Pulvinaria vitis*				*Sphaerolecanium prunastri*			
		0	1	2	3	4	1	2	3	4	1	2	3	4	1	2	3	4	1	2	3	4
C. oblonga	26	96.2	3.9	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
J. regia	390	76.9	0	0	0	0	0	0	0	0	15.1	3.3	0	0	3.9	0.8	0	0	0	0	0	0
M. domestica	195	84.1	0.5	0	0	0	2.1	1.0	0	0	7.7	3.6	1.0	0	0	0	0	0	0	0	0	0
P. armeniaca	552	70.7	0	0	0	0	0	0	0	0	5.8	1.5	0	0	0	0	0	0	6.0	8.0	4.4	3.8
P. avium	105	95.2	0	0	0	0	0	0	0	0	1.9	2.9	0	0	0	0	0	0	0	0	0	0
P. cerasus	28	96.4	0	0	0	0	0	0	0	0	3.6	0	0	0	0	0	0	0	0	0	0	0
P. domestica	47	87.2	0	4.3	0	0	0	0	0	0	6.4	2.1	0	0	0	0	0	0	0	0	0	0
P. persica	39	97.4	0	0	0	0	0	0	0	0	2.6	0	0	0	0	0	0	0	0	0	0	0

0: Clean, 1: Low infestation, 2: Small colony, 3: Large colony, 4: Heavy infestation.

Sphaerolecanium prunastri and *D. unifasciatus* were the most important Coccidae species among the species detected in the survey districts as both could infest their own host plants heavily (density of scale 4), and caused drying of shoot and branches. It is thought that because the number of apricot trees is very high compared to other districts, apricot orchards are established in certain regions, and these orchards are mostly very close to each other, *S. prunastri* can spread and create dense populations in the apricot production areas in the Central and Baskil districts. In many studies carried out in Türkiye, it has been reported that *S. prunastri* substantially gives rise to damage on stone fruit such as peach, plum and apricot (Kılıç & Aykaç 1989; Zeki et al., 2004; Özgen & Bolu, 2009; Akşit & Apak, 2013; Yiğit, 2013; Ayaz et al., 2015). Özgen & Bolu (2009) stated that the highest infestation rate and population density of *S. prunastri* was in the apricot areas in the Yazıhan district of Malatya province. In addition, they detected that 3.14% of the trees examined in the district were infested with *S. prunastri* at low level, 7.05% at medium level and 2.86% at high level. Akşit & Apak (2013) reported that 2.26% of the examined 1942 trees were infested with *S. prunastri* at scale 1 (low infestation), 2.57% at scale 2 (small colony), 3.34% at scale 3 (large colony) and 8.67% at scale 4 (heavy infestation) in the plum orchards of Aydın.

Didesmococcus unifasciatus was previously detected on peach and almond in Eastern and Southeastern Anatolia regions (Kaydan & Kozár, 2010; Bolu, 2012). However, this study revealed that *D. unifasciatus* has the potential to create overpopulation on those host plants and can cause problems especially in almond production areas.

Coccidae species, which is in the all the study districts and have the broadest host range was *P. corni*. Although *P. corni* can form large colonies (scale 3) at limited rates in some hosts such as apple and walnut, it was observed that it cannot form heavy infestation in general and does not cause significant damage to its host plants. However, *Parthenolecanium corni* is a species that should be observed and kept

under control because it is a polyphagous species and can form large colonies in some hosts. *A. pistaciae, E. tiliae, P. vitis, P. bituberculatum* were seen as rare species and they did not cause any economic damage in the surveyed districts.

As a result of this study, seven the soft scale insect species were identified with information on their host plants, distribution areas, densities and infestation rates on the fruit orchards of Diyarbakır and Elazığ Provinces. The findings of this study will contribute to revealing Coccidae fauna of Southeastern Anatolia and Eastern Anatolia Regions, understanding potential damage they can cause and development of measures for their control. It will be of great benefit to carry out detailed studies on the bioecology and control of *S. prunastri* and *D. unifasciatus,* which are were determined to be important pest species in the studied area.

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Original article (Orijinal araştırma)

Tachinid (Diptera: Tachinidae) parasitoids reared from some hemipterous hosts from Türkiye

Türkiye'deki bazı hemipter konukçulardan elde edilen tachinid (Diptera: Tachinidae) parazitoitler

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Abstract

This study was carried out to determine tachinid (Diptera: Tachinidae) parasitoids obtained from some hemipterous hosts in Adana, Ankara, Hatay, Konya and Uşak provinces of Türkiye between 2001 and 2020. For this purpose, adult hemipters were collected from perennial arboreal and annual herbaceous plants. The samples were brought to the laboratory and cultured on the relevant host plant material. As a result of the study, 11 tachinid species were determined from 10 different hemipterous hosts. Of these, four parasitoid-host couples are new records. These were *Aelia rostrata* Boheman, 1852 (Hemiptera: Pentatomidae) and *Scantius aegyptius* (L., 1758) (Hemiptera: Pyrrhocoridae) for *Gymnosoma clavata* (Rohdendorf, 1947) (Diptera: Tachinidae), *Dolycoris baccarum* (L., 1758) (Hemiptera: Pentatomidae) for *Eulabidogaster setifacies* (Rondani, 1861) (Diptera: Tachinidae) and *Piezodorus lituratus (*Fabricius, 1794) *(*Hemiptera: Pentatomidae) for *Cylindromyia rubida (*Loew, 1854) (Diptera: Tachinidae). Also, eight new hosts were reported for the first time in Türkiye. In addition, host information and distributions of each parasitoid are presented in the study.

Keywords: Host records, new record, parasitoid, Tachinidae, Türkiye

Öz

Bu çalışma, 2001 ve 2020 yılları arasında Türkiye'nin Adana, Ankara, Hatay, Konya ve Uşak illerinde bazı hemipter konukçulardan elde edilen tachinid (Diptera: Tachinidae) parazitoitleri belirlemek amacıyla yürütülmüştür. Bu amaçla ergin hemipterler konukçu bitkileri olan çok yıllık ağaçsı ve tek yıllık otsu bitkiler üzerinden toplanmıştır. Örnekler laboratuvara getirilip ilgili konukçu bitki materyali üzerinde kültüre alınmıştır. Çalışma sonucunda, 10 farklı hemipter konukçudan 11 tachinid türü tespit edilmiştir. Yürütülmüş olan çalışmalar ile 10 adet farklı hemipter olan konukçudan 11 adet farklı tachinid türü belirlenmiştir. Bunlardan dört parazitoit-konukçu çifti yeni kayıt niteliğindedir. Bunlar; *Gymnosoma clavata* (Rohdendorf, 1947) (Diptera: Tachinidae) için *Aelia rostrata* Boheman, 1852 (Hemiptera: Pentatomidae) ve *Scantius aegyptius* (L., 1758) (Hemiptera: Pyrrhocoridae), *Eulabidogaster setifacies* (Rondani, 1861) (Diptera: Tachinidae) için *Dolycoris baccarum* (L., 1758) (Hemiptera: Pentatomidae) ve *Cylindromyia rubida (*Loew, 1854) (Diptera: Tachinidae) için *Piezodorus lituratus (*Fabricius, 1794) *(*Hemiptera: Pentatomidae)'dur. Ayrıca Türkiye'de sekiz yeni konukçu ilk kez kayıt edilmiştir. Buna ek olarak, bu çalışmada her bir parazitoitin konukçu bilgileri ve dağılımları sunulmuştur.

Anahtar sözcükler: Konukçu kayıtları, yeni kayıt, parazitoit, Tachinidae, Türkiye

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Introduction

Tachinids (Diptera: Tachinidae) are one of the largest groups of the order Diptera with 8.592 species (O'Hara et al., 2021). This family includes 341 species in Türkiye (Kara et al., 2020). Three more species have been added to this in a study recently published by Soykan & Atay (2022). All tachinids live as endoparasites in various insects. These parasitoid insects were used by inoculative, augmentative or inundative releases and sometimes great successes were obtained (Grenier, 1988). It is known that tachinids play an important role in suppressing the populations of important pests found in both agricultural and forest areas under natural conditions. Their hosts are larvae, nymphs and adults of important pest species, especially those belonging to the orders Lepidoptera, Heteroptera and Coleoptera. In addition, they have a broad oviposition strategy that allows them to parasitize hosts in a variety of habitats (Cerretti & Tschorsnig, 2010; Dindo & Grenier, 2014). For this reason, they have attracted attention in biological control studies and, more than 100 tachinids species have been employed in biological control programs against different insect pests. Some of these programs have been partially or completely successful. For example, since the early 1900s, *Exorista larvarum* (L., 1758) and *Exorista japonica* (Townsend, 1909) (Diptera: Tachinidae) have been introduced several times against *Lymantria dispar* (L., 1758) (Lepidoptera: Lymantridae) into the Northern United States and only *E. larvarum* has established (Sabrosky & Reardon, 1976). Both in our country and many other countries, it has been determined that there is a high parasitization of Sunn pests, *Eurygaster* spp. (Hemiptera: Scutelleridae). For example, in a study conducted in İslahiye district of Gaziantep province in our country, it was reported that overwintered adults collected in wheat fields during the nymphal survey period parasitized at a rate of 40.7% (Tarla, 2002). Similarly, a study carried out in Iran by Amir-Maafi (1991), it was reported that up to 66.9% of Sunn pests were parasitized by adult parasitoids. In this context, studies on host identification and parasitization strategies of tachinid flies may provide useful information for their use as a biocontrol agent today and in the future.

Numerous studies have been conducted on tachinids and their hosts in the Palearctic region, including Türkiye. The most comprehensive catalog of Palearctic region has been recently produced by Tschorsnig (2017). It consists of 827 tachinid species reared from 2672 arthropod hosts belonging to eleven insect orders and one chilopod order. The host-parasitoid pairs of Turkish tachinids are unfortunately not well known. Kara & Alaoğlu (2001), Atay & Kara (2014), Atay et al. (2018) and Aytar et al. (2021) conducted detailed studies to determine the hosts of tachinids in Türkiye. In addition, two detailed catalogs of tachinid-host couples were prepared by Kara & Tschorsnig (2003) and Kara et al. (2014) in Türkiye.

This paper focused on the tachinid parasitoids of some species belonging to the order Hemiptera in different provinces of Türkiye.

Materials and Methods

The study was conducted in Adana, Ankara, Hatay, Konya and Uşak provinces of Türkiye between 2001 and 2020. Hosts were collected from agricultural and forest areas. The collected insects were brought to the laboratory for rearing with the plants they fed on and transferred to distinct rearing boxes and kept at 25±2°C and 60-70% relative humidity. A periodic check of boxes was carried out and the food was replaced when required.

After the adult parasitoids hatched, tachinids were prepared for identification and described according to Herting (1983), Tschorsnig & Herting (1994), Tschorsnig & Richter (1998), Zimin (1966), Gilasian et al. (2013) and Sahebari et al. (2016). The tachinids presented in the study were identified by the second and third authors. Herting & Dely-Draskovits (1993) followed for Tachinidae species nomenclature. New tachinid/ host-couple records were confirmed by Dr. H.-P. Tschorsnig (Staatliches Museum für Naturkunde, Stuttgart, Germany). Pentatomidae and Scutelleridae species were identified by the first author

and Pyrrhocoridae species was identified by Dr. Gülten Yazıcı (Directorate of Plant Protection Central Research Institute, Ankara-Türkiye).

The date of emergence, the number of males and females, hosts, host locations and the plants were presented separately. In addition, the distribution and hosts (in Türkiye) of reared parasitoids were described.

Results and Discussion

As a result of the examination of the samples, 11 different Tachinidae species were reared as parasitoids of 10 hemipteran hosts.

Subfamily: Phasiinae

Tribe: Phasiini

Clytiomya continua (Panzer, 1798)

Reared specimen. 27.V.2020, ♂, reared from *Eurydema ornata* (L., 1758) (Hemiptera: Pentatomidae), collected in Uşak: Sivaslı, 38°29'24"N, 29°41'57"E, 999 m.

Distribution in Türkiye. Tokat (Kara, 1998; Atay, 2011; Atay & Kara, 2014; Lekin, 2014; Lekin et al., 2016), Eskişehir (Aksu, 2005), Sakarya (Balkan, 2014; Balkan et al., 2015), Karabük (Atay, 2017).

Host in Türkiye. *Eurydema ornata* (Atay & Kara, 2014).

Remarks. Pentatomidae (Hemiptera) (especially *Eurydema* spp.) is the usual host family for this parasitoid. In addition, the host range includes several species of Scutelleridae, Coreidae, and Ciydnidae (Hemiptera) (Tschorsnig, 2017).

Clytiomya sola (Rondani, 1861)

Reared specimens. 31.V.2010, ♀, reared from *Ventocoris fischeri* (Herrich-Schaeffer, 1851) (Hemiptera: Pentatomidae), collected in Uşak: Sivaslı, 38°33'00"N, 29°37'03"E, 786 m; 16.III.2011, ♂, reared from *Dolycoris baccarum* (L., 1758) (Hemiptera: Pentatomidae), collected in Uşak: Sivaslı, 38°32'27"N, 29°38'32"E, 830 m.

Distribution in Türkiye. Konya (Tuatay et al., 1972), Manisa, İzmir (Karsavuran & Kara, 2003), Çorum (Uysal, 2018; Uysal & Atay, 2021), Muğla (Lutovinovas et al., 2018).

Host in Türkiye. *Ancyrosoma leucogrammes* (Gmelin, 1790) (Hemiptera: Pentatomidae) (Karsavuran & Kara, 2003), *Carpocoris* sp. (Hemiptera: Pentatomidae) (Tuatay et al., 1972), *Graphosoma lineatum* (L., 1758) (Hemiptera: Pentatomidae) (Kara & Tschorsnig, 2003).

Remarks. *Ventocoris fischeri* and *D. baccarum* are new hosts for *C. sola* in Türkiye. Pentatomidae (Hemiptera) is the usual host family for this parasitoid and commonly was reared host of this family. In addition, the host range includes several species of Reduviidae and Scutelleridae (Hemiptera) (Tschorsnig, 2017).

Ectophasia oblonga (Robineau-Desvoidy, 1830)

Reared specimens. 20.IV.2007, ♀, reared from *Eurygaster integriceps* Puton, 1881 (Hemiptera: Scutelleridae), collected in Hatay: Reyhanlı, 36°15'37"N, 36°30'05"E, 94 m; 27.V.2020, ♂, 02.VI.2020, ♂, reared from *E. ornata*, collected in Uşak: Sivaslı, 38°29'24"N, 29°41'57"E, 999 m and 38°31'58"N, 29°39'55"E, 913 m.

Distribution in Türkiye. Diyarbakır (Dupuis, 1963), Adana (Herting & Tschorsnig, 1993), Ankara (Memişoğlu & Özer, 1994), Tekirdağ (Öncüer & Kıvan, 1995; Kıvan, 1996), Tokat (Kara, 1998; Atay, 2011; Atay & Kara, 2014; Lekin, 2014; Lekin et al., 2016), Gaziantep, Kahramanmaraş, Kilis (İslamoğlu & Kornoşor, 2003, 2007), Eskişehir (Aksu, 2005), Bartın, Karabük, Kastamonu, Zonguldak (Korkmaz, 2007), Adıyaman, Batman, Diyarbakır, Mardin, Siirt, Şanlıurfa (Gözüaçık et al., 2010), Kastamonu (Atay, 2017), Çorum (Uysal, 2018; Uysal & Atay, 2021), Burdur, Muğla (Lutovinovas et al., 2018).

Host in Türkiye. *Eurygaster integriceps* (Dupuis, 1963; Yüksel, 1968; Herting & Tschorsnig, 1993; Öncüer & Kıvan, 1995; Kıvan, 1996; İslamoğlu & Kornoşor, 2003, 2007; Gözüaçık et al., 2010), *Eurygaster maura* (L., 1758) (Memişoğlu & Özer, 1994), *Lygaeus equestris* (L., 1758) (Hemiptera: Lygaedidae) (Kara & Alaoğlu, 1999), *Aelia* sp., *D. baccarum* (Kara & Tschorsnig, 2003); *E. ornata* (Kara & Tschorsnig, 2003; Atay & Kara, 2014).

Remarks. Scutelleridae and Pentatomidae (Hemiptera) are the usual host families for this parasitoid. In addition, the host range includes several species of Lygaeidae, Coreidae, Alydidae, Acanthosomatidae, Reduviidae, Rhopalidae and Stenocephalidae (Hemiptera) (Tschorsnig, 2017).

Gymnosoma clavata (Rohdendorf, 1947)

Reared specimens. 15.IV.2011, ♂, reared from *Aelia rostrata* Boheman, 1852 (Hemiptera: Pentatomidae), collected in Uşak: Sivaslı, 38°29’20”N, 29°41’40”E, 976 m; 19.V.2011, ♀, 23.VI.2016, ♂, 14.III.2019, ♀, reared from *D. baccarum*, collected in Uşak: Central District, 38°41’58”N, 29°20’17”E, 949 m, 38°40’27”N, 29°20’09”E, 896 m and 38°40’19”N, 29°19’17”E, 870 m; 24.V.2011, ♂,12.VI.2016, ♀, reared from *D. baccarum*, collected in Uşak: Sivaslı, 38°29’51”N, 29°41’33”E, 973 m and 38°30’53”N, 29°41’26”E, 956 m; 25.IV.2017, ♀, reared from *Carpocoris* sp. (Hemiptera: Pentatomidae), collected in Uşak: Sivaslı, 38°29’26”N, 29°42’08”E, 1.012 m; 07.VII.2018, ♂, reared from *Scantius aegyptius* (L., 1758) (Hemiptera: Pyrrhocoridae) collected in Uşak: Sivaslı, 38°31’38”N, 29°39’59”E, 928 m.

Distribution in Türkiye. Erzurum (Doğanlar, 1982), İzmir (Karsavuran, 1986; Herting & Tschorsnig, 1993; Karsavuran & Kara, 2003), Tokat (Kara, 1998; Atay, 2011; Atay & Kara, 2014; Lekin, 2014; Lekin et al., 2016), Eskişehir (Aksu, 2005), Antalya, Burdur (Keçeci et al., 2007), Karabük (Korkmaz, 2007; Atay, 2017), Kastamonu (Atay, 2017), Sakarya (Balkan, 2014; Balkan et al., 2015), Muğla (Lutovinovas et al., 2018), Çorum (Uysal, 2018; Uysal & Atay, 2021).

Host in Türkiye. *Dolycoris baccarum* (Karsavuran, 1986; Herting & Tschorsnig, 1993; Kara & Tschorsnig, 2003; Keçeci et al., 2007), *Carpocoris* sp. (Herting & Tschorsnig, 1993), *A. leucogrammes* (Karsavuran & Kara, 2003), *Carpocoris fuscispinus* (Boheman, 1850) (Hemiptera: Pentatomidae) (Atay, 2011; Atay & Kara, 2014).

Remarks. Pentatomidae (Hemiptera) is the usual host family for *G. clavata* and it is commonly reared from *D. baccarum*. *A. rostrata* and *S. aegyptius* are new hosts for this tachinid. In addition, this is the first record of a tachinid being reared from a host belonging to the family Pyrrhocoridae (Tschorsnig, 2017).

Cistogaster globosa (Fabricius, 1775)

Reared specimen. 26.V.2004, ♀, reared from *A. rostrata*, collected in Konya: Sarayönü, 38°17’23”N, 32°29’55”E,1.063 m.

Distribution in Türkiye. Manisa (Soykan, 2021; Soykan & Atay, 2022).

Hosts in Türkiye. Unknown.

Remarks. *A. rostrata* is new host for *C. globosa* in Türkiye. This tachinid commonly parasites *Aelia* spp. (Pentatomidae). In addition, the host range includes only one species of Scutelleridae (Tschorsnig, 2017).

Elomya lateralis (Meigen, 1824)

Reared specimen. 05.IV.2015, ♂, reared from *E. maura*, collected in Ankara, 39°50’06”N, 32°53’26”E,1.164 m.

Distribution in Türkiye. Tokat (Kara & Alaoğlu, 1999; Atay & Kara, 2014), Ankara (Brown, 1962; Memişoğlu & Özer, 1994), Central Anatolia (Dikyar, 1981), Konya (Memişoğlu et al., 1994; Tschorsnig, 2017), Adana (Tschorsnig, 2017), Tekirdağ (Kıvan, 1996; Tschorsnig, 2017), Diyarbakır (Lodos, 1961; Dupuis, 1963; Gözüaçık et al., 2010; Duman & Sertkaya, 2015), Gaziantep, Kilis (İslamoğlu & Kornoşor, 2003), Kahramanmaraş (İslamoğlu & Kornoşor, 2007), Mardin, Siirt, Şanlıurfa (Gözüaçık et al., 2010).

Hosts in Türkiye. *Ceraleptus gracilicornis (*Herrich-Schäffer, 1835). (Hemiptera: Coreidae) *(*Kara & Alaoğlu, 1999), *A. rostrata* (Brown, 1962; Dikyar, 1981; Memişoğlu et al., 1994), *D. baccarum* (Kara & Tschorsnig, 2003), *E. ornata* (Tschorsnig, 2017), *Corizus hyoscyami* (L., 1758) (Hemiptera: Rhopalidae) (Atay & Kara, 2014), *E. integriceps* (Lodos, 1961; Dupuis, 1963; Lodos, 1986; Tschorsnig, 2017; Öncüer & Kıvan, 1995; Kıvan, 1996; İslamoğlu & Kornoşor, 2003, 2007; Gözüaçık et al., 2010; Duman & Sertkaya, 2015), *E. maura* (Memişoğlu & Özer, 1994; Tschorsnig, 2017).

Remarks. *E. lateralis* commonly parasites *Eurygaster* spp. Pentatomidae and Scutelleridae are the usual host families for this parasitoid. In addition, the host range includes several species of Lygaeidae, Coreidae, Acanthosomatidae, Alydidae, and Rhopalidae (Hemiptera) (Tschorsnig, 2017).

Phasia subcoleoptrata (L., 1767)

Reared specimens. 28.IV.2007, ♂, reared from *E. integriceps*, collected in Hatay: Reyhanlı, 36°15’45”N, 36°29’36”E, 96 m.

Distribution in Türkiye. Diyarbakır (Lodos, 1952, 1961; Brown, 1962; Dupuis, 1963; Sun & Marshall, 2003; Duman & Sertkaya, 2015), Konya (Tuatay et al., 1972), Ankara (Memişoğlu & Özer, 1994), Adana, Antalya, Gaziantep, Hatay, Kahramanmaraş, Mersin (Şimşek et., 1994), Tekirdağ (Öncüer & Kıvan 1995; Kıvan, 1996), Gaziantep, Kilis (İslamoğlu & Kornoşor, 2003), Kahramanmaraş (İslamoğlu & Kornoşor, 2007), Antalya, Burdur (Keçeci et al., 2007), Adıyaman, Batman, Diyarbakır, Mardin, Siirt, Şanlıurfa, Şırnak (Gözüaçık et al., 2010), Adıyaman, Gaziantep, Hatay (Gün, 2010), Sivas (Atay, 2011; Atay & Kara, 2014), Şanlıurfa (Duman et al., 2015).

Hosts in Türkiye. *E. integriceps* (Lodos, 1952, 1986; Brown, 1962; Dupuis, 1963; Şimşek et al., 1994; Öncüer & Kıvan, 1995; İslamoğlu & Kornoşor, 2003; Sun & Marshall, 2003; İslamoğlu & Kornoşor, 2007; Keçeci et al., 2007; Gözüaçık et al., 2010; Gün, 2010; Duman & Sertkaya, 2015; Duman et al., 2015; Tschorsnig, 2017), *E. maura* (Lodos, 1961; Tuatay et al., 1972; Memişoğlu & Özer, 1994; Atay, 2011; Atay & Kara, 2014).

Remarks. This tachinid commonly was reared from *Eurygaster* spp. (Tschorsnig, 2017).

Tribe: Leucostomatini

Eulabidogaster setifacies (Rondani, 1861)

Reared specimens. 08.VIII.2010, ♂, reared from *D. baccarum*, collected in Uşak: Sivaslı, 38°27’25”N, 29°41’44”E, 985 m.

Distribution in Türkiye. Tokat (Kara, 1998; Kara & Alaoğlu, 1999; Atay, 2011; Atay & Kara, 2014); Zonguldak (Korkmaz, 2007), Kastamonu (Atay, 2017), Muğla (Lutovinovas et al, 2018).

Hosts in Türkiye. *Corizus hyoscyami* (Hemiptera: Rhopalidae) (Atay, 2011; Atay & Kara, 2014).

Remarks. *D. baccarum* is a new host for this tachinid. The host range of this species is rather narrow. It was reared only from *C. hyoscyami* (Tschorsnig, 2017).

Tribe: Cylindromyiini

Cylindromyia rubida (Loew, 1854)

Reared specimens. 04.X.2001, ♀, reared from *Piezodorus lituratus* (Fabricius, 1794) *(*Hemiptera: Pentatomidae), collected in Adana. Balcalı, 37°03’028”N, 35°21’31”E, 136 m, on *Firaxinus* sp. (Oleaceae).

Distribution in Türkiye. İzmir (Çerçi, 2017).

Hosts in Türkiye. Unknown.

Remarks. *P. lituratus* is a new host for this tachinid. It is also the first record of *C. rubida* reared from a host.

Cylindromyia brassicaria (Fabricius, 1775)

Reared specimens. 19.III.2002, ♀, reared from *D. baccarum*, collected in Adana: Balcalı, 37°03'07"N, 35°21'46"E, 116 m; 27.V.2017, ♂, 16.IV.2019, ♂, reared from *D. baccarum*, collected in Uşak: Central District. 38°41'59"N, 29°20'18"E, 944 m and 38°38'56"N, 29°19'21"E, 815 m.

Distribution in Türkiye. Erzurum (Doğanlar, 1982), İzmir (Karsavuran, 1986), Tokat (Kara, 1998; Kara & Alaoğlu, 1999; Atay, 2011; Atay & Kara, 2014; Lekin, 2014; Lekin et al., 2016), Eskişehir (Aksu, 2005), Antalya, Burdur (Keçeci et al., 2007; Kastamonu (Atay, 2017); Çorum (Uysal, 2018; Uysal & Atay, 2021); Aydın, Muğla (Lutovinovas et al., 2018).

Hosts in Türkiye. *Dolycoris baccarum* (Karsavuran, 1986; Kara & Tschorsnig, 2003; Keçeci et al., 2007; Atay, 2011; Atay & Kara, 2014), *Holcostethus vernalis* (Wolff, 1804) (Hemiptera: Pentatomidae) (Kara & Alaoğlu, 1999).

Remarks. Pentatomidae (Hemiptera) is the usual host family for *C. brassicaria.* It is commonly reared from *D. baccarum*. In addition, it has a few hosts from Scutelleridae (Hemiptera) (Tschorsnig, 2017).

Cylindromyia pilipes (Loew, 1844)

Reared specimens. 17.IX.2002, ♂, reared from *H. vernalis*, collected in Adana: Balcalı, 37°03'07"N, 35°21'34"E, 113 m.

Distribution in Türkiye. Bursa, İstanbul (Herting, 1984; Herting & Dely-Draskovits, 1993), Bartın, Kastamonu (Atay, 2017), Burdur (Lutovinovas et al., 2018), Çorum (Uysal, 2018; Uysal & Atay, 2021).

Hosts in Türkiye. Unknown.

Remarks. *H. vernalis* is new host for *C. pilipes* in Türkiye. *C. pilipes* has a narrow host range and Pentatomidae (Hemiptera) is the only host family for this parasitoid. Tschorsnig (2017) mentioned *D. baccarum*, *P. lituratus* and *Peribalus strictus* (Fabricius, 1803) (syn: *H. vernalis*) (Hemiptera: Pentatomidae) as hosts of this parasitoid.

In this study, tachinid parasitoids of some hemipteran species were determined in Adana, Ankara, Hatay, Konya and Uşak provinces of Türkiye. 11 tachinid species were reared from 10 different hosts. Four parasitoid-host couples were recorded for the first time. These were *A. rostrata* and *S. aegyptius* for *G. clavata*, *D. baccarum* for *E. setifacies*, *P. lituratus* for *C. rubida*. In addition, eight new host records for Türkiye were recorded. More comprehensive studies are required to determine the host-parasitoid couples in various regions and ecosystems in Türkiye.

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Original article (Orijinal araştırma)

Molecular identification and biodiversity of wireworm species, *Agriotes* spp. Eschscholtz, 1829 (Coleoptera: Elateridae) in major potato cultivated areas of Türkiye[1]

Tel kurdu türlerinin, *Agriotes* spp. Eschscholtz, 1829 (Coleoptera: Elateridae) Türkiye'nin başlıca patates ekim alanlarındaki biyoçeşitliliği ve moleküler tanımlanması

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Abstract

Wireworms, *Agriotes* spp. Eschscholtz, 1829 (Coleoptera: Elateridae) are among the most harmful soil-borne insect pests and significantly reduce potato yields under heavy infestations. The presence of wireworm species on potatoes in potato growing areas is not fully known in the provinces of Türkiye. Therefore, this research aimed to identify wireworms molecularly and evaluate their biodiversity in potato growing ecosystems. Here, the first extensive field survey was carried out in Türkiye's principal potato-growing regions in 2019 and 2020 (Afyon, Bolu, İzmir, Kayseri, Konya, Niğde, and Sivas). Species identification of wireworms was performed using DNA barcoding approach based on the fragment of mitochondrial cytochrome c oxidase subunit I (COI). Samples were collected from 400 potato fields, and 510 larval specimens were obtained. The presence of wireworms was confirmed for Afyon, Bolu, Kayseri, Konya, and Sivas provinces, with an average prevalence of 13.5%, while no positive samples were recovered from Niğde and İzmir. *Agriotes sputator* (L.,1758) (Coleoptera: Elateridae) was the most prevalent species in surveyed areas. The Shannon index of wireworm species was found to be as 0.59, which implies a low degree of biodiversity of wireworms in potato fields.

Keywords: *Agriotes* spp., COI, Elateridae, potato, soil-borne pests

Öz

Tel kurtları, *Agriotes* spp. Eschscholtz, 1829 (Coleoptera: Elateridae), en önemli toprak kaynaklı zararlı böcekler arasında yer almakta ve yoğun istilalarda patates üretiminde ciddi verim kayıplarına neden olmaktadır. Türkiye'de patates yetiştirilen alanlarda zararlı tel kurdu türlerinin varlığı tam olarak bilinmemektedir. Bu nedenle bu çalışma, patates yetiştiriciliği yapılan ekosistemlerde tel kurdu türlerinin moleküler olarak tanılanmasını ve biyoçeşitliliğini belirlemeyi amaçlamıştır. Bu çalışmada, 2019 ve 2020 yıllarında Türkiye'nin başlıca patates yetiştirilen bölgelerinde (Afyon, Bolu, İzmir, Kayseri, Konya, Niğde ve Sivas) kapsamlı ilk saha araştırması yapılmıştır. Tel kurtlarının tür tanılaması, mitokondriyal sitokrom c oksidaz Alt Ünite I (COI) geni dizisine dayalı DNA barkodlaması kullanılarak yapılmıştır. Örnekler, 400 adet patates tarlasından toplanmış ve 510 adet larva örneği elde edilmiştir. Afyon, Bolu, Kayseri, Konya ve Sivas illerinde tel kurtlarının yaygınlığı ortalama %13,5 olarak belirlenirken, Niğde ve İzmir illerinden pozitif örnek elde edilmemiştir. *Agriotes sputator* (L., 1758) (Coleoptera: Elateridae) türünün örnekleme yapılan alanlarda en yaygın tür olduğu belirlenmiştir. Tel kurdu türlerinin Shannon indeksi 0.59 olarak bulunmuş olup, patates tarlalarında tel kurtlarının biyolojik çeşitliliğinin düşük olduğu belirlenmiştir.

Anahtar sözcükler: *Agriotes* spp., COI, Elateridae, patates, toprak kaynaklı zararlılar

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Introduction

The larvae of click beetles, *Agriotes* spp. Eschscholtz, 1829 (Coleoptera: Elateridae), known as wireworms, are mostly herbivorous and regarded as one of the most serious pests of crops in the world (Traugott et al., 2015; Poggi et al., 2021). Although the adults of the *Agriotes* spp. feed on the leaves of plants, they rarely cause economic losses. However, the larvae of wireworms are common soil-dwelling insects that seriously harm the roots and seeds of many different crops during their larval development (Ritter & Ritcter, 2013). The damaged plants are generally exposed to secondary infections by various soil-borne pathogens and lose their marketable quality (Susurluk, 2008; Keiser et al., 2012; Traugott et al., 2015). Wireworm damage has increasingly been a problem for Turkish potato growers (Gülperçin & Tezcan, 2021; Kabalak & Sert, 2021). As an underground crop, potato tubers are particularly susceptible to the larval damage of *Agriotes* spp., and yields and quality can drop severely even at low population densities (Parker & Howard, 2001; Furlan, 2014).

Among over 9,000 wireworm species identified worldwide, over 39 species of wireworms are known as potato pests (Kroschel et al., 2020), and nine species; *Agriotes brevis* Candèze, 1863, *Agriotes lineatus* L., 1767, *Agriotes litigiosus* Rossi, 1792) *Agriotes obscurus* L., 1758, *Agriotes proximus* Schwarz, 1891, *Agriotes rufipalpis* Brullé, 1832, *Agriotes. sordidus* Illiger, 1807, *Agriotes. sputator* L., 1758 and *Agriotes. ustulatus* Schaller, 1783 are considered the most devastating ones in Europe (Furlan & Tóth, 2007; Furlan et al., 2021). To date, 483 wireworm species have been reported from various agricultural lands in Türkiye (Kabalak, 2018; Gülperçin & Tezcan, 2021; Kabalak & Sert, 2021). However, majority of these studies are based on morphological characteristics to identify wireworm species. The morphological identification is quite challenging due to the lack of clear distinguishing characters between species (Benefer et al., 2013; Andrews et al., 2020). The mitochondrial cytochrome c oxidase subunit I (COI) provides more accurate identification of the wireworm species and has also been used to construct the phylogeny of the wireworm specimens (Staudacher et al., 2013; Etzler et al., 2014; Zhang et al., 2019; Andrews et al., 2020). Accurately identifying pest species is the first step to achieving a more effective management strategy in integrated pest management (IPM) practices since damage risk and economic threshold levels may vary with the different wireworm species (Furlan, 2014; Furlan et al., 2021). The Shannon diversity index estimates species diversity within a community, and, may indicate the diversity and relative abundance of species in a given community (Shannon, 1948). The main goal of this study was to identify the biodiversity, prevalence, and species composition of wireworms in the major potato-cropping agroecosystems of Türkiye.

Material and Methods

Sampling of *Agriotes* spp.

In 2019 and 2020, soil samples were taken from 400 different potato fields in Afyon, Bolu, İzmir, Kayseri, Konya, Niğde, and Sivas provinces in Türkiye. Sampling was performed when the soil temperature reached 10°C between May and June. The potato plants showing wilt, chlorosis, and stunted growth were located in each sampling area. Using a shovel, the soil was dug up from at least ten separate sites in sampling fields to obtain the larval stages of wireworm species. The samples were taken from 20 cm in diameter and 10 cm in depth from each point (Furlan & Tóth, 2007). Soil samples were sieved through 300 µm to uncover the larval stages of wireworms. The collected wireworm larvae were placed individually into plastic containers containing 95% ethanol and labelled with a specimen code. The samples were then kept in a cooler bag (approximately 15°C) until they were transported to the laboratory.

Molecular analyses

Total DNA extractions were performed using the tissue in the legs and abdominal sections of the larval specimens. Amplification of COI gene fragment were conducted using the polymerase chain reaction (PCR) amplification Kit (Cat. No. PCR-111S; Jena Bioscience GmbH, Jena, Germany) according to the manufacturer's instructions with a universal primer pair of LCO1490 5′-GTCAACAAATCATAAAGATATTGG-3′ and HCO2198 5′-TAAACTTCAGGGTGACCAAAAAATCA-3′ (Folmer et al., 1994). PCR cycle conditions were 94°C for 5 min; 35 cycles of 94°C for 30 s, 54°C for 45 s and 72°C 60 s; and finally, 72°C for 60 s in a T100 thermal cycler (Bio-Rad, Hercules, CA, USA). Sequencing of the PCR amplicons were carried out using one larval specimen for each population. PCR amplicons were purified and bidirectionally sequenced with the same primers by the Macrogen company (Seoul, South Korea).

The resultant sequences were processed using the MegAlign module of DNASTAR v7.1.0 (DNASTAR Inc., Madison, Wisconsin, USA). The obtained sequences were compared to the GenBank database (http://www.ncbi.nlm.nih.gov/genbank) by using the Basic Local Alignment Search Tool (BLAST), and representative species sequences for provinces were deposited in GenBank (*Agriotes sputator*: OP630854, OP630855, OP630856, and OP630857; *A. rufipalpis*: OP630858). The evolutionary history was inferred using the Neighbor-Joining method (Saitou & Nei, 1987). The optimal tree with the sum of branch length = 0.54149612 is shown. The tree is drawn to scale, with branch lengths in the same units as those of the evolutionary distances used to infer the phylogenetic tree. The evolutionary distances were computed using the Maximum Composite Likelihood method (Tamura et al., 2004) and are in the units of the number of base substitutions per site. *Meloidogyne incognita* (Kofoid & White, 1919) was included as outgroups, and sequences were extracted from GenBank. The analysis involved 71 nucleotide sequences. Codon positions included were 1st+2nd+3rd+Noncoding. All positions containing gaps and missing data were eliminated. There was a total of 503 positions in the final dataset. Evolutionary analyses were conducted in MEGA 7 (Kumar et al., 2016).

Diversity Index

The Shannon Diversity Index (H) is a method for measuring diversity of species in a community and calculated as: $H = -\Sigma pi * \ln(pi)$. “Σ”: A Greek symbol meaning "sum"; “ln”: Natural log; “pi”: (Shannon, 1948). The higher the “H” value, the higher the species diversity is in a particular community. The lower the "H" value, the lower the diversity is in a given community (Shannon, 1948). Shannon diversity index (H) is classified according to the following grouping: low ($H < 2$); medium ($2 < H < 4$); and high ($H > 4$) species (Lumeran, 2019). In this study, this index was used to describe variations in wireworm populations and discriminate the biodiversity levels of wireworm species.

Results and Discussion

In total, 510 larvae were collected from 400 samples taken from different potato cultivation locations of sampled provinces. The highest occurrence rate was obtained by Sivas, followed by Kayseri and Bolu provinces (Table 1).

Table 1. Wireworm-positive soil samples collected from potato cultivation areas of seven provinces of Türkiye

Province	Wireworm-positive samples	Wireworm-negative samples	Total	Occurrence rate (%)
Afyon	6	64	70	8.5
Konya	12	58	70	17.1
İzmir	0	60	60	0.0
Sivas	14	36	50	28.0
Bolu	12	38	50	24.0
Kayseri	10	40	50	25.0
Niğde	0	50	50	0.0
Total	**54**	**346**	**400**	**13.5**

No wireworm specimens have been found in the potato growing areas of Niğde and İzmir provinces (Table 2). The majority of the specimens were identified as *A. sputator* (90%), and the rest of the specimens were *A. rufipalpis* (10%), which were encountered only in Afyon (Figure 1). *A. sputator* was the most common species and obtained from all provinces except Afyon (Tables 1 & 2).

Table 2. The list of locations where wireworms were detected

No	Specimen name	Species	No. of larvae extracted	GPS Coordinates
1	AF-1	*Agriotes rufipalpis*	7	38°41'18"K 31°02'47"D
2	AF-2	*Agriotes rufipalpis*	11	38°43'43"K 31°02'30"D
3	AF-3	*Agriotes rufipalpis*	7	38°42'12"K 30°38'37"D
4	AF-4	*Agriotes rufipalpis*	10	38°44'39"K 30°41'14"D
5	AF-6	*Agriotes rufipalpis*	9	38°35'23"K 31°02'52"D
6	AF-8	*Agriotes rufipalpis*	7	38°35'36"K 30°58'19"D
7	BOL-1	*Agriotes sputator*	16	40°45'10''K 31°32'98''D
8	BOL-2	*Agriotes sputator*	7	40°45'12''K 31°32'90''D
9	BOL-3	*Agriotes sputator*	53	40°45'07''K 31°32'00''D
10	BOL-4	*Agriotes sputator*	9	40°45'04''K 31°33'00''D
11	BOL-5	*Agriotes sputator*	24	40°45'14''K 31°33'11''D
12	BOL-6	*Agriotes sputator*	11	40°45'13''K 31°33'13''D
13	BOL-8	*Agriotes sputator*	7	40°45'56''K 31°37'11''D
14	BOL-10	*Agriotes sputator*	4	40°45'83''K 31°37'19''D
15	BOL-12	*Agriotes sputator*	13	40°46'01''K 31°37'19''D
16	BOL-14	*Agriotes sputator*	4	40°46'41''K 31°37'32''D
17	BOL-16	*Agriotes sputator*	7	40°46'39''K 31°37'41''D
18	BOL-18	*Agriotes sputator*	16	40°46'88''K 31°37'74''D
19	KON-1	*Agriotes sputator*	5	37°35'21"K 32°48'40"D
20	KON-2	*Agriotes sputator*	6	37°36'17"K 32°49'50"D
21	KON-3	*Agriotes sputator*	4	37°36'43"K 32°50'00"D
22	KON-4	*Agriotes sputator*	3	37°32'30"K 32°49'40"D
23	KON-5	*Agriotes sputator*	12	37°36'24"K 32°44'03"D
24	KON-6	*Agriotes sputator*	8	37°37'16"K 32°42'52"D
25	KON-10	*Agriotes sputator*	6	38°00'52"K 32°00'38"D
26	KON-12	*Agriotes sputator*	17	38°01'18"K 32°00'49"D
27	KON-14	*Agriotes sputator*	14	38°00'54"K 31°59'25"D
28	KON-16	*Agriotes sputator*	8	38°00'49"K 31°59'59"D
29	KON-18	*Agriotes sputator*	12	37°29'54"K 34°01'34"D
30	KON-20	*Agriotes sputator*	15	37°29'06"K 34°00'07"D
31	SİV-1	*Agriotes sputator*	9	39°10'29"K 36°05'04"D
32	SİV-2	*Agriotes sputator*	13	39°11'26"K 36°05'06"D
33	SİV-3	*Agriotes sputator*	10	39°12'27"K 36°05'59"D
34	SİV-4	*Agriotes sputator*	4	39°13'29"K 36°07'05"D
35	SİV-5	*Agriotes sputator*	6	39°16'19"K 36°11'30"D
36	SİV-6	*Agriotes sputator*	9	39°16'43"K 36°12'16"D
37	SİV-7	*Agriotes sputator*	6	39°17'24"K 36°14'18"D
38	SİV-14	*Agriotes sputator*	7	39°18'00"K 36°23'16"D
39	SİV-16	*Agriotes sputator*	11	39°18'07"K 36°18'53"D
40	SİV-18	*Agriotes sputator*	9	39°19'07"K 35°56'40"D

Table 2. Continued

No	Specimen name	Species	No. of larvae extracted	GPS Coordinates
41	SİV-20	*Agriotes sputator*	7	39°07'13"K 36°05'18"D
42	SİV-22	*Agriotes sputator*	9	39°07'20"K 36°04'51"D
43	SİV-24	*Agriotes sputator*	6	39°12'13"K 36°05'36"D
44	SİV-26	*Agriotes sputator*	8	40°09'30"K 38°07'45"D
45	KAY-1	*Agriotes sputator*	12	38°23'09"K 35°29'37"D
46	KAY-2	*Agriotes sputator*	6	38°21'35"K 35°28'19"D
47	KAY-3	*Agriotes sputator*	5	38°21'19"K 35°28'08"D
48	KAY-4	*Agriotes sputator*	9	38°20'28"K 35°27'49"D
49	KAY-5	*Agriotes sputator*	7	38°19'18"K 35°26'36"D
50	KAY-6	*Agriotes sputator*	3	38°18'58"K 35°26'17"D
51	KAY-8	*Agriotes sputator*	4	38°18'11"K 35°25'58"D
52	KAY-10	*Agriotes sputator*	5	38°16'49"K 35°25'15"D
53	KAY-12	*Agriotes sputator*	6	38°14'32"K 35°26'01"D
54	KAY-14	*Agriotes sputator*	7	38°21'11"K 35°23'26"D

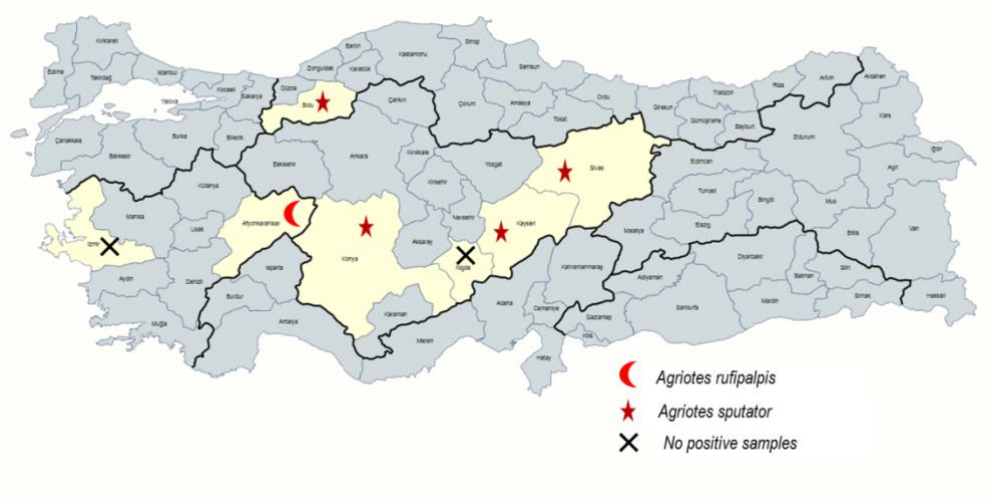

Figure 1. The map of the surveyed locations of wireworm species in potato-growing areas of Türkiye.

The Shannon diversity index (H) was determined to be H= 0.5915 for the wireworm species in potato growing areas. The occurrence of the low "H" index (H < 2) indicates a lack of variation (Table 3).

Table 3. Shannon diversity index "H"of wireworm species in potato fields

Wireworm species	Number	Pi*	ln (pi)	pi*ln (pi)
Agriotes rufipalpis	368	0.721569	-0.32633	-0.23547
Agriotes sputator	142	0.278431	-1.27858	-0.356
Total	510	1	-1.60491	-0.59147
			H	0.59147

* H = -Σpi * ln (pi). "Σ": A Greek symbol meaning "sum"; "ln": Natural log; "pi": proportion of the entire population. Multiplied with negative one.

DNA was extracted from one specimen of each population, and all the extracted specimens were successfully sequenced. Phylogenetic analysis comprised 60 nucleotide sequences and was performed using the Maximum Likelihood approach based on the General Time Reversible model. Beside the branches, the bootstrap test's percentage of duplicate trees where the related taxa are grouped (1000 repetitions) is displayed (Figure 2).

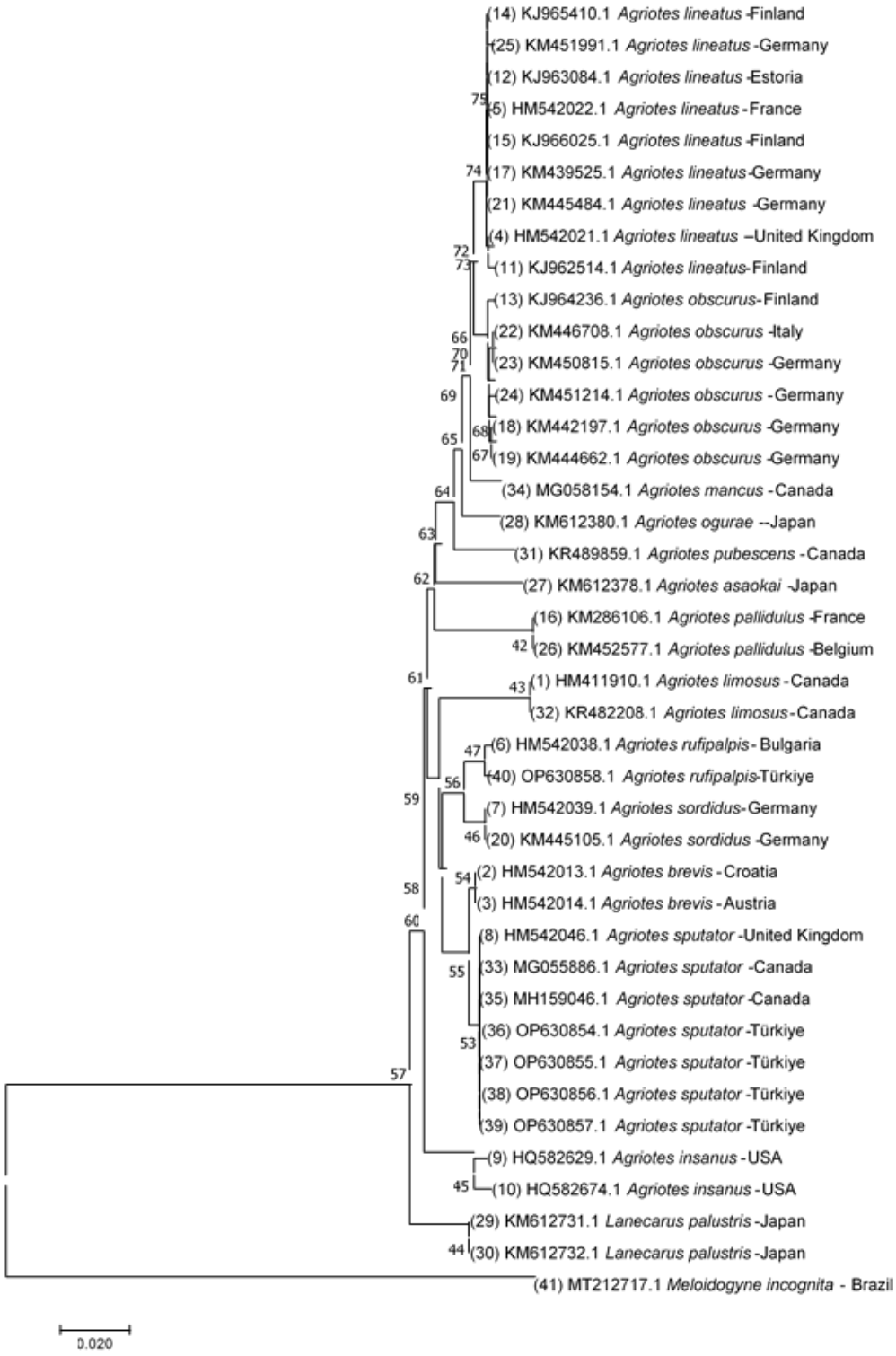

Figure 2. Phylogeny of *Agriotes* specimens generated from Maximum Likelihood analysis of mitochondrial COI sequences, rooted with *Meloidogyne incognita* (Kofoid & White, 1919). Bootstrap support (BS) values >75% are given at the internodes.

Phylogenetic tree shows two distinct clades for the distribution of two Turkish wireworm species (*Agriotes sputator* and *Agriotes rufipalpis*) (Figure 2). Sequencing data confirmed that COI gene sequences showed 100% identity for the populations of *A. sputator,* which were isolated from different areas of Türkiye. The population of A*. sputator* falls under the subclade sharing similarities with the wireworm species found in Canada and United Kingdom. While *A. rufipalpis* species falls under the same subclade of Bulgarian wireworm species. The sequences were deposited in GenBank with the accession numbers for *A. sputator* OP630857.1, OP630856.1, OP630855.1, OP630854.1 and *A. rufipalpis* OP630858.1.

To our knowledge, this is the first study that reports the extensive field survey of wireworm species in the major potato-growing areas of Türkiye. Two wireworm species were detected using molecular methods based on the DNA sequences of COI locus in surveyed locations. *Agriotes sputator* was more common than *A. rufipalpis* and determined in 4 out of 7 surveyed provinces. Similar to our results, *A. sputator* was detected as one of the most widespread species in Türkiye in earlier studies (Kabalak, 2018). Kabalak & Sert (2021) found that *A. sputator* was the most encountered species among 53 wireworm species, with 70 specimens in the Eastern Black Sea Region of Türkiye. In another study, *A. sputator* was one of the abundant species in the Central Anatolian Region of Türkiye (Kabalak & Sert, 2011). Cate (2007) also reported that two wireworm species, *A. lineatus* and *A. sputator*, were the most prevalent species in Türkiye. However, these studies found no specimens belonging to *A. rufipalpis*. Earlier studies suggest 483 described Elaterid species in different parts of Türkiye (Kabalak, 2018). However, only two species were found in potato-growing areas in the current study. Low species diversity found in this study might be due to sampling habitats, collecting methods, seasonal fluctuation of population densities of wireworms, and horizontal and vertical distribution of wireworm species in soil depending on the climatic factors (Kuhar & Alvarez, 2008; Milosavljević et al., 2017). Earlier studies revealed that the feeding activity of wireworms in fields might vary across species according to the types of crops. Cherry (2007) and Kuhar & Alvarez (2008) reported that *Melanotus communis* (Gyllenhal, 1817) (Coleoptera: Elateridae) and *Conoderus* spp. were found less active in the summer months due to unfavorable conditions. Previous studies were conducted at different habitats, including forest, herbaceous plants, and bushes, with varying collection methods such as light traps and insect nets (Kabalak et al., 2013; Kabalak, 2018; Kabalak & Sert, 2021). However, sampling was made by only digging out the soil in this study, and *A. sputator* was the dominant wireworm species in sampling areas. The sample collection time is another factor affecting the acquisition of the wireworm specimens. Previous studies showed that wireworm species are highly active in May and June, which agrees with our sampling time (Kabalak & Sert, 2011; Kabalak, 2018). The low species diversity found in this study may also be attributable to the ability of wireworm species to tolerate seed-applied insecticides. The interviews with the landowners showed that the sampling sites of wireworms were treated with various seed-applied insecticides at least once, which may have affected the species diversity in the sampled fields.

Providing an estimate of species diversity within a community (Shannon, 1948), the Shannon biodiversity index of wireworms was low in potato growing areas in this study (Table 3). This means that in potato fields, wireworm species may be dominant over other species, or that only a few species may possibly have effects on the wireworm community or only a few species may prefer a selective host (potato). This suggests that it may be related to monoculture agriculture in potato growing areas.

Agriotes rufipalpis is a wireworm species known to occur in Türkiye and identified only in Afyon province in this study. Wireworm species have different ecological requirements, such as temperature, soil characteristics, and precipitations (Staudacher et al., 2013). Previous wireworm surveys demonstrated that *A. ustulatus* occurred at warmer and drier climates while *A. brevis* favors higher temperatures and less precipitation (Furlan, 1998; Furlan & Toth, 2007; Lindroth & Clark, 2009; Staudacher et al., 2013). In this regard, our results indicate that *A. rufipalpis* might have found themselves wide range of distribution areas in Afyon.

Conclusions

Morphological identification of wireworm species is quite compelling due to the lack of clear distinguishing characters among highly similar wireworm species. Misidentification of wireworm species might lead to inappropriate management strategies. The results indicated that two wireworm species (*A. sputator* and *A. rufipalpis*) were present in the surveyed potato growing areas and *A. sputator* was the dominant wireworm species in sampling areas. The present study validated the COI region's barcoding significance by successfully discriminating two species in this study from each other and other *Agriotes* species included in phylogenetic analyses.

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Original article (Orijinal araştırma)

Determination of malathion resistance in *Sitophilus oryzae* L., 1763 and *Sitophilus granarius* L., 1758 (Coleoptera: Curculionidae) populations in Türkiye[1]

Türkiye'deki *Sitophilus oryzae* L., 1763 ve *Sitophilus granarius* L., 1758 (Coleoptera: Curculionidae) popülasyonlarında malathion direncinin belirlenmesi

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Abstract

This study was conducted to determine the malathion resistance levels of two important stored grain pest beetles, *Sitophilus oryzae* L., 1763 and *Sitophilus granarius* L., 1758 (Coleoptera: Curculionidae), collected from different provinces of Türkiye in the years 2017-2018. To control these pests, chemical insecticides have been widely used for a long time as a grain protectant in farmer warehouses, flour mills, or silos in many countries, including Türkiye. In the current study, insects were exposed to malathion for 24 hours to determine resistance rates. The highest resistance ratio to malathion in *S. oryzae* was found in the Adana-Kartepe population with 5.73-fold, and the lowest resistance was found in the İstanbul- Büyükçekmece population with 1.57-fold. While the Konya-Alibeyhöyüğü population of *S. granarius* had the highest resistance ratio of 6-fold, the lowest resistance rate of 2.54-fold was found in the population obtained from the same location but from a different warehouse. According to this study, we found that slight resistance developed in the populations of *S. oryzae* and *S. granarius* in Türkiye. In order to prevent the occurrence of resistance due to synthetic insecticides used against stored product pests, it is thought that various insecticide groups with different mechanisms of action should be used.

Keywords: Malathion, resistance, *Sitophilus* spp., Türkiye, warehouse

Öz

Bu çalışma, 2017-2018 yılları arasında Türkiye'nin farklı illerinden toplanan iki önemli depolanmış ürün zararlısı *Sitophilus oryzae* L., 1763 ve *Sitophilus granarius* L., 1758 (Coleoptera: Curculionidae)'un malathion direnç düzeylerini belirlemek amacıyla yapılmıştır. Bu zararlıları kontrol altına almak için insektisitler, Türkiye dahil birçok ülkede çiftçi depolarında, un değirmenlerinde veya silolarda tahıl koruyucu olarak uzun zamandan beri yaygın olarak kullanılmaktadır. Direnç oranlarını belirlemek için böcekler 24 saat boyunca malathion'a maruz bırakılmıştır. Malathion'a karşı en yüksek direnç oranı *Sitophilus oryzae*'de 5.73 kat ile Adana-Çukurtepe (K4) popülasyonunda, en düşük direnç ise 1.57 kat ile İstanbul-Büyükçekmece (R1) popülasyonunda bulunmuştur. *Sitophilus granarius*'un Konya-Alibeyhöyüğü (E3) popülasyonunda en yüksek direnç oranı malathion'a karşı 6 kat iken, en düşük direnç oranı 2.54 kat ile *S. granarius*'un aynı lokasyonun farklı bir deposundan alınan popülasyonunda (E4) bulunmuştur. Bu çalışmaya göre Türkiye'deki *S. oryzae* ve *S. granarius* popülasyonlarında hafif bir direnç geliştiği tespit edilmiştir. Depolanmış ürün zararlısı böceklere karşı kullanılan sentetik insektisitler nedeniyle oluşabilecek direnç vakalarının önlenmesi amacıyla, farklı etki mekanizmalarına sahip çeşitli insektisit gruplarının kullanılması gerektiği düşünülmektedir.

Anahtar sözcükler: Malathion, direnç, *Sitophilus* spp., Türkiye, depo

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Introduction

Wheat, *Triticum aestivum* L. (Poales: Poaceae) ranks first in Türkiye in terms of cultivation area and production, and is considered a strategic product in human nutrition (Aydın, 2022). Notably, in the last two decades, wheat cultivation areas have changed between 6.8-9.4 million hectares and production has changed between 17.2-22.6 million tons in Türkiye (TMO, 2020; Aydın, 2022). Wheat is also the raw material of food products such as bread, bulgur, semolina, and biscuits. It holds strategic importance due to high production amounts, its prolonged storage capacity, and its significance in special situations such as natural disasters, and war.

The granary weevil, *Sitophilus granarius* L., 1758 and the rice weevil, *Sitophilus oryzae* L., 1763 (Coleoptera: Curculionidae), are important primary pest insects of stored grain that cause economic losses by feeding internally and externally on stored grains such as wheat, rice, corn, barley, and etc. These insects have been detected in almost all provinces in Türkiye (Özer, 1957; Coşkuncu, 2004; Işıkber et al., 2005, 2016; Bağcı et al., 2014; Atabay et al., 2018; Koçak et al., 2018; Özder & Toğantimur, 2019; Zengin & Karaca, 2019; Ertürk et al., 2020; Yetkin & Atakan, 2022). Besides, Atabay et al. (2013) reported that *Sitophilus* spp. had the highest incidence rate of 70.4% in stored paddy, rice, and bran in Balıkesir province. It has been reported that in intense Liposcelididae contamination, wheat loses its germination power and insect excrement and wastes cause allergic reactions (Obr, 1978; Kucerova, 2002). *Sitophilus granarius* had the highest density in the wheat warehouses of Kütahya province with a rate of 52.6%, followed by O. *surinamensis* with a rate of 40.2% (Zengin & Karaca, 2020). In addition, it has been reported that these two species are widespread throughout the world (Mason & McDonough, 2012; Correa et al., 2013; Keszthelyi et al., 2021; Nietupski et al., 2021). Grain damaged by these insects decreases in nutritional and market value, as well as in germination percentage and weight. In heavy infestation, molding, heating, odor, and decay occur in the product (Erakay, 1974; Arthur, 1996; Magan et al., 2003; Yiğit et al., 2022). Moreover, high humidity of the grain and poor storage conditions allow insects to enter stored wheat. It has also been reported that if no measures are taken against these pests, the damage caused by insects can reach up to 50% of the harvested crop. Besides, this causes a loss of more than 100 billion USD annually on a global scale (Boxall, 2002; Mebarkia et al., 2010; Asrar et al., 2016).

To minimize the economic loss originating from stored product pests, synthetic insecticides have been used for decades. Malathion, an organophosphate (OP) insecticide, is a widely used insecticide in post-harvest grains in many countries to protect stored products from insect pests (Boyer et al., 2012). Organophosphates are responsible for the inhibition of acetylcholinesterase in the insect nervous system. It causes accumulation of acetylcholine in the synaptic gap due to the inhibited enzyme, impaired neurotransmission, and ultimately death occurs (Attia et al., 2020). In addition, malathion, which was first registered in Türkiye in 1964, is still used today as a residual insecticide against insect pests (Oden & Sahin, 1964; Alagöz & Sağlam, 2022). Factors such as long-term and repeated applications of insecticides, intensive and high-dose applications, and changes in insect populations increase the frequency of insecticide-resistant individuals in insect populations and lead to failure in pesticide applications (Ferizli & Berisli, 2005; Baliota et al., 2022; Hoobdel et al., 2022). In addition, it was determined that stored insect pests showed differences in terms of sensitivity to malathion. (Navarro et al., 1986; Guedes et al.,1996; Mendoza, 1999; Yesir & Koçak, 2017; Baliota et al., 2022). Furthermore, failures in application due to insecticide resistance can lead to control failures, and cause economic losses of several billion dollars worldwide each year (Dennehy, 1987; Elzen & Hardee, 2003). The rapid spread of stored product insect populations through national and international trade, evaluation of used insecticides for resistance, and suggestions for corrective actions are the basic needs. Although long-term storage of grain in local warehouses and silos is common practice in Türkiye, data on the occurrence of resistant populations are scarce. Therefore, the aim of this study was to determine and quantify the resistance status of different field populations of *S. granarius* and *S. oryzae* against malathion, which is used as registered in Türkiye.

Materials and Methods

Field survey, insect populations and rearing condition

For this purpose, adults of *S. oryzae* and *S. granarius* were collected from granaries and mills in Adana, Ankara, Burdur, Isparta, İstanbul, Konya, and Manisa provinces between 2017 and 2018. Besides that, approximately four kg samples were taken from five different points and different depths of the wheat mass in each warehouse using a two-meter probe and the collected samples were brought to the laboratory. Samples were transferred to one-liter glass jars and the jar lids were covered with a fine mesh cloth to allow ventilation. To obtain adults of *S. oryzae* and *S. granarius* individuals, wheat samples were kept in climate cabinets (Nüve ID 501, Türkiye) for 60 days at 25°C temperature and 65±5% relative humidity condition (Işıkber, 2005; Ertürk, 2021). Grain samples were sieved (mesh size 0.85 mm) for isolation and insect species were identified (Freeman, 1980). The insects reared on sterilized whole wheat under the same conditions. The progeny of the insects (F1) was used for bioassays.

Insecticide bioassay

Malathion was selected among widely used insecticides used for chemical control of insect pests in storage facilities. For the determination of median lethal concentration (LC_{50}), a series of at least five different concentrations (3-250 ml/L) of malathion (650 g/L, EC) were prepared with distilled water for *S. granarius* and *S. oryzae*. Glass Petri dishes with a diameter of 9 cm were used for toxicity tests. Filter papers (Whatman No.1) were placed into Petri dishes and sprayed (Spray Tower, Burkard Scientific-BS00281, England) with 2.0 ml of insecticide solutions. Petri dishes sprayed with malathion were kept under a hood to dry for one hour before being used for testing. In the control group, only distilled water was used. Afterward, twenty-five, 1-3-week-old unsexed adult individuals were left in each Petri dish and exposed to the insecticide for 24 hours. To prevent insect escape from the Petri dishes, they were covered with parafilm. After 24 hours, dead and live insects were counted and recorded. Experiments were set up with 3 replications. The test method used in the study was according to IRAC 006 (IRAC, 2009), but no plastic ring was used, and this method was revised and applied in this way.

Statistical analyses

The trial results were initially converted into mortality percentages, followed by analysis using the Polo-PC probit package program (LeOra, 1994). LC_{50} values and 95% confidence intervals were then calculated at a significance level of 5% (Robertson et al., 2003). Resistance ratios (RRs) between populations for all assays were calculated for malathion by dividing LC_{50} values for resistant strains by the LC_{50} value of the most susceptible strain as there was no susceptible strain to assess the resistance factor for these insects (Perez-Mendoza, 1999; Yalçın et al., 2015; Lv et al., 2021).

Results

Sitophilus oryzae and *S. granarius* species samples collected from warehouses in Adana, Ankara, Burdur, Isparta, İstanbul, Konya and Manisa, and the species obtained from wheat samples are presented in Table 1. Different concentrations of malathion were applied to *S. oryzae* populations and their resistance levels are displayed in Table 2.

Within the populations of *S. oryzae*, the LC_{50} value of the Konya/Alibeyhöyük (E5) population was calculated as 8.26 ppm and was considered as the most sensitive population to malathion. The LC_{50} values of other populations were calculated as İstanbul/Küçükçekmece (R1) 13.05 ppm, Ankara/Haymana (B2) 19.97 ppm, Manisa/Saruhanlılar (A2) 26.34 ppm, and Adana/Çukurtepe (K4) 47.33 ppm, respectively. When the toxic effects of different concentrations of the malathion were evaluated according to the E5 population, the R1 population were found 1.57-fold more resistant, while the resistance levels were reported as 2.41-fold for the B2 population, 3.18-fold for the A2 population, and 5.73-fold for the K4 population. Different doses of malathion were applied to *S. granarius* populations collected from the study areas and the resistance levels found are presented in Table 3.

Table 1. Provinces, districts and facilities where *Sitophilus oryzae* and *Sitophilus granarius* individuals were collected

Code	Species	Collection of places	Facilities	Latitude	Longitude
E5	*S. oryzae*	Konya/ Alibeyhöyüğü	Farmer Warehouse	N37°31'52.234"	E 32°39' 44.2"
A2	*S. oryzae*	Manisa/ Saruhanlılar	Farmer Warehouse	N38°40' 18.781"	E27°37' 24.143"
B2	*S. oryzae*	Ankara/ Haymana	Ankara University Faculty of Agriculture Farm	N39°37'1.031"	E32°41'30.703"
K4	*S. oryzae*	Adana/ Çukurtepe	Turkish Grain Board	N37°26'42.616"	E 35°49'7.028"
R1	*S. oryzae*	İstanbul/ Büyükçekmece	Eriş Flour Mill	N41°4'13.427"	E 28°19'9.085"
H1	*S. granarius*	Burdur/ Merkez	Farmer Warehouse	N37°43'32.586"	E30°17'26.387"
D2	*S. granarius*	Elazığ	Hasbek Flour Mill	N38°40'29.338"	E39°13'21.054"
E3	*S. granarius*	Konya/ Alibeyhöyüğü	Farmer Warehouse	N37°31'47.496"	E32°39'37.191"
E4	*S. granarius*	Konya/ Alibeyhöyüğü	Farmer Warehouse	N37°31'57.148"	E32°39'42.659"
G2	*S. granarius*	Isparta/ Merkez	Farmer Warehouse	N37°45'50.941"	E30°34'23.914"
H3	*S. granarius*	Burdur/ Merkez	Farmer Warehouse	N37°43'31.305"	E30°17'26.022"

Table 2. Lethal concentrations of malathion applied to *Sitophilus oryzae* populations

Population Codes	n	Slope±SE	χ^2	LC_{50} (ppm) (95% CL)	RR_{50}	LC_{90} (ppm) (95% CL)	H	p values	Duration of exposure (hour)
E5	450	1.49±0.20	8.65	8.26 (5.88±10.46)	1.00	56.02 (41.96-86.81)	0.72	<0.05	24
A2	450	3.57±0.32	23.99	26.34 22.11±31.49)	3.18	58.83 (47.26-81.86)	1.99	<0.05	
B2	360	4.38±0.43	11.19	19.97 (17.41±22.62)	2.41	39.07 (33.59-48.42)	1.24	<0.05	
K4	450	2.39±0.22	3.56	47.33 (41.17±54.67)	5.73	161.15 (129.90-214.54)	0.28	<0.05	
R1	450	3.05±0.29	15.38	13.05 (11.20±15.21)	1.57	34.39 (27.47-47.92)	1.28	<0.05	

n= The number of individuals used; CL, Confidence limit; RR_{50}, Resistance Ratio; H, Heterogeneity; SE, Standard Error.

Table 3. Lethal concentrations of malathion applied to *Sitophilus granarius* populations

Population Codes	n	Slope±SE	χ^2	LC_{50} (ppm) (95% CL)	RR_{50}	LC_{90} (ppm) (95% CL)	H	p values	Duration of exposure (hour)
H1	450	1.94±0.18	13.02	7.17 (5.84±8.65)	1.00	30.74 (23.44-44.99)	1.09	<0.05	24
D2	540	2.36±0.18	26.33	41.79 (33.70±50.77)	5.82	141.70 (111.01-197.72)	1.76	<0.05	
E3	360	3.10±0.27	20.63	43.07 (33.67±54.94)	6.00	113.58 (86.63-169.73)	2.29	<0.05	
E4	540	1.71±0.14	20.49	18.28 (14.77±22.87)	2.54	92.18 (65.31-149.87)	1.37	<0.05	
G2	450	3.53±0.27	6.11	39.32 (35.40±43.64)	5.48	88.97 (77.55-105.54)	0.51	<0.05	
H3	540	3.06±0.25	16.96	32.60 (28.77±36.61)	4.54	85.63 (72.46-107.12)	1.13	<0.05	

n= The number of individuals used; CL, Confidence limit; RR_{50}, Resistance Ratio; H, Heterogeneity; SE, Standard Error.

Among the *S. granarius* populations, the LC_{50} value of the Burdur/Merkez (H1) population was calculated as 7.17 ppm and was considered as the most sensitive population to malathion. The LC_{50} values of the other populations were calculated as Konya/Alibeyhöyük (E4) 18.28 ppm, Burdur/Center (H3) 32.60 ppm, Isparta/Center (G2) 39.32 ppm, Elazığ (D2) 41.79 ppm and Konya/Alibeyhöyük (E3) 43.03 ppm. It was calculated that the E4 population was 2.54-fold, the H3 population was 4.54-fold, G2 population was 5.48-fold, the D2 population was 5.82-fold and the E3 population was 6-fold more resistant than the H1 population.

Discussion

In this study, we collected grain samples from 11 different localities and warehouses in 2017 and 2018 years. Hill (2002) and Esin (1971) were used to identify the insect species collected from these warehouses and these species were identified as *S. oryzae* and *S. granarius*. The rice weevil and the granary weevil are among the primary pests in wheat warehouses in our country. The genus of *Sitophilus*, which has a cosmopolitan distribution, is common in almost all provinces of Türkiye (Obr, 1978; Kucerova, 2002; Atabay et al., 2013; Zengin & Karaca, 2020). In addition, the distribution of this genus is considered to be related to the domestic grain trade. The fact that these insects are so widespread in the country, as well as the use of insecticides to protect against insect damage in grain trading areas, has raised suspicions about the presence of resistance.

Synthetic insecticides applications are mostly preferred by warehouse enterprises for the control of stored product pests (Yesir & Koçak, 2017). It is also known that the excessive and frequent use of chemical insecticides causes insecticide residues, the death of non-target species and the development of resistance in insecticide applied populations (Isman, 2006). The level of resistance of the populations can be classified into three groups; as high resistance (RR > 10), moderate resistance (5 < RR < 10), and low resistance (3 < RR < 5) (Mazzarri & Georghiou, 1995). In this study, the H1 population of *S. granarius* was found to be the most sensitive population, with an LC_{50} value of 7.17 ppm. Considering the resistance rates among these populations, the RR was highest in the E3 population with 6-fold increment. According to the results of this study, moderate malathion resistance was determined in *S. granarius* D2, G2, and E3 populations. Moreover, low levels of resistance were recorded in E4 and H3 populations. Furthermore, the slope values determined from probit analysis can provide clues about the homogeneity or heterogeneity of the target insect populations of insecticides in terms of resistance. High slope values (>2) indicate that the population is relatively homogeneous, while low slope values (<1) indicate that the population is relatively heterogeneous (Georghiou & Metcalf, 1961; Yu, 2008; Rodrigues et al., 2020). It is noteworthy that the B2 population of *S. oryzae* has the highest slope values suggesting that the B2 populations were homogenous in their response to the malathion. In contrast, the E5 population has the lowest LC_{50} value and can be considered a heterogeneous population. Similarly, the E4 population of *S. granarius* has the lowest slope value, and this population can be considered heterogeneous. On the other hand, the E3 population of granary weevil has the highest slope value and can be considered homogeneous.

Similar to our findings, Kljajić & Perić (2007) reported that the resistance rates obtained for *S. granarius* in the Belgrade Harbor population after 24 hours of malathion exposure were 4.3-fold higher than in the susceptible population. It was also reported that the Apatin population developed a 3.2-fold resistance to malathion in granary weevil populations from different regions of Yugoslavia (Kljajić & Perić, 2006). The lowest LC_{50} value of *S. oryzae* against malathion was obtained from the population of E5. While the R1 population had the lowest resistance rate with 1.57-fold, the K4 population had the highest resistance rate with 5.73-fold. In parallel with this study, Yesir & Koçak (2017) reported that the resistance of *S. oryzae* populations collected from Konya, Manisa, İzmir, and Samsun provinces varied between 1.8 and 8.4-fold in individuals exposed to malathion for 24 hours. Furthermore, Attia et al. (2020) found that the Egyptian populations of the rice weevil *S. oryzae* were 199.6 times resistant to 24 hours of malathion exposure. Karaağaç & Konuş (2015) determined the mortality rates at the dose of 1.5 mg malathion/disc of *Sitophilus zeamais* Motschulsky, 1855 (Coleoptera: Curculionidae) populations collected from Kırıkkale and Samsun as 72.2 and 92.2%, respectively. They reported that cytochrome P450 monooxygenase and glutathione S-Transferase enzymes may have a role in the resistance against malathion. In another study, while the Ankara population of *S. zeamais* showed resistance to malathion, no resistance was detected in the Samsun population. They evaluated that cytochrome P450 monooxygenases play an active role in malathion resistance in the Ankara population of *S. zeamais* (Konuş, 2015). Karaağaç (2015) found that there was no resistance against malathion except for Ankara and Karaman strains of *S. zeamais*. Mutlu et al. (2019), revealed that because of deltamethrin is more frequently used than malathion, the Khapra beetle *Trogoderma granarium* Everts, 1898 (Coleoptera: Dermestidae) has higher resistance ratio for deltamethrin (4-10.7 RR) compared to malathion (1.32-1.92).

The first studies conducted in Türkiye on malathion resistance showed that *Sitophilus* spp., *Tribolium confusum* Jaquelin Du Val, 1868 and *Tribolium castaneum* Herbst, 1797 (Coleoptera: Tenebrionidae) did not develop resistance to malathion (Kalkan et al., 1977; Keyder et al, 1979; Dörtbudak et al., 1987). Similarly, Ribeiro et al. (2003) reported that no resistance cases were observed in *S. zeamais* populations against organophosphate insecticides such as malathion and pirimiphos-methyl, which were widely and frequently used in Brazil in the 1970s and 1980s. However, they reported that malathion was banned due to its use in powder formulation and control failures in *S. zeamais* populations (Santos et al., 1986; Guedes et al., 1994, 1995, 1996). Malathion is actively used in the control of stored product pests in Türkiye, and its use has not been banned yet. However, when the data obtained are evaluated, it is concluded that low and moderate resistance begins to occur in the sampled populations. In addition to these, the lack of knowledge of the insecticide applications previously used in the sample warehouses may be effective in this variation in resistance rates.

As a result, slowing down or preventing the development of resistance in pests or maintaining the effect of pesticides on harmful organisms are very important in terms of managing resistance. Initial toxicity studies of insecticides, determining the presence and level of resistance, comparing them with future resistance studies, and establishing appropriate control methods and resistance distribution maps for the management of pests will contribute to insect resistance management studies.

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Derginin yayın ilkeleri aşağıda özet olarak sunulmuştur. Ayrıntılar için web adresine (www.entomoloji.org.tr) bakınız.

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