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Original article (Orijinal araştırma)

The UV protectant properties of tea extracts on entomopathogenic fungus spores and their lethal effect on *Galleria mellonella* (L., 1758) (Lepidoptera: Pyralidae)

Çay ekstraktlarının entomopatojen fungus sporları üzerindeki UV koruyucu özellikleri ve *Galleria mellonella* (L., 1758) (Lepidoptera: Pyralidae) üzerindeki öldürücü etkileri

Zeynep BAYRAMOĞLU[1]

Abstract

The present study aims to investigate the effectiveness of various tea extracts in providing ultraviolet (UV) protection for entomopathogenic fungi. UV radiation could have detrimental effects on viability of these fungi, which are important biocontrol agents against insect pests. This study was carried out in the Microbiology Laboratory of the Department of Biology Karadeniz Technical University in 2023. We evaluated the UV protective properties of various tea extracts in entomopathogenic fungi [*Beauveria bassiana* (Bals.) Vuil. (Hypocreales: Cordycipitaceae) and *Metharizium flavoviride* (Gams and Rozsypal 1956) (Hypocreales: Clavicipitaceae)] and tea extracts effectiveness against *Galleria mellonella* (L.,1758) (Lepidoptera: Pyralidae) larvae. Our findings demonstrate that certain tea extracts exhibit significant UV protection for entomopathogenic fungi, suggesting their potential application in improving the performance of biocontrol agents in outdoor environments. The highest UV-B protection was observed by adding black and green tea extracts to fungal spores, resulting in a radial growth measurement of 14.6 mm and 14.3 mm, respectively, at the end of 10 days of exposure for 120 minutes. These results contribute to the development of eco-friendly strategies for pest management in agriculture.

Keywords: *Beauveria bassiana*, biological activity, *Metharizium flavoviride*, tea extracts, UV protection

Öz

Bu çalışma, entomopatojenik funguslar için çeşitli çay ekstraktlarının ultraviyole (UV) koruması etkinliğini araştırmayı amaçlamaktadır. UV radyasyonu, böcek zararlılarına karşı önemli biyokontrol etmenleri olan bu fungusların canlılığı üzerinde olumsuz etkilere sahip olabilir. Bu çalışma 2023 yılında Karadeniz Teknik Üniversitesi Biyoloji Bölümü Mikrobiyoloji Laboratuvarında yürütülmüştür. Çay ekstraktlarının entomopatojen funguslar [*Beauveria bassiana* (Bals.) Vuil. (Hypocreales: Cordycipitaceae) ve *Metharizium flavoviride* (Gams and Rozsypal 1956) (Hypocreales: Clavicipitaceae)] üzerindeki UV koruyucu özelliklerini ve *Galleria mellonella* (L.,1758) (Lepidoptera: Pyralidae) larvalarına karşı çay ekstraktlarının etkinliklerini değerlendirilmiştir. Bulgularımız, belirli çay özlerinin entomopatojen funguslar için önemli bir UV koruması sergilediğini göstermekte ve bu ekstraktların açık hava ortamlarında biyokontrol ajanlarının performansını artırmada potansiyel uygulamalarına işaret etmektedir. En yüksek UV-B koruması, fungus sporlarına siyah ve yeşil çay ekstraktları eklenerek elde edilmiş ve 10 günlük 120 dakikalık maruziyet sonunda sırasıyla 14.6 mm ve 14.3 mm'lik bir radyal büyüme ölçümü elde edilmiştir. Bu sonuçlar, tarımda çevre dostu zararlı yönetimi stratejilerinin geliştirilmesine katkıda bulunacaktır.

Anahtar sözcükler: *Beauveria bassiana*, biyolojik aktivite, *Metharizium flavoviride*, çay ekstraktları, UV koruma

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Introduction

Entomopathogenic fungi have been widely utilized for controlling insect pests, playing an incredibly significant role in pest management (Ansari et al., 2011). As a result, they are seen as alternatives to chemical insecticides (Zimmerman, 2007). Fungal pesticides play a crucial part in the biological control of pathogens and insect pests in agriculture and horticulture (Maqbool et al., 2016). *Beauveria bassiana* (Bals.) Vuil. (Hypocreales: Cordycipitaceae), *Metarhizium anisopliae* (Metschn.) (Hypocreales: Clavicipitaceae), and *M. flavoviride* (Gams & Rozsypal 1956) have emerged as key players in the control against many pests, and they are found on various insects worldwide (Feng et al., 1994; Bridge et al., 2005; Faria & Wraight, 2007). Several bioinsecticide products based on entomopathogenic fungi, such as *M. anisopliae, B. bassiana*, and *Isaria fumosorosea* (Wize) Brown y Smith (Hypocreales: Clavicipitaceae) have been effectively employed for insect pest control (Faria & Wraight, 2007).

Applications made in the field have shown negative effects on pests exposed to direct sunlight (Faria & Wraight, 2007; Jackson et al., 2010). The spores of the *B. bassiana* fungus can lose their effectiveness when exposed to ultraviolet radiation (UV) (Fargues et al., 1996; Fernandes et al., 2007). This is considered one of the most significant factors that bound the permanence and effectiveness of entomopathogenic fungi in outdoor environments (Moore et al., 1993). Consequently, it is essential to develop formulations that can extend the survival time of these fungi, which are utilized as microbial control agents. These formulations should incorporate substances capable of providing UV protection.

Various abiotic factors in nature can impede the capability of fungal agents to biocontrol insect pests. Among these factors, UV, including UV-A and UV-B, was considered the primary factors in the environment that influenced the survival of fungi in pest control (Ignoffo & Garcia, 1992; Moore et al., 1993). While certain strains of fungi that are UV-tolerant can be capable of enduring extended periods of direct exposure to solar UV radiation, lasting for several hours, strains that are susceptible to UV could not withstand it. Furthermore, the effects of UV-B exposure on fungi (Fargues et al., 1996; Braga et al., 2001a; Fernandes et al., 2007; Nascimento et al., 2010) or UV-A (Fargues et al., 1997; Braga et al., 2001b) could prolong the germination of surviving conidia and hinder fungal growth. This decrease in fungal resistance and the impact of infective field propagules undermines the control of arthropod pests (Jaronski, 2009). To address these challenges, efforts have been made to select strains with inherent UV tolerance and to develop formulations that incorporate adjuvants capable of absorbing or blocking solar radiation. These strategies aim to protect fungi from the harmful impacts of UV radiation.

Comprehensive studies have been conducted on the UV tolerance of various species of entomopathogenic fungi, including *B. bassiana* (Inglis et al., 1995; Fargues et al., 1996; Morley Davies et al., 1996; Huang & Feng 2009; Posadas et al., 2012), *I. fumosorosea* (Fargues et al., 1996), *Metharizium acridum* (Fargues et al., 1996; Morley Davies et al., 1996; Braga et al., 2001a), and *M. anisopliae* (Fargues et al., 1996; Braga et al., 2001a). These fungi are generally highly sensitive to UV radiation.

Tea is among the most widely consumed beverages globally, second only to water. The different types of tea produced and consumed worldwide include black, green, oolong, and white teas. These teas vary in their polyphenolic content due to the fermentation process during tea manufacturing. Numerous studies have investigated the effects of plant material on pest control, long-term preservation, and protection from sunlight (Abudulai et al., 2001; Shapiro et al., 2007a, b; Shapiro et al., 2008; El Salamouny et al., 2009a, b). Among these studies, the UV protective effects of green and black tea, particularly against nucleopolyhedroviruses, were demonstrated (Shapiro et al., 2008; El Salamouny et al., 2009a, b; El-Husseini et al., 2012; Gifani et al., 2021). Additionally, some research have examined the UV protective impact of tea extracts on entomopathogenic fungi (Kaiser et al., 2018).

In this study, our objective was to evaluate the efficacy of extracts obtained from four different tea extracts in offering protection against ultraviolet (UV) radiation, with the goal of improving the persistence of *B. bassiana* and *M. flavoviride* spores under laboratory condition.

Materials and Methods

Fungal isolates and conidia production

The fungi used in this study, namely *Beauveria bassiana* and *Metharizium flavoviride*, were procured from the entomopathogenic culture collection at Karadeniz Technical University, Department of Biology, Microbiology Laboratory. In previous studies, *B. bassiana* (Pa4) and *M. flavoviride* (As-2) were isolated from *Pristiphora abietina* (Christ, 1791) (Hymenoptera: Tenthredinidae) and *Amphimallon solstitialis* (Linnaeus, 1758) (Coleoptera: Scarabaeidae) in Türkiye, respectively. These fungi exhibited significant insecticidal activity on insect pests (*P. abietina* and *A. solstitialis*) (Biryol et al., 2020, 2021).

Each isolate was inoculated with Sabouraud CAF Agar medium (Liofilchem s.r.l., Italy) in flasks and put in incubator at 25 °C for approximately 2 weeks to supply new conidia from the fungal isolates. The growing fungal spores were harvested using 0.1% Tween 80 (AppliChem) and stock concentrations were calculated with a Neubauer hemocytometer. A concentration of 2×10^7 conidia/ml was used in the trials. We monitored the germination of conidia by introducing them onto Sabouraud CAF Agar medium and then quantifying the count of conidia that had undergone germination following a 24-hour incubation period at a temperature of 25°C. We defined conidia as germinated when their germ tubes reached a length equal to or greater than the width of the conidium.

Preparation of additives

The UV protectant properties of four natural additives, namely the green tea, black tea, oolong tea, and white tea (*Cammellia sinensis* L. Kuntze, Theaceae) extracts, were tested. The tea leaves were infused in sterile distilled water at 70°C for 30 min (2 g of tea leaves with 20 ml of water). The stock solutions were supplemented to the spore suspensions to achieve last UV protectant concentration of 10% v/v in the assays.

Evaluation of UV protection of tea extracts on fungal isolates on agar plates

In the experiment aimed at assessing the resistance of two isolates (*B. bassiana* and *M. flavoviride*) and their combinations with tea extracts to UV-B radiation, the following procedure was followed:

A spore suspension containing 2×10^7 conidia/ml was obtained. Ten microliters (µl) of this suspension were inoculated at three different points on Sabouraud CAF agar plates. Plastic petri dishes with open lids were placed in the sterile cabinet, 20 cm away from the UV-B lamp (15 W, 312 nm). A UV-B fluorescent lamp (Philips, Eindhoven, Holland) with a wavelength of 260-400 nm was used as the radiation source. Petri dishes were exposed to UV-B for 0, 30, 60 and 120 min. This exposure was applied to all Petri dishes containing pure conidia (unformulated control) as well as those with conidia-tea extract mixtures. The radial growth of the fungi was examined on the 5^{th}, 7^{th}, and 10^{th} days after incubation at 28 °C. To produce spores, 100 µl of the fungal spores (2×10^7 conidia/ml) was plated on Sabouraud CAF agar plates. These plates were then exposed to UV-B radiation for 0, 30, 60, and 120 min and incubated at 28 °C. Spores were harvested from the Petri dishes 12 days after incubation, and the concentration of spores was calculated. Trials were carried out in triplicate, with the 0-min exposure serving as the control group (Couceiro et al., 2021). The obtained measurements were recorded and compared using graphs. To assessment the effect of ultraviolet radiation, both the radial growth of the colonies on the agar plates and the count of conidia were measured after exposure to UV-B radiation.

Lethal effect of fungal spores on *Galleria mellonella* after UV exposure

The efficacy of fungal spores harvested after UV exposure was tested on *Galleria mellonella* (L.) (Lepidoptera: Pyralidae) larvae. Laboratory-cultured *G. mellonella* larvae were used in the efficacy experiments. A mixture of beeswax, bran, honey, glycerin, and distilled water was used to feeding *G. mellonella* larvae. Then, the selected larvae were transferred into plastic box (15 cm wide × 8 cm deep) with diet pieces. The fungal spore concentration was calculated as 1×10^7 conidia/ml and applied to the larvae using a sterile sprayer. In the experiments, 30 healthy third instar *G. mellonella* larvae were used in triplicate. All boxes were stored at 25 ± 1°C, 65 ± 5% RH under 12/12 photoperiod for 7 days. The larvae in the control group were only treated with 0.01% aqueous Tween 80. After application, the experiments were monitored for 10 days, and dead larvae were collected. In the control group of *G. mellonella* larvae, only sterile water was used. The dead larvae were transferred to moistened petri dishes and their mycosis was observed. The Schneider-Orelli formula (Püntener, 1981) was utilized to rectify the mortality and mycosis data.

Schneider-Orelli formula:

$$\text{Corrected \%} = \left(\frac{\text{Mortality \% in treated plot} - \text{Mortality \% in control plot}}{100 - \text{Mortality \% in control plot}} \right) * 100$$

Statistical analyses

Data were analyzed using SPSS 25.0 software (IBM Corp., Armonk, NY). The effects of tea extracts on radial growth and spore production tests for two fungal isolates and lethal effect of fungal spores on *G. mellonella* were determined using one-way analysis of variance (ANOVA) and differences between groups by the least significant difference (LSD) tests. Trials with no growth in radial growth and spore production trials were not included in the statistical analysis.

Results

According to the results of the UV-B exposure experiment, it was observed that fungal growth decreased with increasing exposure time. *Beauveria bassiana* isolate of unformulated control (pure conidia) did not show any growth after incubation of 10 days at 120 min of exposure (Figure 1). Fungal spores formulated with black, green, and oolong teas exhibited growth after 120 min of exposure, while fungal spores formulated with white tea did not grow, like the unformulated control. The unformulated *Metharizium flavoviride* isolate showed a radial growth of 4.2 mm at 120 min UV-B exposure, unlike the other isolate. Likewise, the *M. flavoviride* isolate formulated with white tea extract had a radial growth of 4.5 mm at 120 min UV-B exposure (Figure 1). All tested tea extracts showed good compatibility and did not significantly inhibit the growth of *B. bassiana* and *M. flavoviride* spores. The trials without UV-B exposure (0 min) showed the best growth. The two isolates exhibited slightly different responses to UV-B exposure (Table 1).

In the radial growth study, no statistically significant difference was observed in all groups in both isolates at 0 and 30 min exposure times ($F=1.565$; $df=4, 23$; $p=0.265$) (Table 1). It was determined that there was a statistically significant difference between black and green tea and the control group in *B. bassiana* and *M. flavoviride* after 60 min of exposure ($F=77.136$; $df=4, 23$; $p<0.001$). The highest UV-B protection rate was observed with black and green tea extract supplementation to *B. bassiana* spores, resulting in radial growth of 14.6 mm and 14.3 mm, respectively, at the end of 10 days of exposure for 120 min. Treatments containing oolong and white tea also showed significantly increased radial growth compared to the unformulated control after 60 min of exposure ($F=165.985$; $df=4, 23$; $p<0.001$), with measurements of 12.1 mm and 10.9 mm, respectively. Unlike the control, oolong, and white tea formulations of the *M. anisopliae* isolate at 120 min of exposure, black and green tea formulations were statistically in different groups ($F=213.365$; $df=4, 23$; $p<0.001$). After exposure to UV-B radiation, the conidia treated with oolong and white tea displayed noticeably reduced radial growth compared to the control group of unformulated isolates (Table 1).

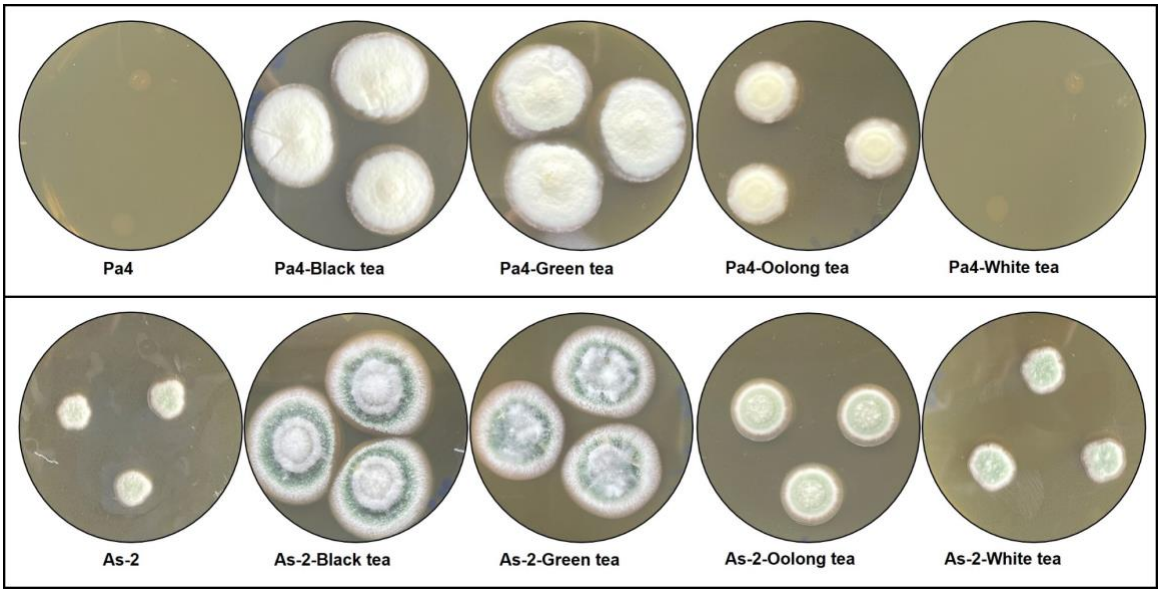

Figure 1. Radial growth of *Beauveria bassiana* Pa4 and *Metharizium flavoviride* As-2 isolates at 120 min UV-B exposure.

Table 1. Effect of UV-B on radial growth diameters (mm±SD) at various exposure times at 10th day

Fungal Isolate	Exposure time (minute)	Unformulated control (pure conidia)		Black tea		Green tea		Oolong tea		White tea	
B. bassiana	0' UV-B	23.6±1.25	aA	23.0±1.36	aA	24.3±2.34	aA	21.6±1.49	aA	24.1±2.46	aA
	30' UV-B	15.2±1.36	bB	20.7±1.65	abA	18.3±0.98	bcA	16.1±1.81	bcAB	15.9±1.03	abB
	60' UV-B	6.3±0.06	bcC	18.2±1.38	cA	16.7±1.03	caB	12.1±0.38	cB	10.9±0.94	cBC
	120' UV-B	NG		14.6±1.26	cA	14.3±1.25	cdA	6.4±0.32	dB	NG	
M. flavoviride	0' UV-B	25.6±0.86	aA	24.5±2.06	aA	23.4±1.94	aA	23.8±1.32	aA	25.1±1.76	aA
	30' UV-B	19.1±1.25	bA	18.6±1.86	bA	16.1±1.05	bA	19.65±1.77	bA	19.5±1.07	bA
	60' UV-B	10.6±1.36	cC	17.3±0.98	bcA	15.8±1.11	bA	12.7±1.68	cBC	12.4±1.09	cBC
	120' UV-B	4.2±0.85	dB	16.12±1.84	bcA	15.6±2.64	bA	5.7±0.85	dB	4.5±0.64	dB

Note: In radial growth, the averages of three replications are presented. The lowercase letters in the columns indicate significant differences between the means according to LSD analysis of UV exposure times ($p<0.05$). Capital letters in the lines indicate significant differences between the means according to the LSD analysis of the unformulated control and fungal spores formulated with tea extracts ($p<0.05$). NG: no growth, SD: standard deviation. 0' UV-B exposure time was considered as the control group.

The effect of UV-B exposure on spore production of strains appears to be highly parallel to the effect of UV-B on radial growth. It was determined that the strains produced the highest number of spores in the application without exposure, and the spore production decreased in antiparallel to the increase in exposure time. *B. bassiana* revealed a more UV-B resistant effect than *M. flavoviride* (Table 2).

The unformulated *M. flavoviride* exhibited higher UV-B resistance compared to *B. bassiana* (Figure 2). Furthermore, the extracts of black and green tea from all the samples tested showed a remarkable protective effect against UV radiation, as evidenced by significantly higher conidial counts compared to the control samples that were not formulated with the extracts.

Table 2. Effect of UV-B on conidia production (conidia/ml×10^7±SD) at various exposure times at 12th day

Fungal Isolate	Exposure time (minute)	Unformulated control (pure conidia)		Black tea		Green tea		Oolong tea		White tea	
B. bassiana	0' UV-B	2.78±1.23	aA	2.75±1.23	aA	2.76±1.52	aA	2.66±1.09	aA	2.76±1.52	aA
	30' UV-B	1.80±0.62	bAB	2.16±0.84	aA	2.19±1.27	aA	1.73±0.25	bAB	1.79±0.61	bAB
	60' UV-B	0.75±0.02	cC	1.47±0.86	bA	1.12±0.34	bA	0.98±0.26	cAB	0.91±0.18	cB
	120' UV-B	0		0.55±0.03	cA	0.46±0.11	cA	0.37±0.01	dB	0	
M. flavoviride	0' UV-B	2.35±1.54	aA	2.37±2.01	aA	2.30±2.12	aA	2.43±0.95	aA	2.50±1.91	aA
	30' UV-B	1.58±0.58	bA	1.56±1.01	aA	1.68±0.69	bA	1.63±0.37	bA	1.62±0.84	bA
	60' UV-B	0.75±0.11	cA	0.80±0.26	cA	0.79±0.11	cA	0.71±0.21	cAB	0.72±0.13	cAB
	120' UV-B	0.09±0.01	dC	0.51±0.09	dA	0.48±0.04	dA	0.29±0.01	dB	0.11±0.02	dBC

Note: The conidia count the averages of three replications are presented. The lowercase letters in the columns indicate significant differences between the means according to LSD analysis of UV exposure times ($p<0.05$). Capital letters in the rows indicate significant differences between the means according to the LSD analysis of tea extracts ($p<0.05$). SD: standard deviation. 0' UV-B exposure time was considered as the control group.

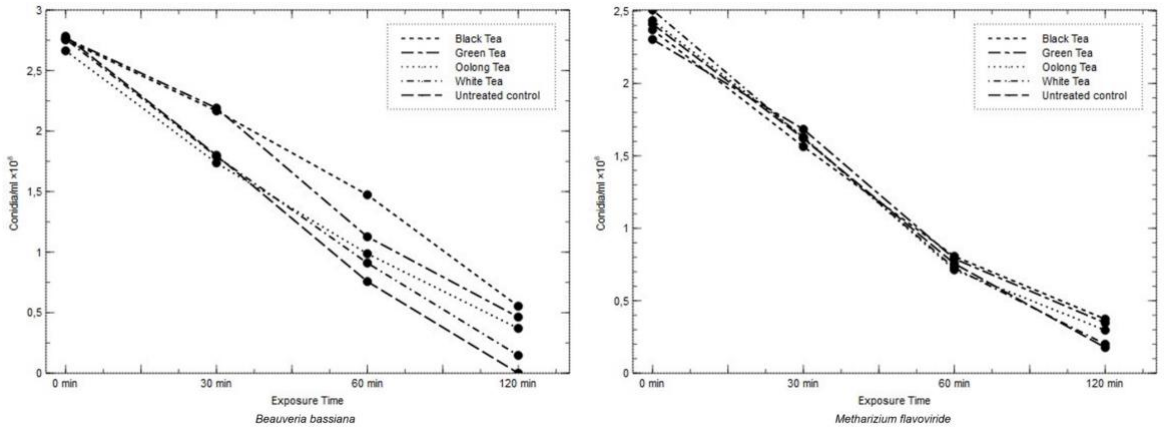

Figure 2. Conidia number of untreated control group (pure spores), black, green, oolong and white tea formulated groups of *Beauveria bassiana* and *Metharizium flavoviride* after UV-B exposure.

Insecticidal activity experiments were conducted using third-stage *G. mellonella* larvae to assess the infectivity of spores from isolates harvested after exposure to UV-B radiation. The mortality percentages of *G. mellonella* larvae infected with fungal spore suspensions (both formulated and non-formulated) following UV-B exposure durations of 0, 30, 60, and 120 minutes are presented in Figure 3.

The study's results revealed that with increasing UV-B exposure duration, lower mortality rates were observed in *G. mellonella* larvae inoculated with non-formulated fungal spores (Figure 3). This corresponded to a decrease in spore germination and infectivity when exposed to UV radiation. In contrast, fungal spores formulated with tea extracts exhibited higher rates of germination and infectivity on *G. mellonella* larvae. This suggests that tea extracts may provide a degree of protection or enhance the survival of fungal spores when exposed to UV-B radiation. No statistically significant difference in mortality rates was observed in fungal spores harvested after 0 and 30 min of exposure to *G. mellonella* ($F=2.105$; $df=4, 23$; $p=0.145$). Regarding the insecticidal activity of the *B. bassiana* isolate after 60 minutes of exposure, formulations containing black tea (68.9%) and green tea (65.2%) were statistically separated from the control group (44.3%) ($F=283.990$; $df=4, 23$; $p<0.001$). In contrast, formulations with oolong tea (51.3%) and white tea (48.2%) were closer to the control group. After 60 minutes of exposure to the *M. flavoviride* isolate, a statistically significant difference was observed between the black tea (62.1%) and green tea (59.2%) formulations and the control group (38.2%) ($F=183.427$; $df=4, 23$; $p=0.618$). Following 120 min of exposure to the *M. flavoviride* isolate, the control group exhibited a mortality rate of 11.2%, while the black tea, green

tea, oolong tea, and white tea formulations recorded mortality rates of 37.3%, 34.6%, 29.1%, and 20.1%, respectively. No death was observed in the control group of *G. mellonella* larvae (Figure 3).

These findings indicate that black tea and green tea extracts have the potential to provide UV protection for fungal spores. In contrast, oolong tea and white tea extracts demonstrated lower UV-protection efficacy, as evidenced by their relatively higher larval mortality rates. Additionally, it was observed that with increased exposure to UV radiation, the radial growth and spore production of the *M. flavoviride* isolate were higher compared to the *B. bassiana* isolate. However, the *B. bassiana* isolate exhibited a higher mortality rate against *G. mellonella* larvae.

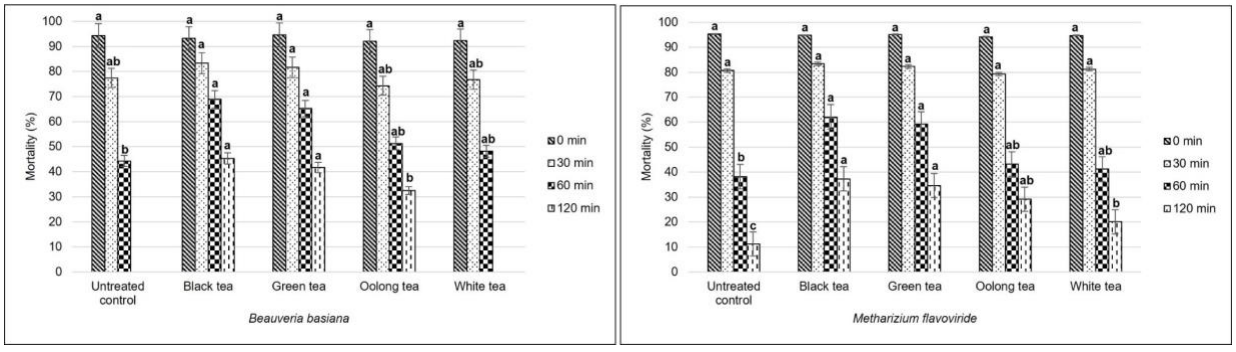

Figure 3. For each isolate, different lower-case letters in each graph column for the same exposure times represent statistically significant differences amongst mortalities according to the least significant difference (LSD) multiple comparison test ($P<0.05$). Mortality indicates the mean of three replications. The bars show the standard deviation of the mean values.

Discussion

Exposure of fungal spores to UV-B radiation, without UV-protective additives, leads to a notable decrease in viability (Kaiser et al., 2018). In the current study, we examined the effectiveness of four tea extracts as UV protectants using *B. bassiana* Pa4 and *M. flavoviride* As-2 isolates and their mortality on *G. mellonella* larvae. These isolates have demonstrated significant potential in previous studies focusing on biological control (Biryol et al., 2020, 2021).

UV-B solar radiation is highly destructive to fungal development and has a considerable impact on their survival and effectiveness in combating environmental insects (Ignoffo & Garcia, 1992; Inglis et al., 2001; Fernandes et al., 2015; Acheampong et al., 2020). Previous research (Braga et al., 2001b, c; Fernández-Bravo et al., 2017) has indicated that a 2-hour exposure to UV-B light significantly reduces the culturability of *Metarhizium* spp. spores, which supports the findings of our study.

The tea plant is widely recognized for its abundant supply of antioxidants and polyphenols, which play a significant role in neutralizing free radicals and reactive oxygen species (Katiyar et al., 2001; Henning et al., 2003; Caffin et al., 2004). Previous studies have demonstrated that green and black tea provide significant UV protection against entomopathogenic viruses (Shapiro et al., 2008; El Salamouny et al., 2009a, b). However, Kaiser et al. (2018) did not detect a similarly potent efficiency for *B. bassiana* spores in their study. Tea extracts are rich in polyphenols, which act as antioxidants against radicals generated by UV radiation. However, they exhibit minimal absorption of UV-A or UV-B (Yusuf et al., 2007). Research demonstrated that the UV protection of viruses and bacteria is partially achieved due to the antioxidants and antioxidative enzymes that counteract the DNA damage induced by radicals (Ignoffo & Garcia, 1978). In the research directed by Kaiser et al. (2018), it was determined that black tea, combined with *B. bassiana*, significantly increased the exposure of the fungus to UV-B in terms of colony-forming units (CFU). However, no effect was observed with green tea. Nevertheless, in the current study, both black tea and green tea demonstrated a significant UV-protective effect on both fungal isolates. In another study, the UV tolerance of *Metharizium* species was investigated, and it was observed that fungal spores in most strains were inactivated after a few hours of UV exposure (Braga et al., 2001b). In the present investigation, there was

a noticeable delay in the development of fungal spores exposed to UV-B compared to the control group (0 min). Additionally, Biryol et al. (2020) examined the UV toleration of *M. flavoviride* As-2 by exposing fungal spores to UV for 30 and 60 min, but significant effects were not observed. Another study result showed that black tea and green tea extracts supply potential UV protectant for the baculovirus, however black tea has the most protectant effect (Ibrahim et al., 2019). Similarly, in this study revealed that black and green tea provided more UV protection in fungal spores. The fact that tea extracts had a higher mortality rate against *G. mellonella* larvae compared to unformulated fungal spores after UV exposure also revealed that they showed a significant protective potential. Ortucu & Algur (2017) reported that there were sudden changes in both radial and spore production, especially at 30 and 60 min of UV-B application. However, in the current study, the radial growth and spore production of fungal spores formulated with black and green tea were higher than the control at 60 and 120 min of exposure. In this study, it was observed that both UV-affected fungal isolates were able to grow and sporulate and maintain their infectivity on *G. mellonella* better than the unformulated control group. An appropriate formulation benefits the application and processing of the bioagent and increases its effectiveness by protecting the active ingredient from adverse environmental factors (Nian et al., 2015).

Although numerous studies have examined the UV tolerance of *B. bassiana* and *M. flavoviride* isolates, there is a scarcity of research on the protective effects of tea extracts as UV protectors for fungal spores. This study presents crucial data by investigating the potential of tea extracts in enhancing the UV protection of fungal spores. The findings of this study demonstrate that the addition of tea extracts as UV-protective additives in formulations can effectively prolong the lifespan of *B. bassiana* and *M. flavoviride* spores when exposed to UV-B radiation.

This study represents the first analysis of the UV-B protection of four distinct tea extracts in laboratory, specifically focusing on their effects on the entomopathogenic fungi *B. bassiana* and M. flavoviride. The present study demonstrated the advantages of black, green, oolong and white tea extracts in UV-B protection of fungal spores and black and green tea have a potential for use as UV protectors under laboratory conditions. The observed decrease in pest mortalities following exposure was attributed to the loss of viable spores due to UV. Hence, the development of formulations to increase persistence of EPF spores under exposure to UV is important in the successful use of EPFs as biological control agents in the fields. Tea extracts have proven UV protection potential in our study and may be an important additive to such a formulation. The developed formulation could be a further valuable tool for fostering the sustainable control of pest insects in the fields.

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Original article (Orijinal araştırma)

The effects of some essential oils on the life table parameters of green peach aphid *Myzus persicae* (Sulzer, 1776) (Hemiptera: Aphididae)

Bazı bitkisel yağların şeftali yeşil yaprakbiti *Myzus persicae* (Sulzer, 1776) (Hemiptera: Aphididae)'nin yaşam çizelgesi parameterleri üzerindeki etkileri

Ali KAYAHAN[1]

Abstract

In this study, essential oils (EOs) of *Citrus limon* (L.), *Citrus sinensis* (L.) (Sapindales: Rutaceae), *Allium sativum* (L.) (Asparagales: Amaryllidaceae) and *Brassica nigra* (L.) (Brassicales: Brassicaceae) were evaluated for their insecticidal effects on the green peach aphid *Myzus persicae* (Sulzer, 1776) (Hemiptera: Aphididae). The lethal and sublethal effects of these EOs on *M. persicae* were studied under laboratory conditions. This study was conducted at Yozgat Bozok University, Faculty of Agriculture, Department of Plant Protection in 2023. The experiments were evaluated at different concentrations for 24 hours after treatment. The lethal concentrations (LC_{50}, LC_{90}) of the EOs were calculated based on the data obtained. The life table parameters of newly born aphids were studied at sublethal concentrations (LC_{40}, LC_{30}) of EOs, and these parameters were calculated using the Euler-Lotka equation. The results show that the mortality rate increases with growing concentration of essential oils. The lethal concentration (LC_{50}) of essential oils were calculated to be 3.47, 4.37, 4.51, and 5.16 µL/L, respectively. The sublethal concentrations (LC_{40}, LC_{30}) of essential oils caused an increase in adult longevity, a decrease in fecundity of surviving aphids and intrinsic rate of increase. From the data obtained, the EOs of *C. limon* and *C. sinensis* were more effective than other EOs in the study. It was found that other essential oils (*A. sativum* and *B. nigra*) may also be effective against *M. persicae*, even if their effect is low.

Keywords: Green peach aphid, intrinsic rate of increase, net reproduction rate, essential oils, lethal concentration

Öz

Bu çalışmada, *Citrus limon* (L.), *Citrus sinensis* (L.) (Sapindales: Rutaceae), *Allium sativum* (L.) (Asparagales: Amaryllidaceae) ve *Brassica nigra* (L.) (Brassicales: Brassicaceae) bitkisel yağlarının, şeftali yaprakbiti *Myzus persicae* (Sulzer, 1776) (Hemiptera: Aphididae) üzerindeki insektisidal etkileri değerlendirilmiştir. Bu bitkisel yağların *M. persicae* üzerindeki öldürücü ve öldürücü olmayan etkileri laboratuvar koşullarında incelenmiştir. Bu çalışma 2023 yılında Yozgat Bozok Üniversitesi, Ziraat Fakültesi Bitki Koruma Bölümünde yürütülmüştür. Denemeler uygulamadan 24 saat sonra farklı bitkisel yağ konsantrasyonları için değerlendirilmiştir. Elde edilen verilere göre bitkisel yağların letal dozları (LC_{50}, LC_{90}) hesaplanmıştır. Bitkisel yağların öldürücü olmayan dozlarında (LC_{30}, LC_{40}) yaşam çizelgesi parametreleri belirlenmiştir ve bu parametreler Euler-Lotka eşitliğine göre hesaplanmıştır. Sonuçlar bitkisel yağ dozlarının artmasıyla ölüm oranın arttığını göstermektedir. Bitkisel yağların *M. persicae* üzerindeki LC_{50} dozları sırasıyla 3.47, 4.37, 4.51 ve 5.16 µL/L olarak hesaplanmıştır. Bitkisel yağların öldürücü olmayan dozları ise (LC_{30}, LC_{40}) ergin ömrünün artmasına, doğurganlığının azalmasına ve kalıtsal üreme oranının azalmasına neden olmuştur. Elde edilen verilere göre *C. limon*, *C. sinensis* bitkisel yağlarının çalışmadaki diğer bitkisel yağlardan daha etkili olduğu görülmüştür. Diğer bitkisel yağların da (*A. sativum* ve *B. nigra*) *M. persicae*'ye karşı düşük de olsa etkili olabileceği belirlenmiştir.

Anahtar sözcükler: Şeftali yeşil yaprakbiti, kalıtsal üreme yeteneği, net üreme gücü, bitkisel yağlar, letal doz

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Introduction

The Green peach aphid *Myzus persicae* (Sulzer, 1776) (Hemiptera: Aphididae) causes damage to more than 400 plants. For this reason, it is one of the most harmful species (Blackman & Eastop, 2000). This harmful insect can increase its population rapidly owing to its parthenogenetic reproduction and short lifespan (Foster et al., 2000). Keeping the population of *M. persicae* under control is only possible with chemical insecticides. Consequently, this harmful insect has gained resistance to different chemicals (Elbert et al., 2008; Bass et al., 2014; Sial, 2019). The use of high amounts of insecticides on agricultural pests creates negative effects on the environment and human health. As a result, the natural equilibrium is disturbed and residue problems arise on the products (Grdıša & Gršić, 2013; Gill & Garg, 2014, Rother, 2018). In recent years, due to such problems of insecticides, it has been searched for compounds of plant origin with little negative effect (Liao et al., 2017; Kunbhar et al., 2018). It is thought that insecticides obtained from plants can be a good alternative to synthetic ones (Isman, 2000; Govindarajan et al., 2016; Khan et al., 2017; Sammour et al., 2018). Volatile compounds in plants do not cause residue problems like other chemicals and their half-lives are quite short in nature. For this reason, compounds derived from plants are preferred in biological control studies (Arnason et al., 1989; Hedin et al., 1997; Regnault-Roger et al., 2012). These compounds are non-lethal to predators, parasitoids and mammals (Scott et al., 2003). In recent years, there have been many studies on the insecticidal activities of essential oils and their components in plants (Isman & Miresmailli, 2011; Ntalli & Menkissoglu-Spiroudi, 2011; Regnault-Roger et al., 2012; Miresmailli & Isman, 2014; Pavela & Benelli, 2016; Chaubey, 2019; Feng et al., 2020; Gaur & Kumar, 2020; Sayed et al., 2021). According to studies conducted in recent years, plant species belonging to 60 families with insecticidal effects can be used as biopesticides (Sukh & Opender, 2017; Isman, 2006). Although there are exceptions such as nicotine, botanical pesticides have a low toxic effect on the environment and essential oils degrade faster in nature than other synthetic chemicals (Moretti et al., 2002; Regnault-Roger & Philogène, 2008). Some researchers have conducted different studies to understand the effect of sublethal concentrations of vegetable oil-based insecticides (Plata-Rueda et al., 2020; Pavela et al., 2020, 2021; Yeguerman et al., 2020; Benelli et al., 2022). The use of essential oils in different concentrations on insects has different effects. Essential oils and the components they contain have different effects, as well as lethal effects, in the adult and pre-adult stages of insects (Alzogaray et al., 2011; Alghamdi, 2018; Abdelaal et al., 2021; Sayed et al., 2022; Al-Harbi et al., 2021). Considering some studies, it is reported that the egg laying rate decreases, adult emergence is suppressed and less damage occurs to the crops (Keita et al., 2001; Rahman & Talukder, 2006). But some studies of citrus EOs have focused on toxicity of aphids. It is known that the extract prepared with lemon (*Citrus limon*) has an insecticidal effect against the rose aphid (*Macrosiphum roseiformis*) (Gupta et al., 2017). In addition, *C. aurantium*, *C. sinensis* and *C. limon* essential oils have been reported to show high toxicity against the woolly beech aphid, *Phyllaphis fagi* (L., 1761) (Hemiptera: Aphididae) (Yazdgerdian et al., 2015). Similar situations apply to vegetable oils obtained from *Allium sativum* and *Brassica nigra*. In other words, they have a toxic effect on different insect species. Considering the studies, it is seen that *A. sativum* has a lethal effect on aphids (Alghamdi, 2018), mosquitoes (Mahanta et al., 2020) and some stored pests (Omar & Zayed, 2021).

In this study, the lethal and sublethal effects of 4 different commercially available vegetable oils [*Citrus limon* (L.), *Citrus sinensis* (L.) (Sapindales: Rutaceae), *Allium sativum* (L.) (Asparagales: Amaryllidaceae), *Brassica nigra* (L.) (Brassicales: Brassicaceae)] on *Myzus persicae* were determined.

Materials and Methods

This study was conducted at Yozgat Bozok University, Faculty of Agriculture, Department of Plant Protection in 2023.

Essential oils (EOs)

The vegetable oils used in this study (*Citrus limon*, *Citrus sinensis*, *Allium sativum*, *Brassica nigra*) were commercially obtained from Botalife®.

Production of pepper plant

The bell pepper plant (*Capsicum annuum* L. var. *grossum*) used in the experiments were grown in 200 mL plastic containers with a 1: 1 soil: peat mixture. The plants were grown in a climate-controlled room at a temperature of 27±1°C, relative humidity of 65±5% and a long daylight photoperiod of 16: 8.

Culture of *Myzus persicae*

The last stage nymph *M. persicae* individuals were transferred to the pepper plants that reached the height (15 cm) and the number of leaves (6 pieces) to be used in the experiments, and they were reproduced in cages (50x50x50 cm) covered with tulle. The initial population of aphids infested to clean plants was obtained from mass production in the laboratory. Aphids were collected on pepper plants in Serik in Antalya and identified by Prof. Dr. İsmail Karaca (Isparta University of Applied Sciences, Isparta Türkiye) in nature were used for the experiments. Aged and decaying plants were replaced with clean plants at weekly intervals to ensure the continuity of mass production. Aphid rearing was performed in climate-controlled rooms at a temperature of 25±1°C, 65±5% proportional humidity and 16: 8 (light: dark) light conditions.

Mixture of fumigant and contact toxicity of essential oils

In the first phase of the study, the lethal effect of different concentrations (0.5, 1, 2, 4, 6, 8, 10, 12 µL/L) of plant oils (*C. limon*, *C. sinensis*, *A. sativum*, *B. nigra*) on *M. persicae* was determined. Petri dishes with filter paper of 9 cm diameter were used for the experiments. The prepared concentrations were included as 1 ml in each Petri dish in the filter paper. The nymphs (2nd and 3rd stages) were transferred to this paper using a thin sable brush. Then, the individuals were put in contact with the concentration on the paper (tarsal, ventral and labial contact), assuming that the plant oils were affected by the toxicity of the fumigant. Then, leaves of bell pepper plants were added to the Petri dish to feed the aphids. After 24 hours, the live and dead individuals were recorded and the effects of the oils were determined. Tween20 (2%) was used to dissolve the oils in the experiments. For each concentration, 10 Petri dishes were used and for each Petri dish, 10 aphids were used. Tween20 (2%) was used as a control. Experiments were conducted in air-conditioned rooms at a temperature of 25±1°C, relative humidity of 65±5%, and a long daylight photoperiod of 16: 8. To determine mortality rates over live and dead individuals, Abbott's formula was used and the percentage of mortality rates was calculated (Abbott, 1925). Analysis of variance (ANOVA) was applied to the results obtained. If the difference between the means was statistically significant, Tukey HSD post-hoc test was used to compare group means ($\alpha<0.05$). The lethal concentrations of the plant oils (LC_{30}, LC_{40}, LC_{50}, and LC_{90}) were determined using the mortality rates obtained in this phase of the study. Probit analysis was used to determine these concentrations.

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

Estimating life table parameters

The effects of LC_{30} and LC_{40} concentrations of plant oils on *M. persicae* were determined. The prepared concentrations 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. Damp cotton is left on the bottom of the filter paper to prevent the leaves from fading. Bell pepper leaves were then laid out as food for the aphids. The daily development of individuals was then monitored; newborns were recorded and removed from the Petri dishes. Counts continued until the aphids died. This part of the experiments was performed with 50 replicates for each concentration. To ensure air circulation in the Petri dish, the lids of the standard size Petri dishes were opened and covered with tulle to prevent escape of the animals. Experiments were performed in air-conditioned rooms at a temperature of 25±1°C, relative humidity of 65±5%, and a long daylight photoperiod of 16: 8.

The data obtained from the experiments were recorded to determine the development of age-related life tables for each temperature used. The parameters of the life tables of *Myzus persicae* were calculated using RmStat-3 software (Özgökçe & Karaca, 2010) according to the Euler-Lotka equation (Birch, 1948), and analyzed separately. In the study, resampling was performed using the bootstrap method and the data obtained here were compared. Tukey multiple comparison test was used to compare the periods with Minitab (Ver. 16) at the level of significant difference $p<0.05$. The following equations were used to calculate the parameters:

Age-related survival rate (l_x), Fertility rate (m_x) (Birch, 1948);

Reproductive value (V_x)

$$V_x = \frac{\sum_{y=x}\left(e^{r_m.y}.\, l_y.\, m_y\right)}{l_x.e^{-r_m.x}}$$ (Imura ,1987);

Net Reproduction Rate (R_0)

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

Intrinsic Rate of Increase (r_m)

$$\sum e^{(-r_m.x)}\, l_x.m_x = 1$$ (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

Toxicity of essential oils on *Myzus persicae*

It was observed that the vegetable oils used in the study were effective on *Myzus persicae*. In addition, especially as the concentration increased, the mortality rate increased ($p<0.05$). The mortality rate at the highest concentration (12 µl/L) of *C. limon* was 94.73%; The mortality rate at the lowest concentration (0.5 µl/L) of *Brassica nigra* was 16.67% (Figure 1).

Considering the lethal concentrations of vegetable oils on *M. persicae*, the lowest LC_{50} (3.47 µl/L) and LC_{90} (9.71 µl/L) values were observed in *C. limon* application, depending on mortality rates. LC_{50} and LC_{90} values of vegetable oils are given in Table 1.

Table 1. Toxicity of different essential oils on *Myzus persicae* after 24 h

Essential oils	N	LC_{30} (µl L^{-1}) (95% Cl)[a]	LC_{40} (µl L^{-1}) (95% Cl)[a]	LC_{50} (µl L^{-1}) (95% Cl)[a]	LC_{90} (µl L^{-1}) (95% Cl)[a]	Slope ±SE[b]	X^2 (df)[c]
Citrus limon	900	0.91 (0.26-1.46)	2.23 (1.70-2.71)	3.47 (3.00-3.92)	9.71 (8.93-10.68)	1.234±0.119	33.56 (7)
Citrus sinensis	900	1.61 (0.97-2.17)	3.04 (2.51-3.53)	4.37 (3.89-4.86)	11.13 (10.24-12.25)	1.218±0.118	22.78 (7)
Allium sativum	900	1.66 (0.99-2.23)	3.13 (2.59-3.64)	4.51 (4.02-5.01)	11.49 (10.55-12.67)	1.179±0.121	24.11 (7)
Brassica nigra	900	2.77 (2.20-3.29)	4.19 (3.69-4.67)	5.16 (5.04-6.02)	12.21 (11.28-13.38)	1.398±0.108	13.49 (7)

[a] 95% confidence intervals; [b] Standart error; [c] Chi-square value (x^2) (Pearson) and degrees of freedom (*df*).

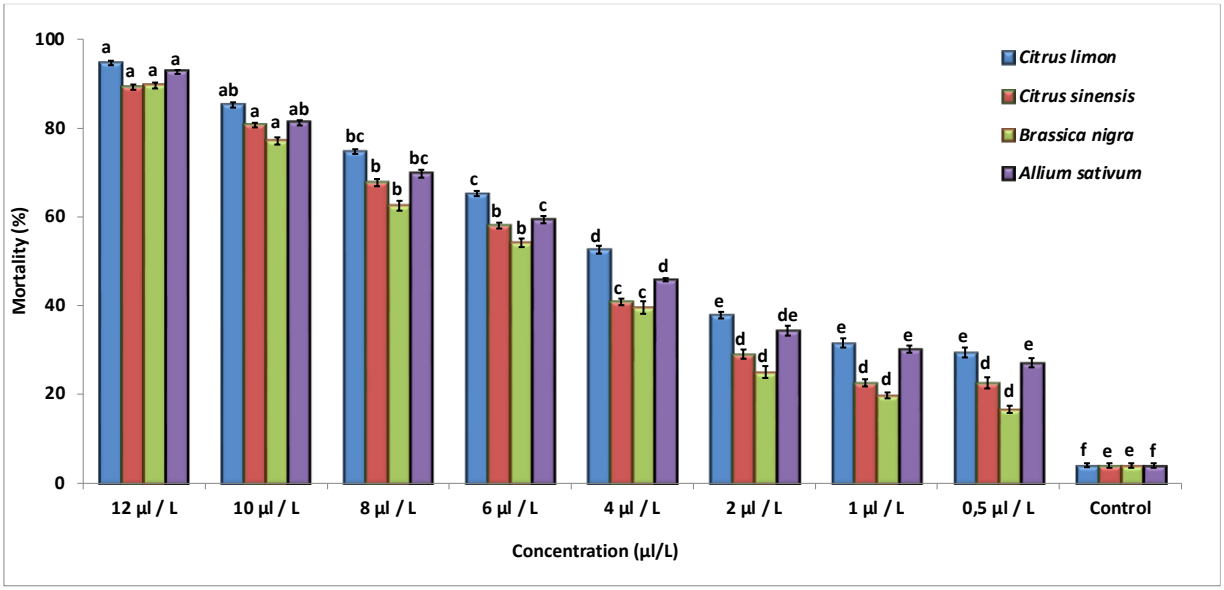

Figure 1. Mortality percentage of *M. persicae* exposed to different concentrations of different EOs (*C. limon*, *C. sinensis*, *A. sativum* and *B. nigra*) for 24 h. Comparisons were made between the application doses of the EOs. Means above columns followed by different letters were significantly different according to Tukey ($F_{C.limon}$: 156.58; $df_{C.limon}$: 8, 86; $P_{C.limon}$: 0.001 / $F_{C.sinensis}$: 125.97; $df_{C.sinensis}$: 8, 86; $P_{C.sinensis}$: 0.001 / $F_{B.nigra}$: 88.17; $df_{B.nigra}$: 8, 86; $P_{B.nigra}$: 0.001 / $F_{A.sativum}$: 88.17; $df_{A.sativum}$: 8, 86; $P_{A.sativum}$: 0.001, Error bars in the figure have shown standard error.).

Sublethal effects of vegetable oils on the life cycle parameters of *Myzus persicae*

Different lethal concentrations (LC_{30} and LC_{40}) were calculated depending on the mortality rates at the end of 24 hours in order to calculate the sublethal effects of EOs (Table 2).

Table 2. Life table parameters of *Myzus persicae* under influence of different concentrations of essential oils (*Citrus limon*, *Citrus sinensis*, *Allium sativum*, *Brassica nigra*)

Parameters*	Control**	*Citrus limon*		*Citrus sinensis*		*Allium sativum*		*Brassica nigra*	
		LC_{30}	LC_{40}	LC_{30}	LC_{40}	LC_{30}	LC_{40}	LC_{30}	LC_{40}
r_m	0.35±0.0041 e	0.27±0.0052 e	0.27±0.0049 e	0.29±0.0033 d	0.29±0.0034 d	0.30±0.0031 bc	0.30±0.0025 c	0.31±0.0034 b	0.31±0.0037 b
R_0	55.95±1.2500 a	26.27±1.5400 e	25.89±1.4100 e	31.07±2.3300 d	30.68±1.9900 d	36.84±1.2100 bc	34.00±1.7600 c	39.55±2.3200 b	38.37±1.5600 b
T_0	11.45±0.0176 h	11.93±0.0031 a	11.65±0.00284 f	11.83±0.00258 c	11.62±0.002 g	11.87±0.0024 b	11.69±0.0023 e	11.89±0.0023 b	11.79±0.0024 d
GRR	62.27±0.0519 a	44.02±0.0188 f	38.59±0.0182 ı	47.76±0.0561 d	40.89±0.0278 h	48.74±0.0458 c	43.66±0.0357 c	51.33±0.0457 b	45.97±0.0193 e
T_2	1.973±0.0035 g	2.529±0.0020 b	2.545±0.0020 a	2.324±0.0011 d	2.353±0.001 c	2.281±0.0104 f	2.298±0.0011 f	2.242±0.0008 g	2.28±0.0009 f
λ	1.421±0.0008 a	1.315±0.0003 g	1.313±0.0003 h	1.347±0.0002 e	1.343±0.0002 f	1.355±0.0002 c	1.352±0.0002 c	1.362±0.0002 b	1.356±0.0002 c
N	50	50	50	50	50	50	50	50	50

* r_m: Intrinsic rate of increase; R_0: Net reproduction rate; T_0: Mean generation time; *GRR*: Gross reproduction rate; T_2: Doubling time; λ: Finite rate of increase.

** Different letters for same parameters in the same row were significantly different according to Tukey (Mean±SE) (F_{rm}: 8942.79; df_{rm}: 8, 41; P_{rm}: 0.001 / F_{Ro}: 34700.45; df_{Ro}: 8, 41; P_{Ro}: 0.001 / F_{To}: 591.17; df_{To}: 8, 41; P_{To}: 0.001 / F_{GRR}: 32985.22; df_{GRR}: 8, 41; P_{GRR}: 0.001 / F_{T2}: 9592.49; df_{T2}: 8, 41; P_{T2}: 0.001 / F_{λ}: 8794.18; df_{λ}: 8, 41; P_{λ}: 0.001).

Calculated concentrations were applied to aphids and their effects were determined. Accordingly, while intrinsic rate of increase (r_m) and net reproduction rate (R_0) values show a decrease compared to the control; mean generation time (T_0) was determined to be higher than the control (p<0.05). The r_m (0.309 and 0.305 nymphs/female/day) and R_0 (39.547 and 38.372 nymphs/female) values close to the control application were calculated in two concentrations of *B. nigra* (p<0.05). The lowest r_m (0.272 nymphs/female/day) and R_0 (25.893 nymphs/female) values were observed in the LC_{40} concentration of *C. limon* (p<0.05). When the gross reproduction rate *GRR* was calculated, it was determined that the lowest value was at the LC_{40} concentration of *C. limon*, and the highest value was at the LC_{30} concentration of *B. nigra* after the control application (Table 2).

As a result of the application of LC_{40} and LC_{30} concentrations of the EOs applied in the study on *M. persicae*, decreases in survival rate (l_x), fecundities (m_x) and reproduction value (V_x) were observed

compared to the control. According to the data obtained, it was determined that the lowest l_x, m_x and V_x values were in *C. limon* concentrations. It was observed that these values increased in other EOs concentrations (Figure 2).

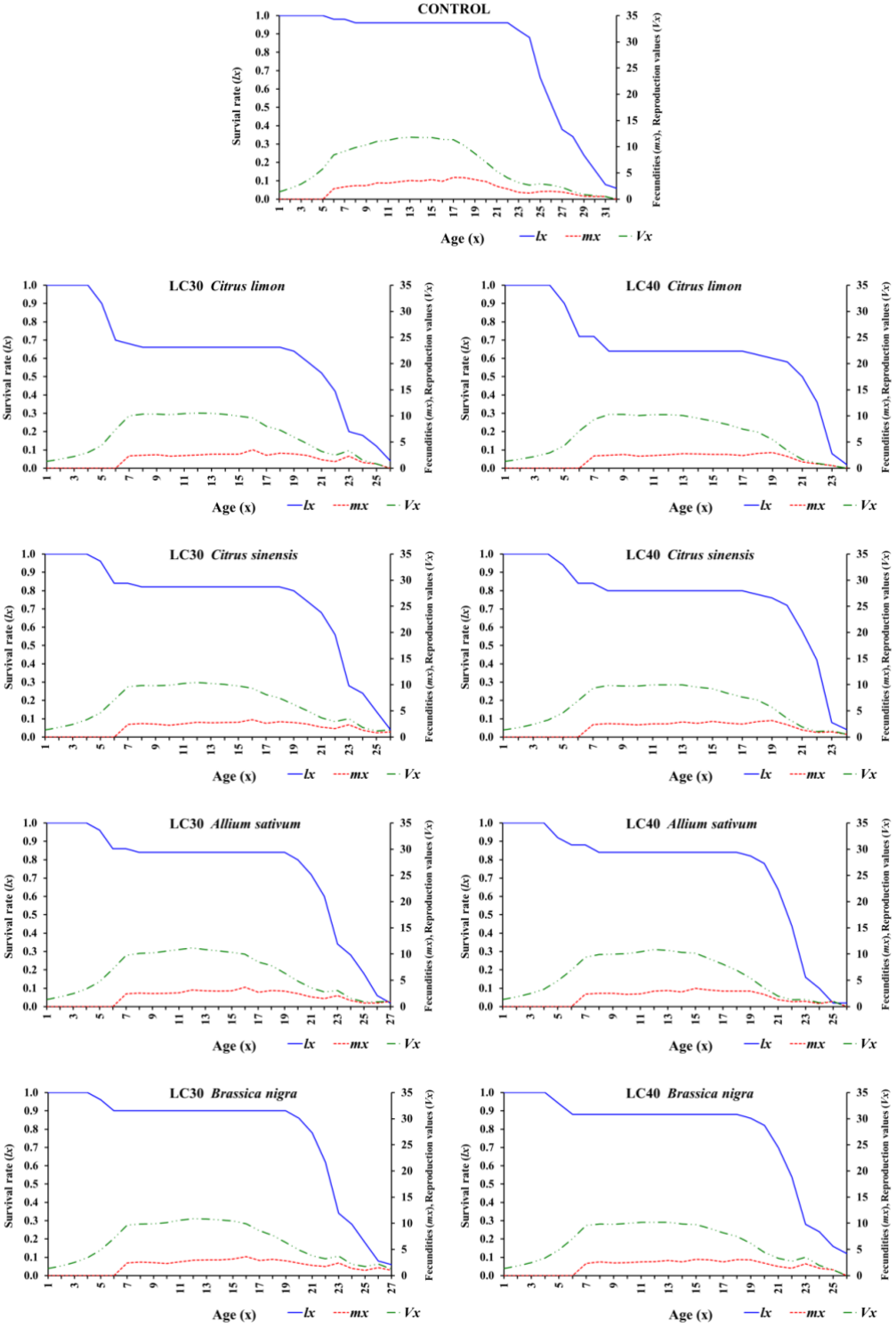

Figure 2. Survival rate, fecundity and reproductive value of *Myzus persicae* under influence of different concentrations of essential oils

Discussion

The data obtained from the experiments show that the EOs used (especially *C. limon* and *C. sinensis*) are effective against *Myzus persicae*. It was noted that in recent years, more studies have been conducted to investigate the effects of plant-based pesticides (from different vegetable families) on pests. (Schoonhoven, 1982; Jacobson, 1989; Isman, 1995; Durmuşoğlu et al., 2011; Sayeda & El-Mogy, 2011; Balcı et al., 2020). Although the effects of biopesticides used in the control of agricultural pests are not fully known, they are shown to have different effects on them. For this reason, the dose, concentration, and application frequency of the bioinsecticides used are very important (Bakkali et al., 2008). In addition, the effects of different plant oils on aphids or other pests have been studied in recent years, and they have been found to be effective on pests, although they vary among species (Işık & Görür, 2009; Górski & Tomczak, 2010; Yazdgerdian et al., 2015; Górski et al., 2016; Albouchi et al., 2018; Benelli et al., 2018; Czerniewicz et al., 2018; Behi et al., 2019; Ravan et al., 2019).

In some studies, extracts from the peels of citrus fruits such as *C. sinensis* and *Citrus paradisi* (L.) (Sapindales: Rutaceae) were found to be quite effective against aphids. It was found that particularly high concentrations of extracts increased the mortality rate (Iqbal et al., 2011; Amiri et al., 2013). Kimbaris et al. (2010) investigated the effect of *C. sinensis* EOs on various aphids (*Aphis fabae* Scopoli 1763, *Macrosiphoniella sanborni* (Gillette, 1908), *Acyrthosiphon pisum* Harris 1776 and *Myzus persicae* (Hemiptera: Aphididae)). In the data obtained, the LC_{50} values were reported to be 1.17, 1.25, 1.92 and 1.43 µL/L, respectively. The results have shown that the plant oil extracted from *C. sinensis* is effective against aphids. Gupta et al. (2017) observed that extracts obtained from the peel of *Citrus limon* were effective on *Macrosiphum roseiformis* (L., 1758) (Hemiptera: Aphididae). According to the LC_{50} values obtained by them, the highest toxicity was calculated to be 6.68 mg/ml and it was found that it could be effective against aphids. In addition, extracts from different parts of citrus plants *Rhyzopertha dominica* (Fabricius, 1792) (Coleoptera: Bostrichidae), *Sitophilus oryzae* Schoenherr, 1838 (Coleoptera: Curculionidae) (Tripathi et al., 2003), *Callosobruchus maculates* (Fabricius, 1775) (Coleoptera: Chrysomelidae) (El-Sayed & Abdel-Razik, 1991), *Tribolium castaneum* (Herbst, 1797) (Coleoptera: Tenebrionidae), *Trogoderma granarium* Everts, 1898 (Coleoptera: Dermestidae) (Zia et al., 2013), *Zabrotes subfasciatus* Boheman, 1833 (Coleoptera: Chrysomelidae) (Zewde & Jembere 2010), *Musca domestica* (L., 1758) (Diptera: Muscidae) (Palacios et al., 2009), *Planococcus ficus* Ben-Dov, 1994 (Hemiptera: Pseudococcidae) (Karamaouna et al., 2013), *Thaumetopoea wilkinsoni* Tams, 1924 (Lepidoptera: Notodontidae) (Çetin et al., 2006), *Attagenus fasciatus* (Thunberg, 1795) (Coleoptera: Dermestidae), *Lasioderma serricorne* (Fabricius, 1792) (Coleoptera: Ptinidae) (Bakr et al., 2010), and mosquitoes (Akram et al., 2010; Effiom et al., 2012) is supported by the literature. Choi et al. (2004) determined the toxicity of 53 vegetable oils to *Tetranychus urticae* C. L. Koch, 1836 (Acari: Tetranychidae). They reported that two citrus oils (bergamot and sweet orange) killed the pest 87% and 61%, respectively. Campolo et al. (2020), in their study on the effect of citrus oils, found that the formulation obtained from sweet orange had a toxic effect on the eggs and larvae of *Tuta absoluta* Stainton, 1856 (Lepidoptera: Gelechiidae). Campolo et al. (2017) found that the effect of formulations based on mandarin and lemon EO was lower than that of orange. When the results obtained are evaluated and compared with the literature, it is seen that the oils obtained from plants belonging to the *Citrus* genus have a toxic effect, especially on aphids. For this reason, it is thought that these oils have potential in controlling these pests.

Alghamdi (2018) determined the effect of essential oil of four different plants [*Moringa oleifera* Lam., 1785 (Brassicales: Moringaceae), *Eruca sativa* (L.), *Raphanus sativus* (L.) (Brassicales: Brassicaceae), *Allium sativum* (L.) (Asparagales: Amaryllidaceae)) on rose aphid (*Macrosiphum rosae* (L., 1758) (Hemiptera: Aphididae)] and field bean aphid (*Aphis fabae*). In this study, conducted with different concentrations, it was found that the number of deaths increased with growing oil concentration. For both aphids, the highest mortality rate was found in arugula oil and the lowest rate in moringa oil. Based on the

results, it was concluded that the oils used in the study could be effective against aphids. In their study, Mahanta et al. (2020) investigated the effects of different plant oils (*A. sativum*, *Ocimum sanctum* L. (Lamiales: Lamiaceae) and *Citrus grandis* (Sapindales: Rutaceae)) on *Culex quinquefasciatus* Say, 1823 (Diptera: Culicidae). It was found that the vegetable oil extracted from *A. sativum* had higher toxic effect on *C. quinquefasciatus* than the others and its LC_{50} value was 18.23 µL/L. Omar & Zayed (2021) investigated the effect of *A. sativum* vegetable oil on *T. castaneum* and *R. dominica* and reported that the EO had toxic effect on the mentioned stored pests. The LC_{50} values calculated via mortality rates were reported to be 0.794 and 0.380 mg/ml, respectively. The *A. sativum* vegetable oil used in our study showed a toxic effect on *M. persicae*, although not as strong as citrus. As per previous studies, it is considered to be particularly effective in controlling aphids.

There are also different studies on *A. sativum*, one of the oils used in the study. Ali & Rodina (2002), in a study, found that a mustard extract obtained with ethanol had high toxicity on the cotton aphid *Aphis gossypii* Glover, 1877 (Hemiptera: Aphididae). At the same time, studies on the effect of the same plant on different pests are also notable. The essential oil extracted from mustard (*Brassica nigra* (L.) (Brassicales: Brassicaceae) showed very high toxicity to *Bruchidius incarnatus* (Boheman, 1833) (Coleoptera: Chrysomelidae), one of the pests of stored products (Sabbour & El-Aziz, 2010). *Callosobruchus chinensis* L., 1758 (Coleoptera: Chrysomelidae) was found to be removed from the environment by the powder extracted from this plant (Li et al., 2008). Ali & Mohamed (2018) determined the effect of *B. nigra* seeds on *Spodoptera littoralis* Boisduval, 1833 (Lepidoptera: Noctuidae). It was reported that the seeds have the ability to prevent feeding on the harmful species. Koneckal et al. (2018) reported that the vegetable oil extracted from *Brassica alba* (L.) (Brassicales: Brassicaceae) has toxic effects on several Lepidoptera pests [*Cydia pomonella* L., 1758 (Lepidoptera: Tortricidae)*, Dendrolimus pini* L., 1758 (Lepidoptera: Lasiocampidae), and *Spodoptera exigua* (Hübner, 1808) (Lepidoptera: Noctuidae)]. The lethal concentration (LC_{50}) values were calculated as 0.422, 11.74 and 11.66 mg/ml, respectively. From the results, the vegetable oil used causes high mortality especially in *C. pomonella* and can be used as a biopesticide in similar lepidopterans. In previous studies, EOs and various substances derived from plants of the genus *Brassica* were found to be particularly effective against stored pests. In our study, *B. nigra* EO, which is used against green peach aphid, was found to have some toxicity, especially at high concentrations, although less than other oils and it is suggested that it may be effective against this type of pest.

Ali & Mohamed (2018) determined the effect of *B. nigra* seeds on *Spodoptera littoralis* Boisduval, 1833 (Lepidoptera: Noctuidae). It was reported that the seeds have the ability to prevent feeding on the harmful species. Koneckal et al. (2018) reported that the vegetable oil extracted from *Brassica alba* (L.) (Brassicales: Brassicaceae) has toxic effects on several Lepidoptera pests [*Cydia pomonella* L., 1758 (Lepidoptera: Tortricidae)*, Dendrolimus pini* L., 1758 (Lepidoptera: Lasiocampidae), and *Spodoptera exigua* (Hübner, 1808) (Lepidoptera: Noctuidae)]. The lethal concentration (LC_{50}) values were calculated as 0.422, 11.74 and 11.66 mg/ml, respectively. Based on the results, it is concluded that the vegetable oil used causes high mortality especially in *C. pomonella* and can be used as a biopesticide in similar lepidopterans. In previous studies, EOs and various substances derived from plants of the genus *Brassica* were found to be particularly effective against stored pests. In our study, *B. nigra* EO, which is used against green peach aphid, was found to have some toxicity, especially at high concentrations, although less than other oils and it is suggested that it may be effective against this type of pest.

There are also studies on the effects of different vegetable oils on *M. persicae*, which is one of the important plant pests and the subject of our study. Kimbaris et al. (2010) studied the effect of different plant oils (*Mentha piperita* L., *Mentha pulegium* L. *Ocimum basilicum* L. (Lamiales: Lamiaceae)and *C. sinensis*) on *M. persicae* and reported that the LC_{50} values were 0.99, 1.12, 1.20 and 1.43 µL/L, respectively. It can be said that the essential oils of the genus *Mentha* are more potent than those of *C. sinensis*. It was found that the plant oils, *Cymbopogon citratus* (DC.) Stapf (Poales: Poaceae), *Cymbopogon winterianus* Jowitt

ex Bor (Poales: Poaceae) and *Eucalyptus citriodora* K.D. Hill & L.A.S Johnson (Myrtales: Myrtaceae) used in different studies with *M. persicae* had a toxic effect on the pest and their LC_{50} values were 2.8, 3.6 and 4.0 mL/L, respectively (Costa et al., 2013; Pinheiro et al., 2013; Costa et al., 2015). Albouchi et al. (2018) studied the effects of *Melaleuca styphelioides* Sm. (Myrtales: Myrtaceae) plant oil on various aphids [*A. gossypii*, *Aphis spiraecola* Patch, 1914 (Hemiptera: Aphididae) and *M. persicae*] and found that it was toxic to them. Based on the data obtained, they calculated LC_{50} values of 3660.99, 619.09 and 756.65 µL/L, respectively. Gouvea et al. (2019) determined the toxicity of aqueous and ethanolic extracts of *Acmella oleracea* L. (Asterales: Asteraceae) on *M. persicae* and *Lipaphis erysimi* (Kaltenbach, 1843) (Hemiptera: Aphididae). Accordingly, ethanol extract caused the death of both aphid species by 90% within 70 hours and reduced their fecundity. Mülayim et al. (2020) studied the fumigation effect of some plant oils [thyme, *Origanum onites* L. (Lamiales: Lamiaceae), anise, *Pimpinella anisum* L. (Apiales: Apiaceae), fennel, *Foeniculum vulgare* (Apiales: Apiaceae), and lavender, *Lavandula angustifolia* L. (Lamiales: Lamiaceae)] against *Aphis craccivora* C.L. Koch, 1854 (Hemiptera: Aphididae) and *M. persicae*. The mortality rate for *A. craccivora* was calculated to be 96.67% in thyme oil at a dose of 60 µl/l air, one of the EOs used. Fennel and thyme essential oils are believed to have the potential to act as biofumigants against *A. craccivora* and *M. persicae*. Nikolova et al. (2021) determined the effects of *Origanum vulgare* subsp. *hirtum* L. (Lamiales: Lamiaceae) on *M. persicae*. In their studies conducted with different concentrations, it was found that the mortality rate increased with increasing concentration and the highest mortality rate was 3 µL/mL. Jasman & Slomy (2021) determined the effect of plant oils from *Mentha longifolia* L. (Lamiales: Lamiaceae) and *Anethum graveolens* L. (Apiales: Apiaceae) on *M. persicae*. At the end of the study, it was found that *M. longifolia* EO was more toxic than *A. graveolens*. Based on the data obtained, it was concluded that *M. longifolia* can be used to control *M. persicae*.

Although vegetable oils, which have a short half-life in nature, have a high toxic effect on pests, their effects on the environment are fully known. For this reason, it is beneficial to use it in low concentrations as in our study and repeat it under field conditions. In addition, it was concluded that the vegetable oils may be useful in controlling the population of *M. persicae* and similar pests. However, the content of the plant oil used must be determined in order to determine from which active ingredient the resulting toxicity is derived. For this reason, it is useful to determine the content of EOs in this study as well as in other studies.

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Original article (Orijinal araştırma)

Insecticide resistance of *Aphis gossypii* Glover, 1877 (Hemiptera: Aphididae) in cotton fields in Çukurova Region (Türkiye)[1]

Çukurova Bölgesi (Türkiye) pamuk alanlarında *Aphis gossypii* Glover, 1877 (Hemiptera: Aphididae)'nin insektisit direncinin belirlenmesi

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Abstract

This study aimed to reveal resistance levels of *Aphis gossypii* Glover, 1877 (Hemiptera: Aphididae) populations against dimethoate, λ-cyhalothrin and chlorpyrifos-ethyl used in cotton fields in Adana province in Çukurova Region (Türkiye). Bioassay, biochemical and molecular methods were used to determine resistance on the populations collected from 16 locations between 2020 and 2021. Six populations were resistant according to the susceptible Toktamış population with leaf dip discriminating dose bioassays. Compared to the susceptible population, four populations were found at decreased susceptibility (DS) resistance levels to dimethoate and one population to chlorpyrifos-ethyl. Only two populations resistance ratio were detected in MR (Moderate resistance) category to chlorpyrifos-ethyl. Resistance levels of other populations were observed as S (susceptible) category. Resistant populations had higher acetylcholinesterase, glutathione-S transferase and cytochrome P450 monooxygenase enzyme activities in biochemical analysis. The carboxylesterase gene transcription levels were higher in resistant populations. S431F and Kdr (knockdown) mutation were determined by the PCR-RLFP method, which is effective in organophosphate and pyrethroid insecticides resistance and 17% and 100% recessive alleles were detected in populations. The biochemical and mutation-induced resistance to dimethoate and chlorpyrifos-ethyl was detected. These results will contribute to developing strategies for resistance management of *A. gossypii.*

Keywords: *Aphis gossypii*, insecticide, resistance, Türkiye, organophosphate, pyrethroid

Öz

Bu çalışma ile, *Aphis gossypii* Glover, 1877 (Hemiptera: Aphididae) popülasyonlarının Çukurova bölgesinde (Türkiye) Adana ilinde pamuk alanlarında kullanılan dimethoate, λ-cyhalothrin ve chlorpyrifos-ethyl'e karşı direnç seviyelerinin ortaya konması amaçlanmıştır. 2020 ile 2021 yılları arasında 16 koordinattan elde edilen popülasyonlar üzerinde bioassay, biyokimyasal ve moleküler yöntemler uygulanmıştır. Yaprak daldırma metodu ile ayırıcı doz çalışmaları yapılarak 16 popülasyon incelenmiş ve Toktamış popülasyonu hassas popülasyon olarak belirlenmiştir. Hassas popülasyona göre, dört popülasyonun dimethoate'a ve bir popülasyon chlorpyrifos-ethyl'e karşı direnci düşük seviye (DD) kategorisindedir. Yalnızca iki popülasyonun chlorpyrifos-ethyl'e karşı direnci orta seviye (OD) direnç kategorisindedir. Diğer popülasyonların direnç oranlarının hassas (D) seviye kategorisinde kaldığı tespit edilmiştir. Dirençli popülasyonların, biyokimyasal analizlerde daha yüksek asetilkolinesteraz, glutatyon-S transferaz ve sitokrom P450 monooksigenaz enzim aktivitelerine sahip oldukları gözlenmiştir. Karboksilesteraz gen ifadesi seviyeleri dirençli popülasyonlarda daha yüksektir. S431F ve Kdr (knockdown) mutasyonları, organofosforlu ve piretroit insektisitlere karşı etkili olan PCR-RLFP yöntemi ile belirlenmiş olup, popülasyonlarda sırasıyla %17 ve %100 oranında baskın alellere rastlanmıştır. Bu çalışmada dimethoate ve chlorpyrifos-ethyl'e karşı biyokimyasal ve mutasyon kaynaklı direnç olduğu düşünülmektedir. Bu sonuçlar, bölgede *A. gossypii*'ye karşı direnç yönetimi stratejilerinin geliştirilmesinde katkı sağlayacaktır.

Anahtar sözcükler: *Aphis gossypii*, direnç, insektisit, Türkiye, organikfosfatlılar, piretroit

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Introduction

Türkiye is one of the vital cotton-growing countries with high yield, considering its ecological characteristics, soil fertility and irrigation capacity (IRAC, 2023). *Aphis gossypii* Glover, 1877 (Hemiptera: Aphididae) is a cotton pest and known as polyphagous species with a broad-spectrum host plant (Cheng et al., 2023). Chemical pesticides, including the commonly used organophosphorus, pyrethroid and carbamates, have been used intensely against various pests, and the resistance problem has been revealed in recent years (Alpkent et al., 2020; Erdoğan et al., 2023). It has been reported that *A. gossypii* developed resistance to more than 50 active ingredients in Türkiye and around the World (Anonymous, 2023). Aphids have rapid reproduction capability, and it is called parthenogenetic telescopic generation (Moran, 1992). This phenomenon caused millions of aphids from one aphid in one season (Moran, 1992; Kersting et al., 1999). This reproduction system has an important effect on resistance development; therefore, rapidly growing generations are exposed to more insecticides and develop resistance fast (Roush & McKenzie, 1987). Carbamate, organophosphate (OP's), organochlorine, and pyrethroid are the most used insecticide groups worldwide. Carbamate and OP's groups are known to inhibit acetylcholinesterase (AChE's) enzymes. These groups impact the nervous system and cause the organism's death (Yorulmaz & Ay, 2010). Pyrethroids affect target site proteins and cause negative activities on sodium ion channels (voltage-gated sodium channel, VGSC) which are active for signal transmission in the nervous system. This channel provides the displacement of sodium ions in the nerve cell membrane, causing action potential and starting neural transmission. However, pyrethroids inhibit channel activation and cause continuous discharge of nerve cells. This situation results with the paralysation and death of the organism (Amad et al., 2003; Marshall et al., 2012). Although new insecticides have been developed for pest control, OP's and pyrethroid have been used widely and intensively. The intensive and uncontrolled use of insecticides against aphids has been causing failure, yield loss, and high resistance development (Pan et al., 2009, 2010). Although chlorpyrifos is banned in cotton-growing areas in Türkiye, dimethoate among the OP's and synthetic pyrethroids (SP's) have been used for 40 years against aphids by farmers (Wang et al., 2021). In resistance management, determining the level of resistance against different insecticides over time is important for updating the integrated pest management (IPM) strategy. The introduction of new active ingredients into the market in the last 30 years, especially the intensive use of neonicotinoid group insecticides, has led to a decrease in the use of old actives. The level of resistance allele and susceptibility of the harmful organism in agricultural areas is an important fact that must be followed in order to guide resistance management strategy. For this purpose, in Çukurova region, determining the resistance status against OP's and SP's insecticides, which have been used extensively, will make positive contributions to insecticide resistance management (IRM) and IPM. According to the results of the previous studies, aphids develop resistance against OP's and SP's rapidly, and this resistance occurs owing to various mechanisms (O'Brien & Graves, 1992; Shang et al., 2012). Depending on increasing metabolic activity, carboxylesterases (CarE's), glutathione S-transferases (GST's) and cytochrome P450 enzymes may cause insecticide resistance in *A. gossypii* (Pan et al., 2009; Carletto et al., 2010; Shang et al., 2012). In addition, it has been reported that point mutations carrying AChE's may be the reason for resistance (Li & Han, 2004; Pan et al., 2010). Generally, CarE's combines with OP's and hydrolyzes the insecticide esters into non-toxic products. Thus, preventing OP's from reaching the target zone AChE's (Pan et al., 2009). It has been reported that higher CarE's levels (E4 and FE4) owing to gene duplications and amplifications cause increasing detoxification of OP's, carbamates, and SP's on *Myzus persicae* (Sulzer, 1776) (Hemiptera: Aphididae) (Field & Devonshire, 1998; Field, 2000). Similarly, it was reported that the increase in gene amplification or transcription level in CarE's played a role in OP's resistance in *A. gossypii* (Devonshire & Moores 1982; Cao et al. 2008a; Pan et al., 2009, 2010; Lokeshwari et al., 2016). In addition, the structural changes (mutations) in the CarE's gene have also been reported to be associated with OP's resistance (Sun et al., 2005; Pan et al., 2009). GST's provide the excretion of metabolism, detoxification, and many pesticide and plant toxins by catalyzing the conjugation of a diverse array of electrophilic compounds with glutathione,

unlike CarE's (Liu et al., 2006). The occurring products are less toxic, more soluble in water, and excreted quickly from cells compared to non-GSH conjugated substrates. GST's and CarE's play essential roles in the OP's resistance to various insecticides (Abel et al., 2004). Monooxygenases, which are catalysed by cytochrome P450, are multifunctional and the most effective enzyme system for the detoxification of insecticides (Pan et al., 2009). Due to the broad substrate spectrum, it can affect insecticide classes and cause resistance (Scott, 1999). There was some report about resistance connected with monooxygenase activity for *Culex pipiens* L., 1758 (Diptera: Culicidae) (Shen et al., 2003), *Plutella xylostella* (L. 1767) (Lepidoptera: Plutellidae) (Bautista et al., 2009), *Bemisia tabaci* (Gennadius, 1889) (Homoptera: Aleyrodidae) (Karunker et al., 2008) and *M. persicae* (Puinean et al. 2010). The resistance against SP's and OP's associated with increasing monooxygenase activity in *A. gossypii*, has been reported (Shang et al., 2012). Another insecticide resistance mechanism for *A. gossypii* is target site mutation that occurs at the S431F (known as ACE1 resistance) position on acetylcholinesterase. It causes specific and cross-resistance in Carbamates (pirimicarb), OP's (omethoate and dimethoate) (Andrews et al., 2004; Toda et al., 2004). S431F mutation on the ACE1 gene causes resistance by using OP's insecticide against *A. gossypii* in Australian Cotton vegetation areas (Herron & Rophail, 2001; Herron et al., 2003; McLoon & Herron, 2009). Similarly, Knockdown Resistance (Kdr) was first revealed against houseflies (*Musca domestica*) in 1951 as a resistance mechanism based on target site insensitivity to pyrethroids (Busvine, 1951). Two point mutations (Leu1014 to Phe and Met918 to Thr) on the Voltage-Gated Sodium Channel (VGSC) gene reduce the binding of the insecticide to its target site, resulting in the emergence of the Kdr phenotype in some insect species (Williamson et al., 1996; Lee et al., 1999). Marshall et al. (2012) reported the occurrence of Kdr mutations with PCR-RFLP methods in *A. gossypii*. *Aphis gossypii* populations resistance situations from different coordinates in the Çukurova region of Adana province was studied against dimethoate (OP's), chlorpyrifos-Ethyl (OP's) and λ-cyhalothrin (SP's).

The objective of this study was to determine the resistance levels and mechanisms in *A. gossypii* cotton populations. For this purpose, bioassay studies with discriminant dose, metabolic enzyme assays, the occurrence of S431F and KDR mutations, and some CarE's gene expression levels were determined.

Materials and Methods

Collecting *Aphis gossypii* populations

Aphis gossypii populations were collected from 16 different *Gossypium hirsutum* L. (Malvaceae) vegetation coordinates in Çukurova Region in Adana, Mersin (Türkiye) (Figure 1, Table 1). Cotton leaf samples were plucked from cotton plants and brought with in paper bag to Adana Biological Control Research Institute climate rooms. Populations were reared and cultured on cotton plants in cages with insect net separately at 22±1°C, % 65±5 RH, 16:8 LD in climate rooms. *A. gossypii* were identified and classified with morphological methods by Dr. Işıl Özdemir in Kocaeli University Agriculture Faculty, Plant Protection Department, Kocaeli (Türkiye).

Insecticides and bioassays

Commercial products of dimethoate (400 g/l:EC), chlorpyrifos-ethyl (480 g/l:EC) and λ-cyhalothrin (50 g/l:EC) were used during this study. Insecticide Resistance Action Committee (IRAC) No: 019 leaf dipped method used in bioassay studies (IRAC, 2023). Discriminating dose bioassays were applied to distinguish resistant and susceptible *A. gossypii* populations. For this purpose, the most susceptible population against insecticides were accepted as a reference during the first-year surveys. After that, a discriminating dose study was performed based on the LC_{90} (lethal concentration to kill 90 % of the test population) values of the reference population for resistant/susceptible distinction of all the collected populations. The LC_{90} dose obtained for each insecticide from the reference population was applied to all other populations. If the mortality rate was >90%, it was considered susceptible, if the mortality rate was

<90%, it was regarded as a resistant population. Bioassay experiments were performed on all populations considered resistant. In insecticide bioassay studies, leaves from cotton plants were cut into 4 cm diameter discs. These discs were dipped in the insecticide solution for 10 seconds and then placed in petri dishes containing agar after drying. Twenty wingless individuals of aphid from the rearing colonies were taken, placed into Petri dishes with a fine brush and then transferred into climate rooms. Bioassay studies were done according to randomized parcel experimental design, and six different doses with three replications were used for each insecticide in this study. Each experiment included at least three control petri; if the mortality rate of control petri aphids was <20%, experiments were repeated. After 72 hours of application, counting was done by touching the specimens with a fine brush under the stereo microscope to determine if they are dead or alive. Dead /alive individuals were recorded. LC_{50} and LC_{90} values for populations were calculated via probit analysis using the POLO Plus software (Leora Software, 1987).

Acetylcholinesterase (AChE) enzyme activity

According to Kranthi (2005), 10 individuals of wingless aphids were homogenized within 300 µL 0.05 M, %0.1 Triton X-100 phosphate buffer (pH: 7.0) by a homogenizer. Sample tubes were centrifuged at 10000 g +4°C for 20 minutes, and the supernatant was used as an enzyme source. 2.86 ml sodium phosphate buffer (0.1 M pH 8.0), 10 µL 0.1 M DTNB (0.01 M, in pH 8.0 sodium phosphate buffer) and 30 µL of 0.1 M acetylcholine iodide (0.1 M, pH 8.0, in sodium phosphate buffer) were added into the supernatant (100 µL) and the reaction was initiated. Under the same conditions, the enzyme-free blank sample was prepared, 250 µL samples were transferred to microplates, and the alteration in absorbance was recorded at 412 nm wavelength at room temperature for 30 minutes in Thermofisher MultiscanGo spectrophotometer device.

Glutathione S-Transferase (GST) enzyme activity

Thirty wingless adult aphids were homogenized in 300 µl 0.1 M pH 6.5 sodium phosphate buffer, centrifuged at 10000 g +4°C for 20 minutes, and the supernatant was used as an enzyme source. 50 µl 50 mM CDNB, 150 µl 50 mM reduced glutathione (0.1 M, pH 6.5 in phosphate buffer) were added to the 30 µl enzyme sample. The enzyme mixture was diluted in 2.77 ml of phosphate buffer (0.1 M pH 6.5), and 250 µl of the diluted mixture was transferred to microplates for spectrophotometer readings. The enzyme-free blank sample was measured as a control without homogenate. The change in absorbance was recorded by reading at 340 nm wavelength for 10 minutes in Thermofisher MultiscanGo spectrophotometer device. (Habig et al., 1974; Kranthi, 2005).

Cytochrome P450 monooxygenase enzyme activity

Hansen & Hodgson (1971) method was used to determine P450 enzyme activity. Two hundred wingless adult aphids were homogenized in 300 µl 0.1 M pH 6.5 sodium phosphate buffer, centrifuged at 10000 g +4°C for 20 minutes, and the supernatant was used as an enzyme source. According to this method, 90 µl enzyme source and 100 µl 2mM p-nitroanisole (PNOD) as a substrate were mixed into each microplate cell. The reaction was initiated by adding 10 µl 9.6 mM nicotinamide adenine dinucleotide phosphate (NADPH) after incubating at 27°C for 2 minutes. The changes in enzyme activity were read at 27°C and 405 nm wavelength for 30 minutes with a microplate reader in Thermofisher MultiscanGo spectrophotometer device. All protein readings and quantifications after the determination of activity were performed according to Bradford (1976).

Detection of the presence of S431F mutation on the AChE enzyme

To detect the presence of S431F mutation on the AChE enzyme gene in *A. gossypi* populations, DNA isolation was performed with a kit (Thermo Scientific) and five wingless adult aphids were used DNA extraction. S431F mutation, which is one of the effective resistances to the CarE gene in populations, was determined by the PCR-RLFP method. As stated in the method, 36 replications and 180 individuals were

done from all resistant populations. Isolated DNA samples and ACE-F - (5'-CAA GCC ATC ATG GAA TCA GG-3'), ACE-R- (5'-TCA TCA CCA TGC ATC ACA CC-3') primers were performed to a polymerase chain reaction (PCR) (Chen et al., 2013). The conditions of PCR were followed by denaturation at 94°C, 5 min 1 cycle, 40 cycles at 94°C for 30 seconds, 53°C for 30 seconds, 72°C for 45 seconds and at 72°C for 10 minutes extension. After the PCR products were visualized by agarose gel electrophoresis (1.5%), 20 µL of PCR product was cut by incubating with four units of SspI restriction endonuclease enzyme at 37ºC for 3 hours and the condition of the bands was observed in agarose gel electrophoresis (1.5%) (Figure 4).

Detection of the presence of knockdown resistance (Kdr) mutation

According to Marshall et al. (2012) the PCR-RFLP method was applied to detect the presence of Kdr mutation, which is effective in the resistance of pyrethroid insecticides in *A. gossypii* individuals. DNA isolation was performed with the help of a kit (Thermo Scientific) with at least six replications, each including five wingless aphid individuals. Isolated DNA samples and Kdr-DP1 (5'- TCTTGGCCCACACTTAATCTTT-3'), Kdr-DP4 (5'-CTCGCCGTTTGCATCTTATT-3') primers were performed to a polymerase chain reaction (PCR) (Marshall et al., 2012). PCR conditions were followed by denaturation at 94°C, 2 min one cycle, 35 cycles at 94°C for 30 seconds, 48°C for 1 minute, 72°C for 90 seconds, and final elongation at 72°C for 5 minutes. After the PCR products were visualized by agarose gel electrophoresis (1.5%), 40 µL of PCR product was cut by incubating with five units of BstEII cutting enzyme at 60ºC for 6 hours and the condition of the bands was observed in agarose gel electrophoresis (1.5%) (Figure 4).

Expression profiles of carboxylesterase (CarE) enzyme gene

At least eight replications and 50 wingless adult aphid individuals for each population were used to perform the Quantitative Real-time PCR study. Fresh aphid samples were frozen at -80°C, homogenized with the help of buffer and their total RNA was extracted according to the Thermo Scientific RNA purification kit. After extraction, the RNA amounts of the populations were measured by nano-drop and diluted with TE buffer to obtain equal concentrations of 50 ng/µl for each of them. In the Quantitative real-time PCR study, the expression profile of the carboxylesterase gene was examined based on the relative activity of the CarE gene (Accession No. AB016720). P1-F-5'-CATACCCTACGCTCAACCAC-3', P2-R-5'-GCAATCTTCACTTCCAACGA-3' primers were used specifically for the CarE gene (Cao et al., 2008b). Primers of *β-actin* gene Forward 5'-AGCTCTATTCCAACCTTCCTTCT-3', Reverse 5'-TGTATGTAGTCTCGTGGATACCG-3' were used as the housekeeping gene. PCR temperature table 50°C 15 min 1 cycle, 95°C 15 min 1 cycle, 40 cycles at 95°C for 20 seconds at 59°C for 30 seconds and at 72°C for 2 minutes and final elongation 72°C with one cycle of 10 min was followed. The Quantitative Real-time PCR was performed with the help of Thermo Fisher Scientific, One-Step qRT-PCR kit, USA. The average of Obtained Ct values recorded, and ΔΔCt calculations were done according to susceptible populations to determine the relative activity levels of the CarE gene (Livak & Schmittgen, 2001).

Statistical analysis

Dose-response regressions were calculated with the Polo-plus software. LC_{50} values of resistant populations were rated with LC_{50} values of susceptible populations to determine the resistance rate. Other statistical analyses were done with SPSS 23 (SPSS Inc., Chicago, IL, USA). One-way ANOVA was used to determine the differences between means and Duncan's multiple comparison tests were applied to compare groups between aphid populations collected from Adana during this study ($p<0.05$). The classification was done according to the World Health Organization (WHO, 1980) standards. Susceptible (RR<3 times) (S), Decreased Susceptible (DS) (RR=3 to 5 times), Lower Resistance (LR) (RR=5 to 10 times), Medium Resistance (MR) (RR=10 to 40 times), Higher Resistance (HR) (RR=40 to 160 times) and Extremely High Resistance (RR>160) are classified as resistance levels. The percentage of sensitive and resistant alles rate was calculated from total PCR-RFLP analysis samples.

Results

Aphis gossypii populations were collected from 16 different cotton fields between 2020 and 2021 in this study (Table 1, Figure 1).

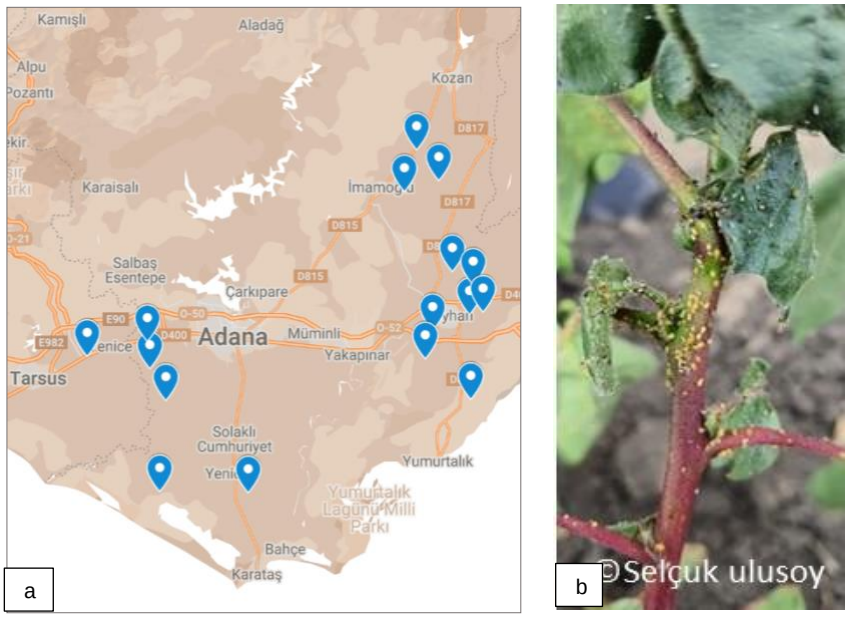

Figure 1. a) Locations of *Aphis gossypii* populations from cotton fields in Adana, Türkiye; b) Cotton plant infested with *Aphis gossypii* individuals.

Table 1. *Aphis gossypii* populations collected from cotton growing fields

Population	Location	Coordinate	Date
1	Çiftlikler, Ceyhan	37°01'49.9"N 35°51'24.2"E	June, 2020
2	Hamzalı, Yumurtalık	36°52'44.2"N 35°51'35.0"E	May, 2020
3	Helvacı, Karataş	36°42'46.4"N 35°21'40.7"E	May, 2020
4	Karagöçer, Karataş	36°42'50.3"N 35°09'49.4"E	May, 2020
5	Karayusuflu, Seyhan	36°52'39.7"N 35°10'39.4"E	June, 2020
6	Küçükçıldırım, Seyhan	36°56'03.8"N 35°08'29.0"E	May, 2020
7	Toktamış, Ceyhan	37°00'11.2"N 35°46'27.9"E	June,2020
8	Yeniköynazımbey, Ceyhan	36°57'10.8"N 35°45'29.4"E	June, 2020
9	Değirmendere, Ceyhan	37°02'09.2"N 35°53'15.4"E	May, 2021
10	Gokçeler, Seyhan	36°58'59.9"N 35°08'14.8"E	June, 2021
11	Hamitbeybucağı, Ceyhan	37°06'27.2"N 35°49'04.6"E	June, 2021
12	İncetarla, Ceyhan/Adana	37°04'59.7"N 35°51'50.1"E	June, 2021
13	Konaklar, Tarsus	36°57'25.5" 35°00'09.4"E	June, 2021
14	Sokutaş, İmamoğlu	37°16'13.8"N 35°47'11.9"E	June, 2021
15	Üçdutyeşilova, Ceyhan	37°15'03.9"N 35°42'38.7"E	June, 2021
16	Ufacıkören, İmamoğlu	37°19'28.0"N 35°44'19.8"E	May, 2021

Bioassay analysis

Dimethoate, chlorpyrifos-ethyl and λ-cyhalothrin resistant aphid populations were distinguished with discriminating dose bioassay. LC_{50} and LC_{90} values of all resistant populations were determined (Table 2). All populations' resistance levels were classified according to WHO (1980) scale. With discriminating dose bioassay, except for the Toktamış population, six *A. gossypii* populations were identified as resistant. Compared to the susceptible population, four populations showed DS level resistance to dimethoate, and one population to chlorpyrifos-ethyl insecticides. Only two populations showed MR levels to chlorpyrifos-ethyl. Other populations were observed as S levels within this study (Table 2, Figure 2). LC_{50}: 11.4 ppm for Dimethoate, LC_{50}: 165.3 ppm for λ-cyhalothrin and LC_{50}: 108.7 ppm for chlorpyrifos-ethyl were found in the Toktamış population. Çiftlikler, Helvacı, Karagöçer, Hamzalı, Hamitbeybucağı, İncetarla, Değirmendere, Üçdutyeşilova, Gökçeler and Konaklar populations were detected as susceptible and eliminated according to the discriminating dose. Yeniköynazımbey, Küçükçıldırım, Karayusuflu, Sokutaş and Ufacıkören populations were detected as resistant populations. Yeniköynazımbey and Küçükçıldırım populations were the most resistant to dimethoate and chlorpyrifos-ethyl, RR_{50} was found 5.81 and 10.9 times, respectively. Sokutaş population was observed as the most resistant (RR_{50}: 3.7) population for λ-cyhalothrin (Table 2).

Table 2. Bioassay of Dimethoate, λ-cyalothrin, Chlorpyrifos in test populations of *Aphis gossypii*

Insecticide	Population	n	LC_{50} (mg/L) (CL)	LC_{90} (mg/L) (CL)	Slope (±SE)	X^2	RR_{50}	Resistance level
Dimethoate	Toktamış*	360	11.4 (8-14.7)	32.1 (23.6- 56.9)	2.86±0.5	0.55	-	-
	Yeniköynazımbey	380	66.5 (47.1-107)	352.3 (178.5-2397.1)	1.77±0.4	1.08	5.8	LR
	Küçükçıldırım	365	51.2 (33.2-83.2)	293.9 (150-1523.5)	1.68±0.3	4.13	4.4	DS
	Karayusuflu	360	46.1 (26.5-81)	568.2 (237.2-4270.6)	1.17±0.2	2.19	4.0	DS
	Sokutaş	370	42.2 (32.3-55.4)	106.7 (73.8- 290.6)	3.18±0.8	1.22	3.7	DS
	Ufacıkören	360	47.6 (36.3-66.6)	128.5 (83.9-439.7)	2.97±0.7	1.75	4.1	DS
λ-Cyhalothrin	Toktamış*	360	165.3 (122.3- 203.1)	337 (263.5- 578.9)	4.14±0.9	2.64	-	-
	Yeniköynazımbey	360	370.7 (240.7-508.7)	1594.7 (1013.7-4363.4)	2.02±0.4	2.54	2.2	S
	Küçükçıldırım	365	437.0 (345.5-528.3)	797.7 (631.2-1443.1)	4.90±1.2	2.20	2.6	S
	Karayusuflu	380	387.7 (259.8-536.8)	1717.9 (1066.5-4902.8)	1.98±0.4	1.88	2.3	S
	Sokutaş	360	370.6 (276.1-439.7)	646 (511.9-1896.8)	5.31±1.8	3.77	2.2	S
	Ufacıkören	360	306.5 (201.8-413)	938 (598.8-5383.1)	2.63±0.8	2.54	1.8	S
Chlorpyrifos	Toktamış*	360	108.7 (73.9- 140.2)	335.2 (243.3-638.6)	2.62±0.5	1.37	-	-
	Yeniköynazımbey	380	1130.4 (770-1301.9)	2624.3 (1901.4-5454.5)	3.14±0.7	2.11	10.3	MR
	Küçükçıldırım	365	1184.8 (780.3-1454.1)	3451.6 (2268.8-8863.6)	2.52±0.5	0.36	10.8	MR
	Karayusuflu	360	525.3 (311-746.9)	2005.6 (1299.5-5132.7)	2.20±0.5	3.12	4.8	DS

CL: Confidence Limits; *: Susceptible population; RR_{50}: Resistance Rate R_{50} ; n: Individual number

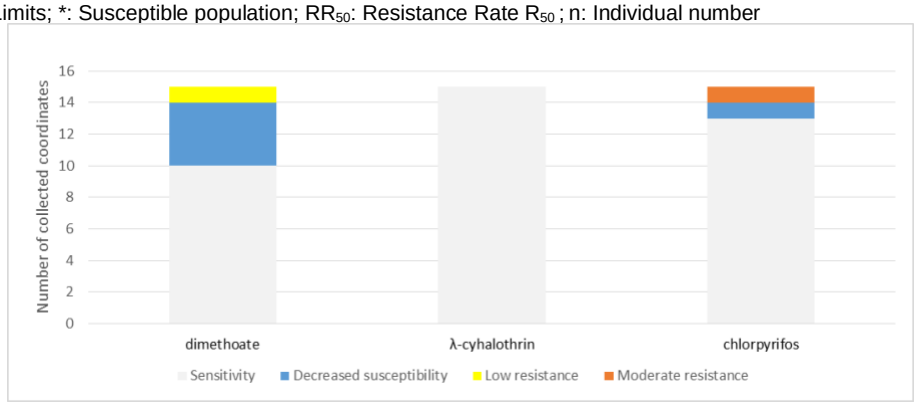

Figure 2. The distribution of Resistance levels for *Aphis gossypii* to dimethoate λ-cyhalothrin and chlorpyrifos-ethyl from the 16 different locations in Adana, Türkiye.

The enzyme and gene expression analysis

According to the enzyme analysis, enzyme activity values were relatively lower in the Toktamış population than other resistant populations. Activities were higher and at different rates according to the enzyme type in other populations (Table 3). When gene expression level of the CarE gene was examined, all populations were more regulated than the susceptible population. 1.25 times more gene regulation was seen in the Sokutaş population (Figure 3).

Table 3. Acetylcholinesterase, glutathione S-transferase (GST), cytochrome P450 monooxygenase (P450) enzyme activities of resistant and susceptible *Aphis gossypii* populations

Populations	Specific Enzyme activities U.mg^{-1}.min					
	Acetylcholinesterase		GST		P450	
Toktamış*	19.08±3.0	a	6.45±1.44	a	0.02±0.01	a
Yeniköynazımbey	52.21±6.40	bc	17.02±3.42	ab	0.10±0.03	ab
Küçükçıldırım	76.81±7.84	d	27.69±7.72	b	0.05±0.02	ab
Karayusuflu	32.82±6.16	ab	23.39±4.40	b	0.05±0.01	ab
Sokutaş	65.70±7.47	cd	17.60±2.79	ab	0.13±0.05	ab
Ufacıkören	66.89±9.67	cd	67.31±1.90	c	0.10±0.01	b

* Susceptible population, the difference between average number of specific enzyme activity of *Aphis gossypii* were statistically significant ($p<0.05$), Duncan multiple comparison tests were applied; ±: standart error

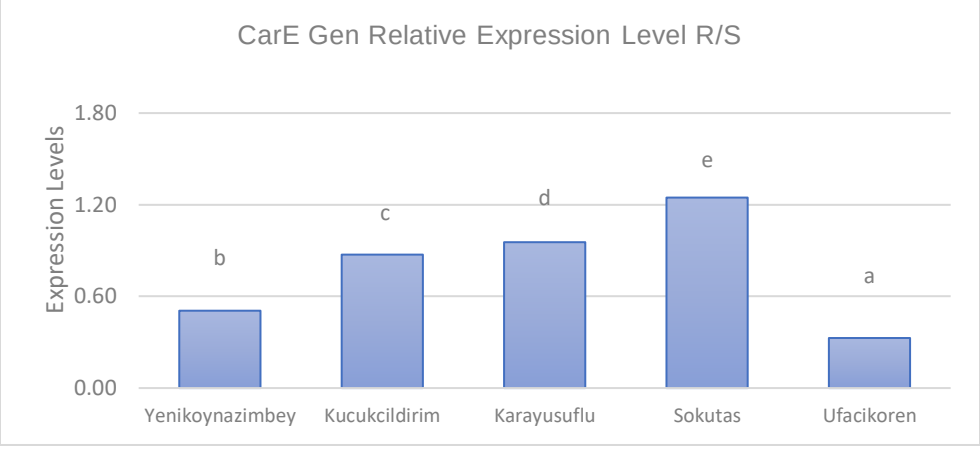

Figure 3 Relative expression levels (R/S) of Carboxylesterase gen (CarE) in *Aphis gossypii* populations (R/S: Resistant population/susceptible population).

The difference between average number of CarE gen relative expression levels of *Aphis gossypii* were statistically significant ($p<0.05$).

Mutation screening analysis

The recessive allele was found only in the Toktamış population (Figure 4). Approximately 17% of recessive alleles were detected for S431F mutation. A total of 36 replications was scanned from all populations for Kdr mutation (Table 4). It was seen that enzyme cutting occurred in all populations by PCR-RLFP method (Figure 4). All populations were found to have 100% recessive alleles for Kdr mutation. All populations were detected as susceptible in terms of Kdr mutation.

Table 4. S431F and KDR mutation frequency *in Aphis gossypii* populations

Mutation	Recessive alleles %	Resistant alleles %
S431F mutation	17	83
KDR mutation	100	-

Figure 4. *Aphis gossypii* PCR-RFLP agarose gel electrophrosesis photograph. M: 100 bp ladder, 1: S431F PCR product (667 bp), 2: S431F PCR cut by SspI RLFP product (Resistant allel: 667 bp, uncut by SspI); 3: S431F PCR cut by SspI RLFP product (Susceptible allel: 335 bp, cut by SspI); 1a: Knockdown resistance (Kdr), Negative control PCR product 500 bp; 2a: Kdr PCR cut by BstEII RLFP product (Susceptible allel: 325 bp, cut by BstEII).

Discussion

Resistance management is a key factor to prevent the development of resistance in pests and to maintain insecticide activity against the harmful organism (Alpkent et al., 2023). Insecticide resistance is an important problem for *A. gossypii* (Ulusoy et al., 2018; Ullah et al., 2020; Erdogan et al., 2023; Cheng et al., 2023). The resistance levels of 16 populations in the Çukurova region were observed in this study; four populations showed DS level resistance against dimethoate, and one population against chlorpyrifos-ethyl. Only two populations against chlorpyrifos-ethyl showed MR-level resistance. Other populations were found susceptible. Considering this aspect, moderate and low levels of resistance was observed in the region and it was not detected at high and extremely high resistance levels (Figure 2). The reference population is of significant importance in determining resistance levels. In this study, susceptible aphids that were cultured in the laboratory environment for many years could not be obtained for various reasons. Instead, the susceptible population was obtained from cotton growing areas. This may have caused resistance levels to be lower. In addition, the fact that the Çukurova region is suitable for polyculture agriculture and extensive agricultural practices throughout the year may bring about higher resistance level expectations. However, the widespread use of neonicotinoids or new active groups in the last 30 years may have reduced the use of organophosphate and pyrethroid insecticides. The reducing excessive insecticide consumption and using different insecticide groups is an important factor that reduces resistance. Before, Ulusoy et al. (2018) reported high-level resistance against neonicotinoid for *A. gossypii* owing to exposure to more insecticides in the same region. According to the results of enzyme analysis, all activities were found to be relatively lower in the Toktamış population compared with other populations. Activities in other populations were observed as higher and different rates. As observed in the previous studies, AChE's and GST enzyme activities were found at high rates in organophosphate, carbamate and pyrethroid resistance populations (Devonshire & Moores, 1982; Lokeshwari et al., 2016; Ulusoy et al., 2018). As in this study, monooxygenase P450 enzyme activity, which is responsible for many metabolic activities, was observed at high levels in each resistant population (Shang et al., 2012; Seyedebrahimi et al., 2015). There was a general increase in metabolic enzyme activities compared to the susceptible population with the evaluation of all enzyme analyses. Moreover, the relative expression activity of the CarE gene increased compared to the susceptible population in this study. In parallel with this study, it has been reported that the transcription

level increased in populations of *A. gossypii,* which showed a resistant organophosphate population (Hawkes, 2002; Cao et al., 2008a; Wang et al., 2021). CarE's have a significant role in the detoxification of exogenous harmful ingredients in arthropods (Ma et al., 2018). Also, the overexpression of carboxylesterase gene activity in resistant *A. gossypii* populations against λ-cyhalothrin was reported (Sial et al., 2018; Wang et al., 2021). Regarding the presence of S431F mutation, a high percentage of resistant alleles were found between populations. Parallel to these findings, it was seen that dimethoate and chlorpyrifos-ethyl resistant populations resistance levels were higher than λ-cyhalothrin resistant populations. Similar studies have reported the mutation relationship between the AChE gene with organophosphate and pirimicarb-resistant populations (Benting & Nauen, 2004; Chen et al., 2013; Hlaoui et al., 2022; Nam et al., 2022). In this study, recessive alleles were found in all populations in terms of Kdr mutation. The low levels of resistance to λ-cyhalothrin in all populations support this case. Similar studies have reported a relationship between Kdr mutation and resistance to synthetic pyrethroids in *A. gossypii* and different aphid species (Marshall et al., 2012; Wang et al., 2021; Valmorbida et al., 2022; Fontaine et al., 2023). It is possible that the presence of lower resistance levels of λ-cyhalothrin can be associated with metabolic resistance. The higher enzyme activities in biochemical analysis support this phenomenon (Lokeshwari et al., 2016). In conclusion, moderate and low resistance rates against dimethoate and chlorpyrifos-ethyl insecticides in *A. gossypii* were observed in sixteen different populations in the Çukurova region of Adana provinces. Recessive Kdr mutation in aphid populations overlaps with low λ-cyhalothrin resistance.

It has been concluded that the S431F mutation, overexpression of the CarE gene and high biochemical metabolic activities are effective in the occurrence of higher rates of resistance levels to dimethoate and chlorpyrifos-ethyl. These results should be taken into account in resistance management. In chemical control, the same group of insecticides should be reduced and selection of aphid populations with resistant alleles should be prevented. In Türkiye, resistance monitoring studies should be accelerated and chemical control programs should be carried out regionally and temporally.

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Original article (Orijinal araştırma)

A survey in sunflower fields in Tekirdağ (Türkiye) to determine soil health with nematode-based diversity indices

Tekirdağ ili (Türkiye) ayçiçeği tarlalarında nematod çeşitlilik indeksleri ile toprak sağlığının belirlenmesine yönelik bir araştırma

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Abstract

In this study conducted in 2021-2022, nematode community structure was investigated in sunflower fields in Tekirdağ to determine sampled fields' physical characteristics based on nematode biodiversity indices. For this purpose, soils collected from 37 sunflower fields were examined for nematode presence. In the soils, 34 genera of fungivore [3 genera, e.g., *Aphelenchoides* Fischer, 1894 (Aphelenchida: Aphelenchoididae)], bacterivore [9 genera, e.g., *Acrobeloides* Cobb, 1924 (Rhabditida: Cephalobidae)], omnivore [4 genera, e.g., *Dorylaimus* Dujardin, 1845 (Dorylaimida: Dorylaimidae)], predator [2 genera, e.g., *Seinura* Fuchs, 1931 (Aphelenchida: Aphelenchoididae)], and plant-parasitic [17 genera, e.g., *Pratylenchus* Filipjev, 1936 (Tylenchida: Pratylenchidae)] nematodes were recovered by the modified Baermann Funnel method. The dominant nematodes were fungivores and plant-parasitics occurring in all fields. Among 17 plant-parasitic nematodes identified at a species level, the most economically important species were *Longidorus elongatus* Mikoletzky, 1922 (Dorylaimida: Longidoridae)*, Ditylenchus dipsaci* (Kühn, 1857) Filipjev,1936 (Tylenchida: Anguinidae)*, Pratylenchus thornei* Sher & Allen 1953, and *Pratylenchus zea* Graham, 1951 (Tylenchida: Pratylenchidae). Maturity indices calculated to estimate soil characteristics in fields were 2.33±0.36, and the value was determined to be <3 in most fields except for two fields. Food web analysis revealed that 76.3% of soils had worsened physical characteristics and a high C: N ratio. The characteristics of soils in two fields were enriched, and the others were fertile.

Keywords: *Helianthus annuus*, nematode community analysis, soil health

Öz

2021-2022 yıllarında yürütülen bu çalışmada, Tekirdağ ili ayçiçeği tarlalarında nematod biyoçeşitlilik indeksleri kullanılarak örneklenen tarlaların fiziksel özelliklerini belirlemek için nematod kommünite yapısı incelenmiştir. Bu amaçla 37 ayçiçeği tarlasından toplanan topraklarda nematod varlığı incelenmiştir. Toprakta 34 cins fungivor [3 cins, örneğin *Aphelenchoides* Fischer, 1894 (Aphelenchida: Aphelenchoididae)], bakterivor [9 cins, örneğin *Acrobeloides* Cobb, 1924 (Aphelenchida: Aphelenchoididae)], omnivor [4 cins, örneğin *Dorylaimus* Dujardin, 1845 (Dorylaimida: Dorylaimidae)],, predatör [2 cins, örneğin *Seinura* Fuchs, 1931 (Aphelenchida: Aphelenchoididae)] ve bitki paraziti [17 cins, örn., *Pratylenchus* Filipjev, 1936 (Tylenchida: Pratylenchidae)] nematodları içeren 34 cins nematod modifiye edilmiş Baermann Huni yöntemiyle izole edilmiştir. Tüm alanlarda fungivor ve bakterivor türler baskın bulunmuştur. Tür düzeyinde tespit edilen 17 bitki paraziti nematod arasında ekonomik açıdan en önemli türler *Longidorus elongatus* Mikoletzky, 1922 (Dorylaimida: Longidoridae), *Ditylenchus dipsaci* (Kühn, 1857) Filipjev,1936 (Tylenchida: Anguinidae), *Pratylenchus thornei* Sher & Allen 1953 ve *Pratylenchus zea* Graham, 1951 (Tylenchida: Pratylenchidae) olmuştur. Tarlalarda toprak özelliklerini tahmin etmek için hesaplanan olgunluk indeksleri 2.33±0.36 olup, iki alan dışında çoğu alanda değer <3 olarak belirlenmiştir. Besin ağı analizi, toprakların %76.3'ünün fiziksel özelliklerinin kötüleştiğini ve yüksek bir C:N oranına sahip olduğunu ortaya çıkarmıştır. İki tarlada ise toprak özellikleri zenginleştirilmiş, diğerleri ise verimli olarak saptanmıştır.

Anahtar sözcükler: *Helianthus annuus*, nematod kommünite analizi, toprak sağlığı

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Introduction

Sunflower is the most-grown oil seed plant in Türkiye with the large cultivation area. The plant is preferred due to its adaptation to dry and irrigated soil conditions and suitability to mechanization during the vegetation period. Sunflower oil corresponds to 69% of the vegetable oil production in the country and constitutes 32% of the total consumed oil (Yağmur et al., 2021). Each plant has 22-55% total oil content, and the oil contains saturated and unsaturated fatty acids (Akkaya, 2018). In addition to processing it into oil, sunflower seeds are consumed as a snack or animal feed. An essential part of the sunflower fields planted in Türkiye is located in provinces such as Edirne, Tekirdağ, and Kırklareli in Thrace.

As with many cultivated plants, there are many organisms in sunflower soils, and soil health in cultivation areas can be determined using their diversity and interaction with the environment. Soil health is the soil's continuous function in the ecosystem. It is described as the continuous processing potential of the soil to maintain biological productivity in cultural soils, improve the quality of the soil environment, and protect living organisms. Besides affecting the development of plants, soil health affects the life cycles of beneficial microorganisms in the soil. It causes tremendous damage to the living things that consume sunflower products (Doran & Zeiss, 2000).

There are many indicators of soil health worldwide, and nematodes are considered among them. Nematodes have an important place regarding the number of species and population. An average of 385 nematode species are named annually, and 25.000 identified species are included in 2.271 genera and 256 families (Anderson, 2008; Hodda, 2011). They are classified into different trophic groups according to their feeding patterns. Nematodes with different trophic groups are associated with plants, bacteria, fungi, and other soil faunas and are considered an essential component of the soil food web. For instance, by moving nematodes in the soil, the permeability and water infiltration increase, allowing the soil and organic residues to mix. Soil nematodes are responsible for recycling nutrients and minerals in the environment. The soil physical condition was assessed using Diversity indexes such as Shannon-Weiner, Basal, Structure, and Enrichment calculated using the trophic structures and functional guilds of nematodes (Bongers, 1990).

Many nematode species in sunflower fields are mentioned in the literature, and the most damaging species to plant growth are *Meloidogyne* (Tylenchida: Heteroderidae) species. The other genera found in sunflower plantations include several ectoparasitic and endoparasitic nematodes like *Helicotylenchus* Steiner, 1945 (Tylenchida: Hoplolaimidae), *Pratylenchus* (Tylenchida: Pratylenchidae), *Quinisulcius* (Tylenchida: Belonolamidae), and *Xiphinema* (Dorylaimida: Longidoridae) (Fourie et al., 2017). In our country, in the study conducted in 2000, 30 plant-parasitic species belonging to 9 families and 24 genera were identified. Still, free-living species have not been studied (Kepenekçi, 2001).

The current study investigated plant-parasitic and free-living nematode diversity, and the soil physical properties were determined by calculating some diversity indices. This is the most recent study in which nematode community structure was determined together with free-living nematodes.

Materials and Methods

The study area and soil collection

An intensive nematode survey was conducted between 2021-2022 in 11 districts in Tekirdağ province of Thrace, Türkiye. During the study, randomly selected 37 sunflower fields with at least 15km distance were visited (Figure 1, Table 1). The latitudes of the fields were between 40°52'-41°19'S, longitudes were between 27°6'-28°0'W, and elevations ranged between 3 and 258 meters. The large-scale non-irrigated sunflower production was dominant in the visited fields. The weather temperature during the survey was 25-28°C, and the average monthly total precipitation was 24.2 mm. Six soil subsamples were collected from different points in each field by moving in a zigzag pattern. The soil depth of sampling was 0-60 cm, and an average of 1 kg of soil was taken per field.

Figure 1. Map representing study area in Tekirdağ.

Table 1. The total production, survey locations, and total acreage of sunflower fields in Tekirdağ

Provinces/ID	Districts	Production area (ha)	Total production (ton)
Çerkezköy (Çer)	Merkez	1.896	3.092
Çorlu (Çor)	Sarılar, Seymen, Yenice	12.619	21.929
Ergene (Erg)	Ahimehmet, Misinli, Velimeşe	14.388	27.733
Hayrabolu (Hay)	Dambaslar, Soylu, Susuzmüsellim	36.108	69.666
Kapaklı (Kap)	Bahçeağıl, Yanık ağıl	4.128	7.171
Malkara (Mal)	Evrenbey, İbribey, Karamurat	33.483	64.729
Marmara Ereğlisi (Mar)	Türkmenli, Yakuplu, Yeniçiftlik	4.782	10.1162
Muratlı (Mur)	Arzulu, İnanlı, Kırkkepenekli, Yurtbekler	13.431	35.253
Saray (Sar)	Büyükyoncalı	14.351	29.060
Süleymanpaşa (Sül)	Barbaros, Bıyıkali, İnecik, Köseilyas, Mahramlı	31.882	61.598
Şarköy (Şar)	Beyoğlu, İshaklı	2.688	5.177

Nematode recovery and identification

The nematodes in each field soil were extracted from 100 cm^3 of the sample by the Baermann Funnel technique. In this method, soil cores were placed in sieves with a single layer of filter paper, filled with water, and incubated for 24 hours. The next day, nematodes that migrated to water were collected by sieving on 400 mesh sieves. The nematodes were counted with 1 ml of the extracted suspension at 10X magnification under the microscope. Free-living nematodes were identified at the genus level, and plant parasitic and Aphelenchids were identified at the species level by examining the female's morphologic features like stylet shape, vulva position, tail shape, and longitudinal striations. The slides of nematode females were prepared by heat-killing and processing in TAF [(7%) formaldehyde + (2 %) triethanolamine + (91%) distilled water)], Seinhorst I (1 ml glycerin + 79 ml distilled water), and Seinhorst II (5 ml glycerin + 95 ml ethanol) solutions. Processed nematodes were fixed on a glycerin-dropped slide by the wax ring method (Seinhorst, 1959). Plant parasitic species were identified with published references (Geraert & Raski, 1987; Brezski, 1991; Loof & Luc, 1993; Castillo & Volvas, 2005, Handoo et al., 2007).

The nematode trophic groups, diversity indices, nematode-soil health relation

Nematodes extracted from sunflower fields were subjected to several diversity and food web analyses to determine the health status of soils in sampled areas. The Shannon-Weiner Diversity Index, Evenness, and Richness were calculated to evaluate the diversity index of nematode fauna in fields. The formulas used to calculate the indices were as follows (Pielou, 1966; Neher & Darby, 2009). Additionally, Principal component analysis (PCA) was performed with the XSLSTAT 2022 software to the data obtained from the soils taken from each field. Abundance and frequency were analyzed with PCA.

Shannon-Weiner Index (H′): $H' = -\sum[(pi) \times \log(pi)]$

Pielou's Evenness Index (J′): $J' = H'/\ln(S)$

Genera Richness Index (GR): $GR = S-1/\ln N$

Pi: the proportion of individuals in genera; S: the number of genera; N: the number of all identified nematodes.

The extracted nematodes were divided into bacterivore, fungivore, predator, omnivore, and plant-parasitic based on the feeding habitat. Soon they were classified by colonizer-persister values. The nematode community and soil food web were analyzed with Maturity (MI), Maturity (MI)2-5, Plant-parasite (PPI), Basal (BI), Channel (CI), Enrichment (EI), and Structure (SI) indices calculated according to Ferris et al. (2001). MI was used to assess disturbance in soil; lower values were considered as more disturbed, and higher values as less disturbed soils. The MI2-5 value was used to indicate soil health status. Other indices were computed to determine the state of the food web in the soil. While the EI indicates nutrient enrichment and availability, the SI value gives information about the food web structure. The BI also shows the soil food web; higher values indicate worsened conditions. CI was used to indicate whether organic matter decomposition was related to bacterial or fungal feeder nematodes. Fungivore nematodes dominated the decomposition at high CI values, while bacterivores played a primary role in decomposition at low CI values (Ferris et al., 2001).

Nematode Indicators Joint Analysis (NINJA) online software was used to prepare the c-p triangle and soil food-web scheme (Sieriebriennikov et al., 2014). Based on nematode diversity the fields were placed in different Quadrats (Figure 2) (Ferris, 2001)

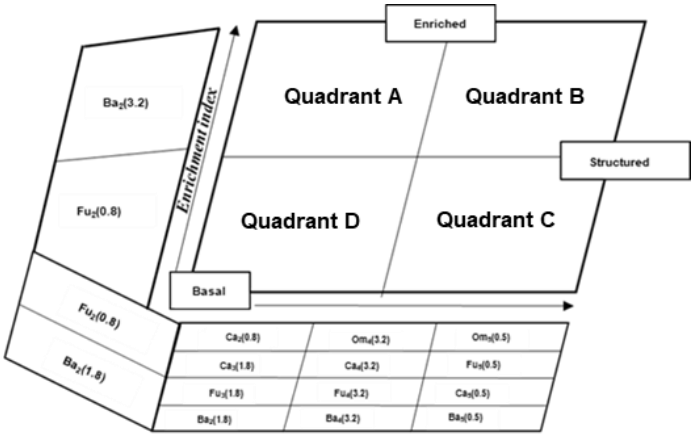

Figure 2. Scheme representing soil food-web. Quadrant A. disturbed and enriched; Quadrant B: enriched and maturing; Quadrant C: Matured; Quadrat D: Degraded (Ferris et al., 2001).

Results

The nematode genera, distribution, and trophic groups

In this study, 37 genera of nematodes were identified in 37 sunflower fields in Tekirdağ. Nematodes belonged to 8 orders (Aphelenchida, Chromadorida, Dorylaimida, Rhabditida, Mononchida, Triplonchida, and Tylenchida), 8 suborders, and 22 families (Figure 3).

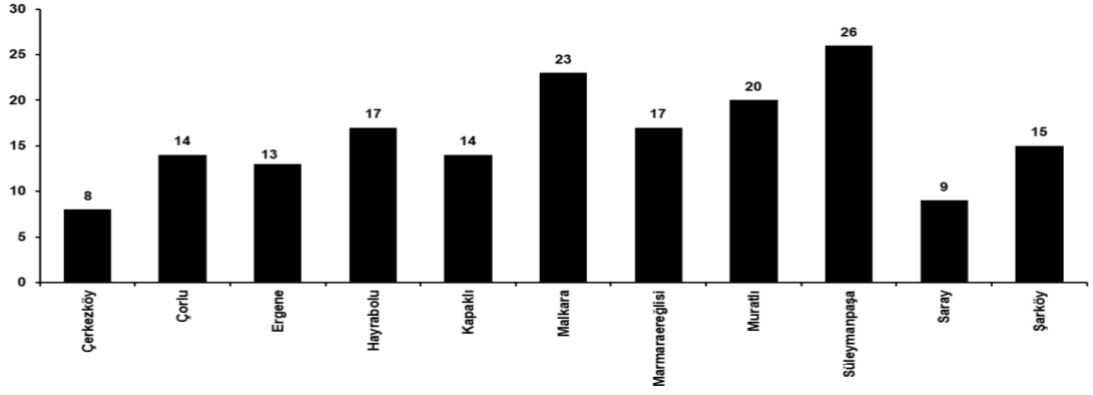

Figure 3. The number of genera identified in sunflower fields in 11 districts.

Specimens were classified into five trophic groups by feeding habitat. Of these, nine genera were bacterivores, three were fungivores, six were predators, and three were omnivores. Fungivore and bacterivores, omnivores, and predators were grouped under free-living nematodes. The plant-parasitic nematodes belonged to 17 genera and included a plant-parasitic species (*Ditylenchus dipsaci* Kühn, 1857) from the fungivore genera *Ditylenchus*. The most common nematodes in sunflower fields were bacterivores, followed by fungi feeders, plant-parasitic, omnivores, and predators. In the sunflower fields, fungivores dominated three samples, while bacterivores were prominent in five and plant feeders in eight samples. A higher population of fungi and bacterivores were found in Süleymanpaşa and Kapaklı (Figure 4). The lowest rate of plant parasitic nematodes in soil samples was 3.6%, and the highest was 69.1%. Omnivores were found only in 86.5 % and predators in 43.2% of the fields. In some locations, the free-living nematode population was high and plant-parasitic nematode were low in number.

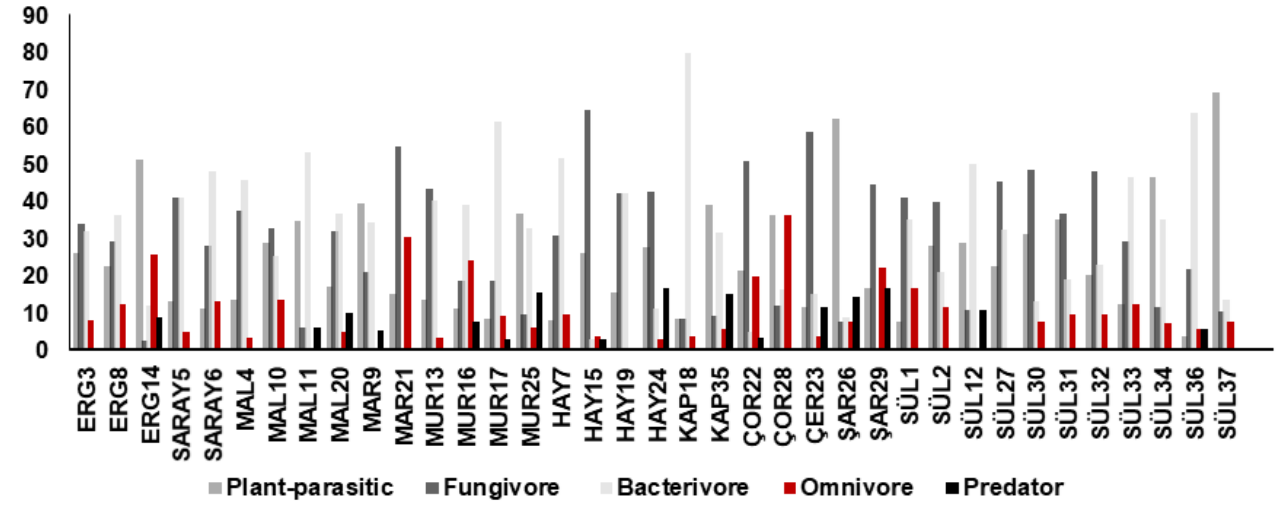

Figure 4. The % proportion of nematodes with different feeding habitats in 37 sunflower fields surveyed in Tekirdağ.

Identified free-living nematodes were classified under nine suborders and 14 families (Table 2). The greater abundance of *Ditylenchus* Filipjev, 1936 (fungivore), *Acrobeloides* Cobb, 1924 (bacterivore), *Cephalobus* Bastian, 1865 (Rhabditida: Cephalobidae (bacterivore), *Mesodorylaimus* Andrássy, 1959 (Dorylamida: Dorylaimidae) (bacterivore) was observed in sampled areas. An average of 69.3±35.8 (23-234) free-living nematode individuals were found per 100 cm^3 of soil in each soil sample.

Twenty-two plant-parasitic nematode species belonging to two suborders and 11 families were identified in sunflower fields in Tekirdağ (Table 3). The abundance of plant parasitic nematodes in all soil samples ranged from 41 to 234 individuals per 100 cm^3 soil. Of the recovered species, *Filenchus filiformis* Ebsary, 1991 (Tylenchida: Tylenchidae), *Geocenamus tesellatus* (Goodey, 1952) Brzeski, 1991 (Tylenchida: Merliniidae), *Tylenchorhynchus annulatus* Cobb, 1913 (Tylenchida: Belonolaimidae) and *Pratylenchus zeae* Graham, 1951 were the most widespread. In contrast, *Longidorus elongatus*, *Paratrophurus acristylus* Siddiqi & Siddiqui, 1983 (Tylenchida: Telotylenchidae), and *Xiphinema pachtaicum* (Dorylaimida: Longidoridae) were found to be rare.

Table 2. Data representing taxonomic classification, c-p values, the occurrence, and abundance of free-living nematodes in sunflower fields in Tekirdağ (ba: bacterivore; fu: fungivore; om: omnivore; pr: predator)

Genera / species	Functional guild	Suborder	Families	Occurrence (%)	Abundance (min-max nematodes/ 100 cm^3 soil)
Achromadora Cobb,1913	Ba3	Chromadorina	Achromadoridae	5.4	1-2
Acrobeles Linstow, 1877	Ba2	Cephalobina	Cephalobidae	27.0	3-21
Acrobeloides Cobb, 1924	Ba2	Cephalobina	Cephalobidae	81.0	2-20
Alaimus de Man, 1880	Ba4	Dorylaimina	Alaimidae	10.8	1-3
Cephalobus Bastian, 1865	Ba2	Rhabditina	Cephalobidae	67.5	3-53
Cervidellus Thorne, 1937	Ba2	Cephalobina	Cephalobidae	17.1	2-3
Monhystera Bastian, 1865	Ba2	Monhysterina	Monhysteridae	8.1	2-5
Plectus Bastian, 1865	Ba2	Chromadorina	Plectidae	2.7	2
Rhabditis Dujardin, 1845	Ba1	Rhabditina	Rhabditidae	14.3	2-11
Wilsonema Cobb, 1913	Ba2	Chromadorina	Plectidae	5.4	2
Aphelenchoides clarus Thorne & Malek, 1968	Fu2	Aphelenchina	Aphelenchoididae	8.2	3-15
Aphelenchoides sacchari Hooper, 1958	Fu2	Aphelenchina	Aphelenchoididae	35.1	7-27
Aphelenchoides obtusus Thorne & Malek, 1968	Fu2	Aphelenchina	Aphelenchoididae	5.4	3-7
Aphelenchus avenae Bastian, 1865	Fu2	Aphelenchina	Aphelenchidae	48.7	3-14
Ditylenchus geraerti (Paramonov, 1970)	Fu2	Tylenchina	Anguinidae	70.2	3-21
Aporcelaimellus Heyns, 1965	Om5	Dorylaimina	Aporcelaimidae	24,3	2-3
Dorylaimus Dujardin, 1845	Om4	Dorylaimina	Dorylaimidae	40.5	1-4
Eudorylaimus Andrássy, 1959	Om4	Dorylaimina	Qudsianematidae	16.2	2-8
Mesodorylaimus Andrássy, 1959	Om4	Dorylaimina	Dorylaimidae	59.4	3-28
Prodorylaimus Andrássy, 1959	Om4	Dorylaimina	Dorylaimidae	5.4	2-3
Clarkus Jairajpuri, 1970	Pr4	Mononchina	Mononchidae	2.7	7
Seinura Fuchs, 1931	Pr4	Aphelenchina	Aphelenchoididae	8.1	2
Tripyla Bastian, 1865	Pr3	Tripylina	Tripylidae	8.1	4-10

As well in Principle Component Analysis (PCA), nematode species nematodes were grouped based on frequency as dominant (4 genera), frequent (6 genera), infrequent (15 genera), and rare (12 genera) (Figure 5).

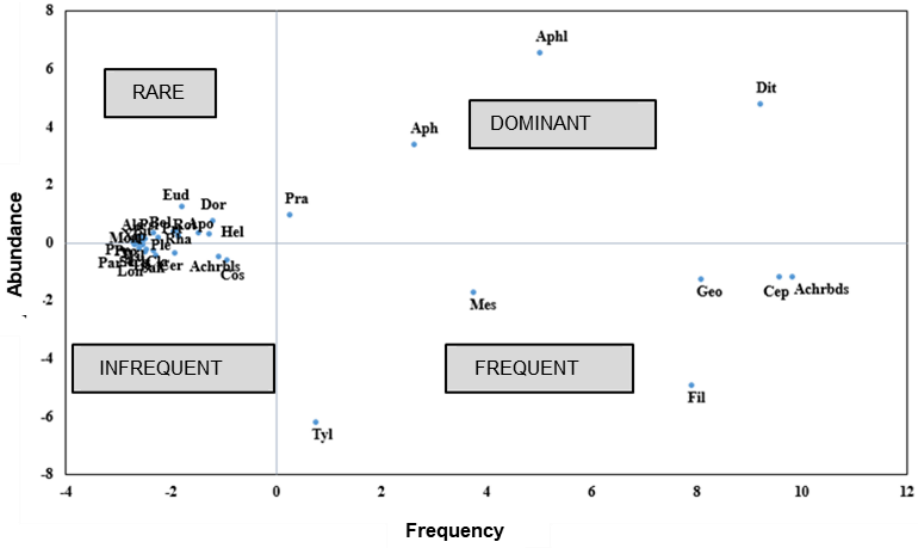

Figure 5. Principle component analysis (PCA) of nematode distribution pattern in sunflower fields in Tekirdağ. Abundance and frequency scheme of nematode genera. (Ach: *Acromodora*; Achrbls: *Achrobeles*; Achrbds: *Achrobeloides*; Ala: *Alaimus*; Aphl: *Aphelenchoides;* Aph: *Aphlenchus*; Bit: *Bitylenchus*; Bol: *Boleodorus*; Cep: *Cephalobus*, Cer: *Cervidellus*; Cos: *Coslenchus*; Dit: *Ditylenchus*; Dor: *Dorylaimus*; Eud: *Eudorylaimus*; Fil: *Filenchus*; Geo: *Geocenamus*; Hel: *Helicotylenchus*; Lon: *Longidorus*; Mes: *Mesodorylaimus*; Mon: *Monhystera*; Pro: *Prodorylaimus*; Psi: *Psilenchus*; Par: *Paratylenchus*; Pry: *Paratylenchus*; Ple: *Plectus*; Pra: *Pratylenchus*; Prt: *Pratylenchoides*; Rha: *Rhabditis*; Rot: *Rotylenchus*; Sak: *Sakia*; Sei: *Seinura*; Tyl: *Tylenchorhynchus*; Tri: *Tripyla*; Xip: *Xiphinema*.

Table 3. Data representing taxonomic classification, c-p values, the occurrence and abundance of plant-parasitic nematodes in sunflower fields in Tekirdağ (Pp: Plant-parasitic; RHF: Root hair feeder)

Genera / species	Functional guild	Suborder	Families	Occurrence (%)	Abundance (min-max nematodes/ 100 cm^3 soil)
Bitylenchus parvus Jairajpuri 1982	Pp3/RHF	Tylenchina	Dolichodoridae	5.4	3-6
Boleodorus thylactus Thorne, 1941	Pp2/RHF	Tylenchina	Boleodorinae	16.2	2-3
Coslenchus alacinatus Siddiqi, 1981	Pp2/RHF	Tylenchina	Tylenchidae	27.0	2-5
Ditylenchus dipsaci (Kühn, 1857) Filipjev,1936	PP2	Tylenchina	Anguinidae	18.9	3-17
Filenchus filiformis Ebsary, 1991	Pp2/RHF	Tylenchina	Tylenchidae	51.3	7-23
Filenchus thornei (Andrassy, 1954) Andrassy, 1963	Pp2/RHF	Tylenchina	Tylenchidae	24.3	3-5
Filenchus cylindricus (Thorne & Malek, 1968)	Pp2/RHF	Tylenchina	Tylenchidae	13.5	3-6
Geocenamus tesellatus (Goodey, 1952) Brzeski, 1991	Pp3	Tylenchina	Merliniidae	43.2	2-32
*Geocenamus brevidens (*Allen, 1955) Siddiqi	Pp3	Tylenchina	Merliniidae	24.3	4-60
Helicotylenchus digonicus Perry, 1959	Pp3	Tylenchina	Hoplolaimidae	21.6	8
Helicotylenchus tunisiensis Siddiqi, 1964	Pp3	Tylenchina	Hoplolaimidae	5.4	3-4
Longidorus elongatus Mikoletzky, 1922	Pp5	Dorylaimina	Longidoridae	2.7	2
Paratrophurus acristylus Siddiqi & Siddiqui, 1983	Pp3	Tylenchina	Telotylenchidae	2.7	2
Pratylenchus thornei Sher & Allen 1953	Pp3	Tylenchina	Pratylenchidae	13.5	2-17
Pratylenchus zeae Graham, 1951	Pp3	Tylenchina	Pratylenchidae	29.7	4-7
Pratylenchoides alkani Yüksel, 1977	Pp3	Tylenchina	Pratylenchidae	11.4	2-3
Psilenchus hilarulus de Man, 1921	Pp2	Tylenchina	Boleodorinae	8.1	1-2
Rotylenchus buxophilus Golden, 1956	Pp3	Tylenchina	Hoplolaimidae	10.8	3-4
Paratylenchus rotundicephalus Bajaj, 1988	Pp3	Tylenchina	Paratylenchidae	5.4	2
Sakia allii Suryawanshi, 1971	Pp2/RHF	Tylenchina	Boleodorinae	5.4	5
Tylenchorhynchus annulatus Cobb, 1913	Pp3	Tylenchina	Belonolaimidae	29.7	5-105
Xiphinema pachtaicum Tulaganov, 1938	P-P5	Dorylaimina	Longidoridae	2.7	3

Omnivore, predator, fungivore, and bacterivore free-living nematodes recovered from sunflower fields in Tekirdağ were grouped from 1 to 5 depending on the colonizer-persister (c-p) values. Genera from c-p2 were prominent among free-living nematodes. The % fraction of c-p2 nematodes in 37 soil samples ranged between 43.75 and 100. In the sampled fields in Tekirdağ, enrichment opportunist nematodes in the c-p1 class were found only in 6 fields (Figure 6). Furthermore, nematodes with c-p3 values were detected in 6 areas, c-p4 in 34 areas and c-p5 values in 9 areas.

Plant-parasitic nematodes belonged to three c-p groups. The prevalence of c-p2, c-p3, and c-p5 among all sampled fields were 45.9%, 100%, and 5.4%, respectively. The nematodes in 20 fields belonged to c-p3 only. Parallel to the frequent occurrence of the c-p3 class in survey areas, the population was also higher in soils. *Geocenamus* was the dominant genus in c-p3, while *Filenchus* was the most common genus in c-p2. The c-p5 nematode was detected only in two locations in Süleymanpaşa, and two species *Longidorus elongatus,* and *Xiphinema pachtaicum,* from this class were identified (Figure 7).

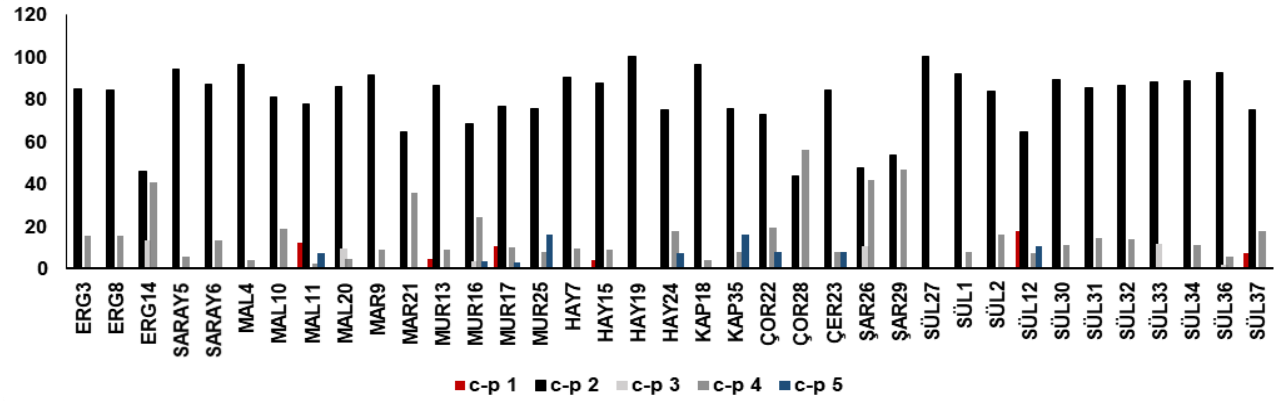

Figure 6. The % proportion of free-living nematodes with different colonizer-persister values in 37 sunflower fields surveyed in Tekirdağ.

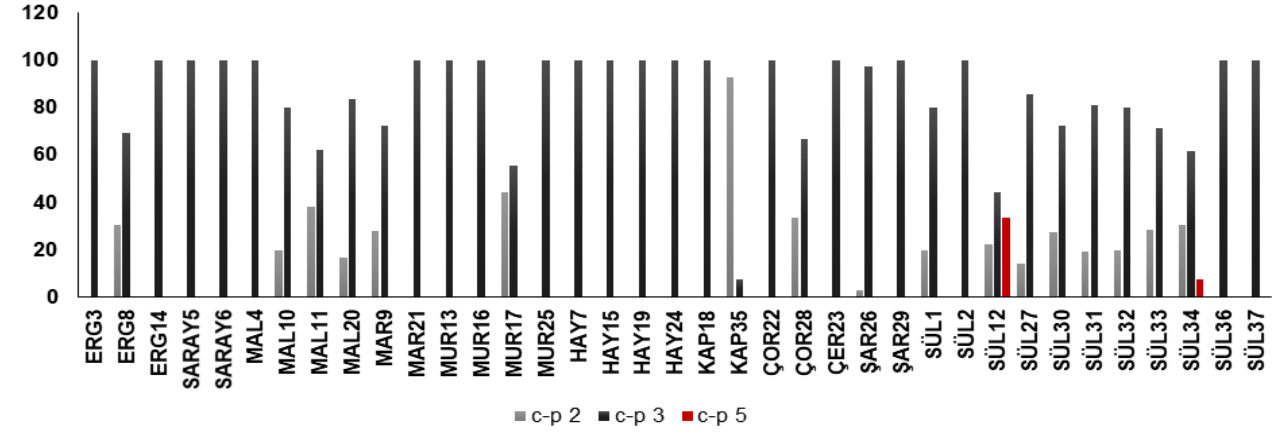

Figure 7. The % proportion of plant-parasitic nematodes with different colonizer-persister values collected from 37 surveyed sunflower fields in Tekirdağ.

Nematode diversity indices, soil food web, soil health

Several indices indicating nematode diversity and soil health were calculated for sunflower fields. As seen in Figure 8, The Maturity index (MI) and, Maturity index $(MI)_{2-5}$ in fields differed; the mean value was 2.35±0.26 (2-3.12) for MI and 2.37±0.28 (2-3.12) for (MI)2-5. The MI value of all fields was ≥ 2 in. The lowest value (2) was observed in two, and the highest was (3.12) in one field. Consequently, as the soils with low MI index were examined, the abundance of bacterivore and fungivore from the c-p 1-2 group was high compared to the other trophic groups. On the contrary, in soil with an MI value above 3, the ratio of plant, fungivore, and bacterivore nematodes was counted as 36%, 12%, and 16%. The mean PPI value [2.91±0.15 (2.6-3.4)] was also found to be low in sunflower fields where endoparasitic, semi-endoparasitic, and large-sized species like *X. pachtaicum* from Longidoridae are not common.

The Shannon-Weiner Diversity Index (H′) was 1.9±0.3 (1-2.57). Pielou's Evenness Index (J′) was 0.89±0.06 (0.7-0.98). The genera richness ranged between 4-17 (Figure 9).

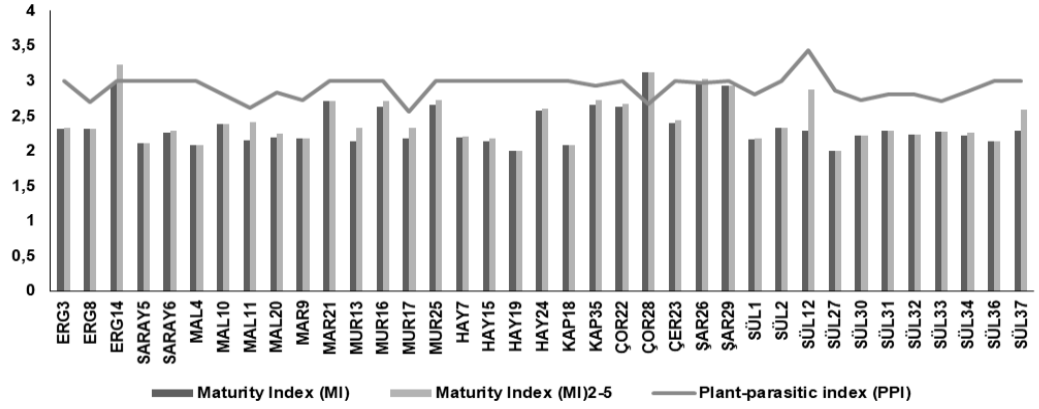

Figure 8. Maturity index (MI), Maturity index $(MI)_{2-5}$, and Plant-parasitic (PPI) values of 37 sunflower fields surveyed in Tekirdağ.

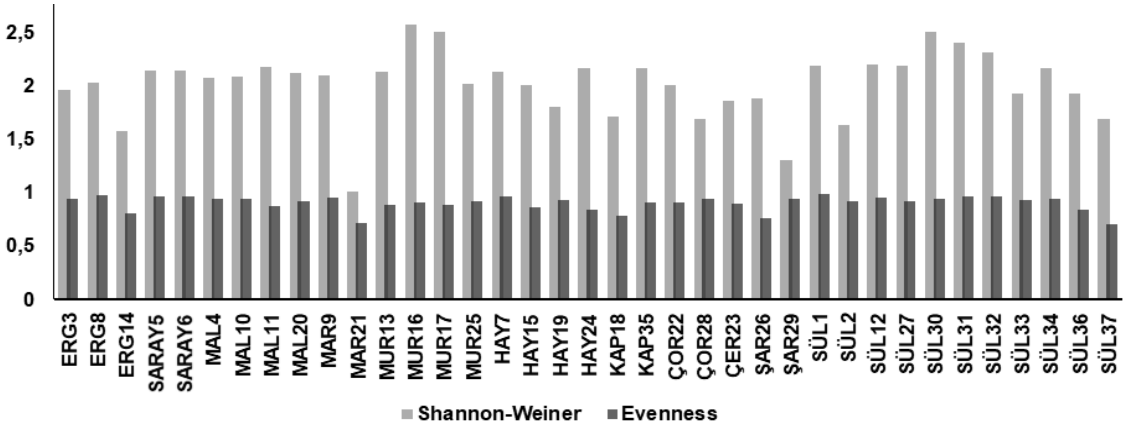

Figure 9. Shannon-Weiner diversity (H′) and Evenness indices of nematode diversity of 37 sunflower fields surveyed in Tekirdağ.

In each field, Basal (BI), Channel (CI), Enrichment (EI), Plant parasite (PPI), and Structure (SI) indices were calculated (Figure 10). The mean CI was determined as 92.7±18.6 (33.3-100), which was calculated as >50 in 91.9% of the fields. On the other hand, the average BI was found to be 41.6±14.8 (15.2-66.7). This value was calculated as ≤30 in 11 fields, 30-60 in 22 fields, and ≥60 in five fields. The mean EI indicating nutrient enrichment was 37.49±10.2 (13.6-51.1), and 64.9% of fields had values under 40. Again, our average Structure Index value, determined as 43.13±21.3 (0-83.7), was very low in 11 fields.

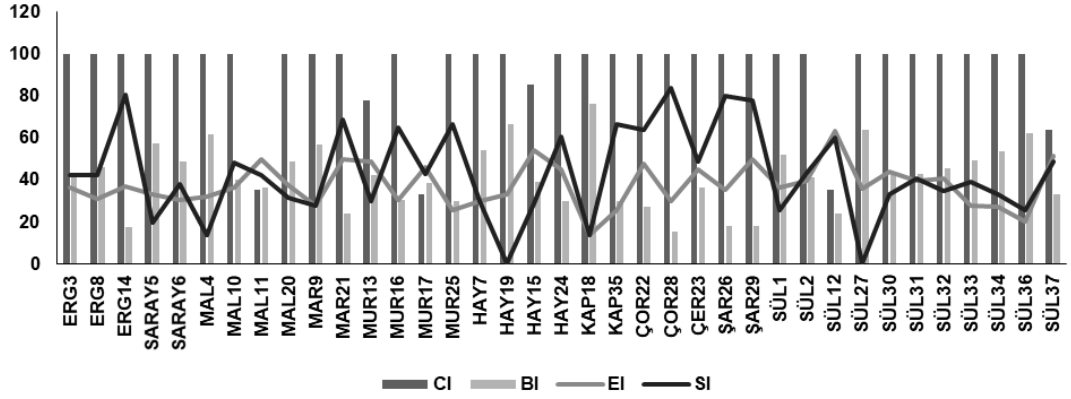

Figure 10. Channel (CI), Basal (BI), Enrichment (EI), and Structure (SI) Indices of nematode diversity of 37 sunflower fields surveyed in Tekirdağ.

The c-p triangle plot prepared using c-p values of nematodes revealed that most of the nematodes in sunflower fields in Tekirdağ belonged to the c-p2 and c-p3-5 classes. As the soil food web scheme was examined, the difference in the soil-food web in surveyed sunflower fields in Tekirdağ can be seen. The fields were replaced in four quadrats. Twenty-three (62.1 %) fields are located in the D quadrate. For instance, fields in Süleymanpasa, Saray, Çerkezkoy, Malkara, and Marmara Ereğlisi were placed in group D. Based on the explanations of the scheme of Ferris et al. (2001), the soil conditions of 23 fields in this quadrate were considered degraded, and stressed, containing high levels of C: N and the fungal decomposition is dominant. In this study, the species and population numbers of nematodes feeding on fungi and bacteria have come to the fore in the fields located in the D quadrate, confirming this. Fungivore *D. geraerti, A. avenae*, and *Aphelenchoides species* have been detected commonly in these fields. However, seven (18.9 %) fields in quadrat C were undisturbed and fertile, containing more bacterivore and fungivore species. On the contrary, fields in quadrat A (Hay15, Mal 11, Sül37) and quadrat B (Mar21, Sül12, Şar29) had strongly or slightly disturbed soils with N-enriched characteristics. In these soils, bacterivore nematodes were dominant (Figure 11).

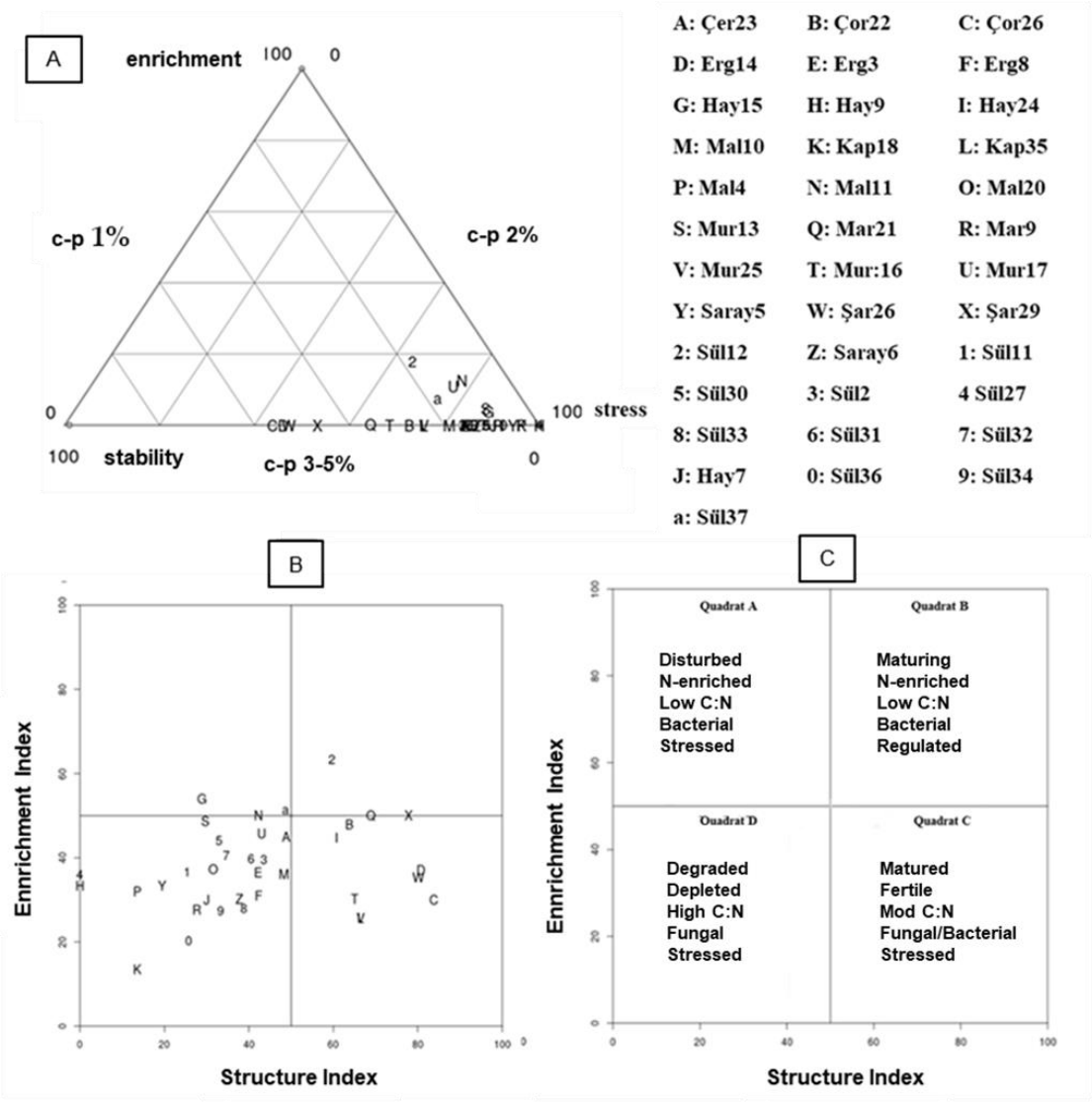

Figure 11. A) c-p triangle representing soil status of sunflower fields based on c-p group; B) the metabolic footprint of surveyed sunflower fields; C) structure and enrichment status of the soil food web from sunflower fields.

Discussion

Nematodes in Phylum Nematoda are one of the most abundant organisms in the soil ecosystem. Their number of genera and species is also higher than other pests. Ritz et al. (2009) identified 183 biological indicators to assess soil conditions, including parameters such as the biodiversity indices of nematodes, number of taxa, and abundance. Since at least one genus of nematodes is present in every soil, they can be used to measure the state of the structure of the soil food web (Pothula et al., 2022).

In the study carried out in the sunflower fields in Tekirdağ province, free-living bacterivore and fungivore nematodes, which adapt well to different soil conditions, were commonly distributed. At least one genus of these nematodes was detected in each sunflower field. Accordingly, *Acrobeloides, Cephalobus*, and *Aphelenchus*, a highly occurred genera in Tekirdağ, were reported to be common in agricultural areas in several countries as well as in Türkiye (Bongers, 1990; Yıldız, 2007; Yıldız et al., 2017; Çetintaş, 2017). Identified free-living soil nematodes have been reported to have different contributions to organic material decomposition and structure improvement in soil. Nematodes restore minerals and other nutrients to the soil, which they decompose from bacteria, and other substrates by feeding. This affects the soil's amount of elements like N, P, K, and Mg (Yeates et al., 1993; Yadaw et al., 2018). For instance, *Tripyla* and *Clarkus*, which have a prevalence of 8.1% and 2.4% in sunflower fields in Tekirdağ, respectively, feed on the soil microbes and lead to N release. There is a balance in fertile, well-developed soils in the populations of these nematodes, which are in different trophic groups and have different functions (Tahat et al., 2020).

In addition to free-living species, several plant parasitic nematodes were present in soil samples in Tekirdağ. According to the feeding strategy, nematodes were grouped as ectoparasites, semi-endoparasites, and migratory endoparasites. Among ectoparasites *Boleodorus, Filenchus, Paratylenchus*, and *Sakia* feed on plant root hairs, and feeding these species with several fungi has been mentioned in several studies (Yeates, 1993). Ectoparasites, detected in 94.6% of all examined areas, were found to be the most common in survey areas. Endoparasites were found only in 20 samples. Of the recovered species, *F. filiformis* Ebsary, 1991, *G. tesellatus* (Goodey, 1952) Brzeski, 1991, *T. annulatus* Cobb, 1913 and *P. zeae* Graham, 1951 were the most widespread. Commonly detected nematodes were weak plant-parasitic species that did not cause plant damage or yield loss, while populations of all species remained below the economic damage threshold. For example, *P. zea* has been reported as a poor host of sunflower genotypes, and the population of the nematode was found low (33/250 cm^3 soil) in previous studies. For this reason, rotation with sunflower is recommended in the control of P. zeae, which is very harmful to corn plants (Bolton & Waele, 1989). Likewise, in a previous study dating back to 2001 by Kepenekçi, 19 plant parasitic nematode species were identified in sunflower fields in Türkiye, and as in our study, *G. tesellatus, P. zeae,* and *C. allacinatus* were the most common species in survey locations. Unlike the study of Kepenekçi (2001), in our study, species from plant-parasitic *B. thylactus*, *D. dipsaci, G. brevidens,* and *G. tesellatus*, *L. elongatus*, *P. acristylus*, *P. hilarulus*, and *X. pachtaicum* were found for the first time in sunflower fields in Tekirdağ. Among them, *L. elongatus* has been reported as a vector of viruses in some plants, but no data were found about its damage in sunflowers. (Brown et al., 1995). *D. dipsaci,* can parasitize more than 450 plants, especially onions and garlic (Greco et al., 1993).

The soil conditions of surveyed sunflower fields in Tekirdağ were estimated based on identified soil nematode diversity and related indices. In most of the fields calculated, MI and MI2-5 values were under 3. Values between 2-3 represent disturbance, stress, and a weak food web structure, and an MI of more than three indicates quality structured soil with a good food web (Du Preez et al., 2022). As a matter of fact, 21 fields we sampled in Tekirdağ were found to have degraded structures. The Channel index (BI) value of more than 50 in 34 fields indicates organic matter decomposition, mainly with fungivore nematodes (Sánchez-Moreno & Ferris (2007). Indeed, in sunflower fields, fungivore nematodes such as *Aphelenchus, Aphelenchoides*, and *Ditylenchus* genus dominate soils with a CI value of 50 and above. Meanwhile,

according to Sánchez-Moreno & Ferris (2007), a higher Enrichment index (EI) indicates the bacterial decomposition of organic material, and a value of around 35 was found in 24 fields. Again, Structure and Basal indices were found to be less than 60. Even if the SI value was 0 in two fields, assuming that an index closes to 30 or low is considered a disrupted food web and high values as improved. In soils with higher EI and lower SI values, fertility is high. In contrast, soils with higher SI values are more suppressive to opportunistic species (Sánchez-Moreno & Ferris, 2018).

According to the c-p triangle, most of the nematodes in survey areas belonged to the c-p2 group. Short lifecycles and high reproduction characterize the identified c-p2 and c-p3 nematodes (Zhou et al., 2023). The c-p 1 group nematodes were low in numbers and not highly distributed. The abundance of these nematodes in the soil can be used to predict organic matter content and nitrogen mineralization in the soil food web. Since soils with low c-p1 nematode species and individuals are characterized as poor in organic matter (Ciobanu & Popovici, 2017). According to scheme of Ferris (2001), surveyed sunflower fields in Tekirdağ were generally located in Quadrats C and D in the soil food web scheme. Fungivores were common in Quadrat D and fungivores/bacterivores in Quadrat C. In these soil diversity conditions C: N amount is quite similar to the food source, and nearly half of C is consumed mostly by fungivores in respiration (Ferris, 1998). Additionally, bacterivore and fungivore nematodes increase the nitrogen content in the soil (Ferris, 2010). After the nematodes feed on organic residues, CO_2 and NH_4 are released, thus increasing soil fertility. The nematodes retain 16% of the nitrogen, and 84% is released into the soil. Of the released N bacterivore nematodes consume much more for survival (Khanum & Mahmood, 2021).

The present study provides information on the nematode community status and the relationship between the present nematode species, soil structure, and soil food web in sunflower fields in Tekirdağ. Knowing the nematode community structure in agroecosystems will promote the development of new policies to protect soil health.

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Original article (Orijinal araştırma)

Contributions to the knowledge of Turkish saproxylic beetle fauna of Anatolian sweetgum forests[1]

Anadolu sığla ormanlarının Türkiye saproksilik böcek faunasına katkıları

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Abstract

The aim of this study was to determine the saproxylic beetle species (Coleoptera) present in Anatolian sweetgum, *Liquidambar orientalis* Miller (Saxifragales: Altingiaceae) forests, to reveal the contribution of this endemic tree species to insect biodiversity. Beetles were sampled in two areas in Muğla province of Türkiye, where *L. orientalis* is the most widely distributed tree species. Five old trees (diameter at breast height 36.0-51.9 cm) were selected in each area and two trap types were used on each tree. Traps were set on March 13, 2021, and checked once a month for six months. At the end of the study, 1,302 individuals belonging to 108 species from 33 families were obtained. Fifteen beetle species were new records for the fauna of Türkiye. The families with most individuals were Curculionidae (555), Anobiidae (325) and Tenebrionidae (104). Families with the highest number of represented species were Anobiidae (15), Elateridae (13), Curculionidae (8) and Tenebrionidae (8). According to the IUCN Mediterranean Red List, *Ectamenogonus montandoni* (Buysson, 1889) (Coleoptera: Elateridae) is classified as endangered (EN) and *Propomacrus bimucronatus* (Pallas, 1781) (Coleoptera: Euchiridae) as vulnerable (VU).

Keywords: Coleoptera, dead wood, hollow trees, *Liquidambar orientalis*, saproxylic beetle

Öz

Bu çalışmada, Anadolu sığla ağacı, *Liquidambar orientalis* Miller (Saxifragales: Altingiaceae) ormanlarındaki saproksilik böcek türlerinin (Coleoptera) belirlenmesi ve endemik olan bu ağaç türünün böcek biyolojik çeşitliliğine katkısının ortaya konulması amaçlanmıştır. Çalışma, *L. orientalis* türünün en geniş yayılış yaptığı Türkiye'de Muğla iline bağlı iki örnek alanda gerçekleştirilmiştir. Böcekleri örneklemek amacıyla, her iki alanda beşer adet yaşlı (göğüs yüksekliği çapı 36.0-51.9 cm) ağaç seçilmiş ve her ağaçta iki tuzak tipi kullanılmıştır. Tuzaklar 13 Mart 2021 tarihinde kurulmuş ve altı ay boyunca ayda bir kere kontrol edilmiştir. Çalışmanın sonunda 33 familyadan 108 türe ait 1302 adet birey elde edilmiştir. 15 böcek türünün Türkiye faunası için yeni kayıt olduğu belirlenmiştir. Curculionidae (555), Anobiidae (325) ve Tenebrionidae (104) en yaygın bulunan familyalardır. Tür sayısı en yüksek familyalar ise Anobiidae (15), Elateridae (13), Curculionidae (8) ve Tenebrionidae (8)'dir. IUCN'in Akdeniz kırmızı listesine göre *Ectamenogonus montandoni* (Buysson, 1889) (Coleoptera: Elateridae) tehdide açık (EN) ve *Propomacrus bimucronatus* (Pallas, 1781) (Coleoptera: Euchiridae)'un duyarlı (VU) sınıfında yer aldığı belirlenmiştir.

Anahtar sözcükler: Coleoptera, ölü odun, kovuklu ağaçlar, *Liquidambar orientalis*, saproksilik böcek

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Introduction

The presence of dead standing trees or wood helps to increase forest productivity by providing moisture, nutrients, organic matter and regeneration areas, and biodiversity by providing habitat and nutrition for many species (Kirby et al., 1998; Nilsson et al., 2001; Aakala et al., 2008; Thorn et al., 2020), reducing soil erosion and minimizing the negative impacts of climate change by sequestering carbon (Krankina & Harmon, 1995; Chambers et al., 2000; WWF, 2004). Many species depend on the micro-habitats formed by dead trees for their basic needs such as feeding, hiding, sheltering and nesting (Kaila et al., 1997; WWF, 2004). Species of saproxylic insects inhabiting these habitats are amongst the most important groups of endangered invertebrates associated with old and dead trees in Europe (McLean & Speight, 1993; Sama et al., 2011). Many species are also included in the European Red List (Nieto & Alexander, 2010; Avgın et al., 2014).

Saproxylic insects contribute directly or indirectly to important ecosystem services associated with wood fragmentation and decomposition (Stokland, 2012; Ramírez-Hernández et al., 2019), nutrient cycling (Samuelsson et al., 1994; Sverdrup-Thygeson & Ims, 2002), forest pest management and pollination (Ulyshen, 2016; Micó, 2018). These insects bring their associated predators and fungi to the areas where they occur (Hammond et al., 2001; Vanderwel et al., 2006; Dennis et al., 2018), thus indirectly accelerating wood degradation (Müller et al., 2002; Dennis et al., 2018). The IUCN European Red List gives 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 (Cálix et al., 2018). Sixty-one 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).

The genus *Liquidambar*, in the Altingiaceae, has four species globally (Ickert-Bond et al., 2005). *Liquidambar orientalis* Miller (Saxifragales: Altingiaceae), Anatolian sweetgum tree is a relict and endemic species from the third (Tertiary) period dating back to approx. 65 million years ago (Yıldırım Şenyer, 2014). In Türkiye, it is distributed in Denizli (Günlük Brook, Gerenis Brook, Gölcük village), Antalya (Aksu Valley) and Isparta (Sütçüler), and is common in Muğla (Marmaris, Fethiye, Köyceğiz and Ula) (Efe, 1987, 2008; Velioğlu et al., 2008; Göçmen, 2010; Ürker & Yalçın, 2011; Teker, 2013). Since *L. orientalis* is an endemic species with a narrow distribution, it is of great importance in terms of protecting biodiversity and ensuring the continuity of the forest ecosystem. The forest area of *L. orientalis,* one of the two species producing sweetgum oil in the world (Öztürk, 2008), has decreased from approximately 7000 hectares in 1947 to 900 hectares today (Huş, 1949; Topçuoğlu, 1968; Özmen, 2011). *Liquidambar orientalis* was considered to be in the category "Species under High Threat in the Medium-Term Future in Nature" in the list prepared according to the IUCN Threat Categories in 2000, and in 2001, it was recognized by EUFORGEN as a species that should be protected throughout Europe and included in the 'Valuable Leaves' status. In 2018, it was upgraded from 'vulnerable-sensitive (VU-Vulnerable)' to 'threatened (EN-Endangered)' status in the IUCN Red List, which classifies the threat status of species on a global scale (Ekim et al., 2000; Alan & Kaya, 2003; Kavak & Wilson, 2018).

Studies on *L. orientalis* are a required part of the necessary measures for the conservation and continuity of this species to future generations. As an important part of this process, Coleoptera saproxylic insect species in *L. orientalis* forests in Muğla province were determined and the contribution of this endemic tree species to insect biodiversity revealed.

Materials and Methods

Study area

The study was conducted in Muğla province in southwestern Türkiye, where *Liquidambar orientalis* has a wide distribution. Two sample areas [Köyceğiz/Kavakarası (36°53'59.00"N, 28°42'42.00"E), Fethiye/ Küçük Kargı Nature Park (36°43'1.00"N, 29°1'17.00"E)] include dense stands of old, hollow, cavity trees and were

found by utilizing the management plans of Muğla Regional Directorate of Forestry (Figure 1). The study areas are at 0 to 15 meters altitude. *Liquidambar orientalis* is the dominant trees species in the study areas and their surroundings. *Smilax excelsa* L. (Liliales: Smilacaceae), *Hedera helix* L. (Apiales**:** Araliaceae), *Vitex agnus-castus* L. (Lamiales: Lamiaceae), *Pinus brutia* Ten. (Pinales: Pinaceae), *Quercus ilex* L. (Fagales: Fagaceae), *Ruscus aculeatus* L. var. *angustifolius* Boiss (Asparagales: Ruscaceae) and *Nerium oleander* L. (Gentianales: Apocynaceae) are also present in the same forests (Akbaş, 2012; Anonymous, 2021).

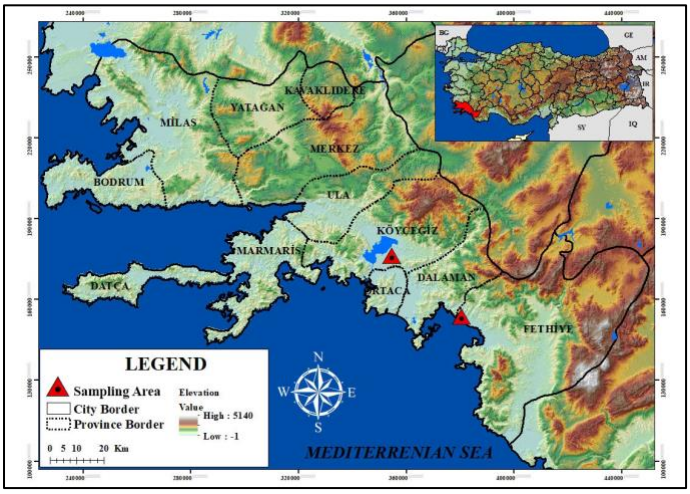

Figure 1. The location of study area.

Selection of trees and sampling of beetles

In the study areas, five old, hollow, cavity trees were located, and pitfall and window traps (Jansson & Lundberg, 2000; Ranius & Jansson, 2002; Milberg et al., 2014) were set in each of them (Figure 2). The traps were set at the beginning of the vegetation period, on 13/03/2021 and were checked once a month for six months (April-September).

Figure 2. Window and pitfall traps used to collect saproxylic beetles in the study (a, b).

Date, location, trap type and trap number were recorded during the sampling times. In Küçük Kargı Nature Park, beetles were obtained by using the light trap (Stork et al., 2016) on 22/07/2021. Ultraviolet and white light were used together in the trap (García-López et al., 2011). In addition, during the field studies, the hollows, parts and other cavities of the fallen, leaning, and old trees in the field were examined and beetles collected manually with an insect aspirator (Neumann et al., 2013). Beetles were brought to the laboratory and placed in plastic tubes filled with 70% alcohol. The tubes were labelled by recording the sampling area, date of collection, coordinates, trap type and number. Familial distinction was made with the help of experts and using identification keys. Photographs of the prepared insect specimens were taken with LEICA Z16 APO.

The species obtained in the study were classified according to the IUCN Europa and Mediterranean Red List (Cálix et al., 2018; Garcia et al., 2018), and saproxylic beetles classified into trophic categories according to their location in the tree and their feeding patterns (Carpaneto et al., 2015; Ulyshen, 2018) (Table 1).

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

CO	Commensal of SX/XY or of other saproxylic insects
MB	Mycetophagous on carpophora of large fungi (mostly Polyporales) growing on veteran trees or on old stumps
MM	Myrmecophilous or melittophagous inside hollow trees or stumps hosting colonies of ants or of other social Hymenoptera
MY	Mycophagous (developing on ifae of saproxylic fungi or on micromycetes, yeasts and Myxomiceta)
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 its decomposition, including the wood mould inside hollow trees
UN	Trophic category unknown
XY	Xylophagous (fresh wood or bark but also developing on healthy trees)

Results and Discussion

During sampling, 108 species and 1 302 individuals belonging to 33 families of Coleoptera were obtained (Table 2). Fifteen species were new records for Türkiye (Figure 3). Publications, especially Avgın et al. (2014); Koçak (2014); Löbl & Smetana (2006, 2007, 2008, 2010, 2011, 2013); Löbl & Löbl (2015, 2016, 2017); Gülperçin & Tezcan (2016); Tezcan (2020) and Alonso-Zarazaga et al. (2017) were utilized to determine whether the species had been recorded previously in Türkiye. The *Dorcatoma* sp. was determined to be a new species to science and identification studies continue. The newly recorded species of saproxylic beetle fauna for Türkiye were from 11 families; the Curculionidae (Scolytinae) and Eucnemidae (Macraulacinae and Melasinae) had three species each and the Scraptiidae (Anaspidinae and Scraptinae), with two species, were particularly notable.

Table 2. Species and individual numbers by family (*New records for Türkiye)

Family / Species Name	Number of Individuals	Presence Rate (%)	IUCN Category Europa (Mediterranean)	Trophic Category
ADERIDAE				
Aderus populneus (Creutzer in Panzer, 1796)	1	0.08		SX
Gompelia ruficollis (Rossi, 1794)	5	0.38		
ANOBIIDAE				
Anobiinae				
Anobium punctatum (De Geer, 1774)	132	10.14		XY
Dorcatoma farbiaki Zahradník, 1998	8	0.61		MB
Dorcatoma sp.	18	1.38		MB
Falsogastrallus unistriatus (Zoufal, 1897)	15	1.15		XY
Lasioderma sp.	20	1.54		
Mesocoelopus niger (P. W. J. Muller, 1821)	28	2.15		XY
Metholcus phoenicis (Fairmaire, 1859)	27	2.07		XY
Oligomerus ptilinoides Wollaston, 1854	4	0.31		XY
Ptilinus pectinicornis (L., 1758)	1	0.08		XY
Stagetus elongatus (Mulsant & Rey, 1861)	27	2.07		SX
Xyletinus sp.	1	0.08		XY

Table 2. Continued

Family / Species Name	Number of Individuals	Presence Rate (%)	IUCN Category Europa (Mediterranean)	Trophic Category
Ptininae				
Dignomus frivaldszkyi (Reitter, 1884)	1	0.08		
**Dignomus urbanus* Borowski, 1999	40	3.07		
Ptinus (*Bruchiptinus*) sp.1	2	0.15		
Ptinus (*Bruchoptinus*) sp.2	1	0.08		
ANTHRIBIDAE				
Anthribinae				
Noxius curtirostris (Mulsant & Rey, 1861)	8	0.61		XY (SX, MY)
BOSTRICHIDAE				
Bostrichinae				
Scobicia chevrieri (Villa & Villa, 1835)	3	0.23	LC	XY
CERAMBYCIDAE				
Cerambycinae				
Axinopalpis gracilis (Krynicki, 1832)	1	0.08	LC	XY
Gracilia minuta (Fabricius, 1781)	1	0.08	LC	XY
Stromatium unicolor (Olivier, 1795)	1	0.08	LC	XY
Lamiinae				
Anaesthetis testacea (Fabricius, 1781)	1	0.08		XY
Niphona picticornis Mulsant, 1839	1	0.08		XY
Prioninae				
Aegosoma scabricorne (Scopoli, 1763)	2	0.15	LC	XY
Rhaesus serricollis (Motschulsky, 1838)	16	1.23	NT	
CIIDAE				
**Cis tomentosus* Mellié, 1849	4	0.31		MB
Cis villosulus (Marsham, 1802)	1	0.08		
CLERIDAE				
Clerinae				
Clerus mutillarius Fabricius, 1775	8	0.61		PR
Tillinae				
Denops albofasciatus (Charpentier, 1825)	1	0.08		PR
COLYDIDAE				
Colydiinae				
**Colydium filiforme* Fabricius, 1792	1	0.08		
Synchita mediolanensis Villa & Villa, 1833	3	0.23		
CORYLOPHIDAE				
Corylophinae				
Arthrolips sp.	1	0.08		MY
CURCULIONIDAE				
Cossoninae				
Melicius cylindrus (Boheman, 1838)	6	0.46		SX
Stenoscelis (*Stenoscelis*) *submuricata* (Schoenherr, 1832)	33	2.53		SX
Scolytinae				
**Ambrosiodmus rubricollis* (Eichhoff, 1875)	2	0.15		MY
Hypothenemus eruditus (Westwood, 1834)	5	0.38		
Kissophagus vicinius (Comolli, 1837)	3	0.23		
**Scolytus orientalis* Eggers, 1910	1	0.08		
Xyleborinus saxesenii (Ratzeburg, 1837)	492	37.79		MY
**Xylosandrus crassiusculus* (Motschulsky, 1866)	13	1.00		MY

Table 2. Continued

Family / Species Name	Number of Individuals	Presence Rate (%)	IUCN Category Europa (Mediterranean)	Trophic Category
DASYTIDAE				
Dasytinae				
Dasytes (*Dasytes*) *tardus* Schaufuss, 1872	8	0.61		
Rhadalinae				
Aplocnemus (*Aplocnemus*) *rufipes* Miller, 1862	2	0.15		PR
DERMESTIDAE				
Megatominae				
Anthrenus (*Florilinus*) *verbasci* (L., 1767)	7	0.54		
Thorictus grandicollis grandicollis Germar, 1842	1	0.08		
Trogoderma glabrum (Herbst, 1783)	3	0.23		
ELATERIDAE				
Agrypninae				
Adelocera pygmaea (Baudi, 1871)	2	0.15	EN	
Lacon punctatus (Herbst, 1779)	2	0.15	LC	PR
Dendrometrinae				
Elathous emrei Platia et al., 2011	1	0.08		
Elathous rufobasalis Wurst, 1994	6	0.46		
**Hypnoidus riparius* (Fabricius, 1792)	2	0.15		
Elaterinae				
Ampedus cinnabarinus Eschscholtz, 1829	1	0.08	LC	PR
Agriotes acuminatus (Stephens, 1830)	1	0.08		
Agriotes brevis Candèze, 1863	16	1.23		
Agriotes paludum Kiesenwetter, 1859	1	0.08		
Agriotes sputator (L., 1758)	13	1.00		
Ectamenogonus montandoni (Buysson, 1889)	1	0.08	NT (EN)	PR
Haterumelater fulvago (Marseul, 1868)	28	2.15		
Pittonotus theseus (Germar, 1817)	26	2.00		
ENDOMYCIDAE				
Anamorphinae				
Symbiotes gibberosus (Lucas, 1846)	3	0.23		MB
EUCHIRIDAE				
Euchirinae				
Propomacrus bimucronatus (Pallas, 1781)	5	0.38	NT (VU)	
EUCNEMIDAE				
Macraulacinae				
**Dromaeolus simplicifrons* Otto, 2016	2	0.15		
Melasinae				
**Brevisegmentus miyatakei* (Hisamatsu, 1955)	3	0.23		
**Melasis balwanti* Fleutiaux, 1934	2	0.15		
HISTERIDAE				
Dendrophilinae				
Cyclobacanius soliman (Marseul, 1862)	1	0.08		PR
Histerinae				
Platysoma (*Platysoma*) *compressum* (Herbst, 1783)	3	0.23		PR
Tribalinae				
Epierus comptus Erichson, 1834	2	0.15		PR
Tribalus anatolicus Olexa, 1980	1	0.08		
Pseudepierus italicus (Paykull, 1811)	1	0.08		PR

Table 2. Continued

Family / Species Name	Number of Individuals	Presence Rate (%)	IUCN Category Europa (Mediterranean)	Trophic Category
LANGURIIDAE				
Cryptophilinae				
**Cryptophilus integer* (Heer, 1841)	2	0.15		
LATRIDIIDAE				
Corticariinae				
Corticaria sp.	1	0.08		MY
Melanophthalma (*Melanophthalma*) *distinguenda* (Comolli, 1837)	4	0.31		MY
Latridiinae				
Enicmus rugosus (Herbst, 1793)	1	0.08		MY
Latridius minutus (L., 1767)	5	0.38		MY
LUCANIDAE				
Lucaninae				
Dorcus parallelipipedus (L., 1785)	12	0.92	LC	SX
MALACHIIDAE				
Malachiinae				
Troglops sp.	2	0.15		
MONOTOMIDAE				
Monotoma sp.	1	0.08		MY
MORDELLIDAE				
Mordellinae				
Mordellistena sp.1	5	0.38		SX
Mordellistena sp.2	2	0.15		SX
Tomoxia bucephala (Costa, 1854)	1	0.08		SX
MYCETOPHAGIDAE				
Mycetophaginae				
Mycetophagus decempunctatus Fabricius, 1801	1	0.08	LC	MY
NITIDULIDAE				
Nitidulinae				
Amphotis orientalis Reiche, 1861	3	0.23		MM
Omosita discoidea (Fabricius, 1775)	5	0.38		
OEDEMERIDAE				
Oedemerinae				
Oedemera (*Oncomera*) *flavicans* (Fairmaire, 1860)	9	0.69		SX
PHALACRIDAE				
Phalacrinae				
Stilbus sp.	1	0.08		
PYROCHROIDAE				
Hemidendroides ledereri (Ferrari, 1869)	3	0.23		
SCIRTIDAE				
Prionocyphon ornatus Abeille de Perrin, 1881	8	0.61		
SCRAPTIIDAE				
Anaspidinae				
Anaspis (*Anaspis*) *lurida* Stephens, 1832	12	0.92		SX
Anaspis (*Anaspis*) *thoracica* (L., 1758)	2	0.15		
**Anaspis* (*Silaria*) *varians* Mulsant, 1856	6	0.46		
Scraptiinae				
**Scraptia ferruginea* Kiesenwetter, 1861	1	0.08		

Table 2. Continued

Family / Species Name	Number of Individuals	Presence Rate (%)	IUCN Category Europa (Mediterranean)	Trophic Category
SPHINDIDAE				
Sphindinae				
Aspidiphorus orbiculatus (Gyllenhal, 1808)	4	0.31		MY
**Sphindus dubius* (Gyllenhal, 1808)	3	0.23		MY
STAPHYLINIDAE				
Pselaphinae				
Batrisodes sp.	1	0.08		
Staphylininae				
Hesperus auricomus Schillhammer et al., 2007	3	0.23		
Quedius (*Raphirus*) sp.1	1	0.08		
Quedius (*Raphirus*) sp.2	3	0.23		
Tachyporinae				
Tachinus laticollis Gravenhorst, 1802	6	0.46		
Tachyporus hypnorum (Fabricius, 1775)	3	0.23		
Tachyporus nitidulus (Fabricius, 1781)	1	0.08		
Xantholininae				
Xantholinus sp.	3	0.23		
TENEBRIONIDAE				
Alleculinae				
Allecula estriata Seidlitz, 1896	24	1.84		
Mycetochara quadrimaculata (Latreille, 1804)	59	4.53	NT	SX
Prionychus ater (Fabricius, 1775)	10	0.77	LC	SX
Diaperinae				
Diaperis boleti (L., 1758)	2	0.15	LC	MB
Tenebrioninae				
Bolitophagus reticulatus (L., 1767)	2	0.15		MB
Nalassus plebejus (Küster, 1850)	2	0.15		SX
**Uloma ferruginea* (Piller & Mitterpacher, 1784)	5	0.38		
TOTAL	1302	100		

Dignomus urbanus Borowski, 1999 (Coleoptera: Anobiidae) was first identified in Saudi Arabia and described in 1999 (Löbl & Smetana, 2007). With this study, Türkiye became the second known country of distribution of this species, particularly notable because a high number of individuals (40 individuals) were found among the new records.

Cis tomentosus Mellie, 1848 (Coleoptera: Ciidae) is distributed in Europe, Asia, and North Africa (Jelínek, 2008; Amini et al., 2020). It appears to be associated with fungi in the genus *Trichaptum* and reports from *Alnus* sp. (Betulaceae) and *Fagus* sp. (Fagaceae) are available (Amini et al., 2020). *Colydium filiforme* Fabricius, 1792 (Coleoptera: Colydidae) is distributed in Europe and Asia (Węgrzynowicz, 1999; Otero & Ghahari, 2020). No data on host records were found.

Three species of the subfamily Scolytinae are new records for the fauna of Türkiye. Among these species, *Ambrosiodmus rubricollis* (Eichhoff, 1875) is commonly found in the Far East and is endemic to Asia, although it also occurs in Australia (Wood & Bright, 1992), North America (Bright, 1968) and Europe (Italy and Slovenia) (Faccoli et al., 2009; Gomez et al., 2018). It has a wide host range and is mostly found on broad-leaved trees and shrubs (Faccoli et al., 2009; EPPO, 2023). It has been detected on *Liquidambar styraciflua* L. (Saxifragales: Altingiaceae) in the state of Florida in the United States of America (You et al., 2015). *Scolytus orientalis* Eggers, 1910 has been reported from Russia, Bulgaria, Ukraine, Iran, and Turkmenistan (Petrov, 2021; Alonso-Zarazaga et al., 2017). It was detected on *Ulmus glabra* Huds., *U. laevis* Pall., *U. pumila* L. (Rosales: Ulmaceae) and *Zelkova carpinifolia* (Pall.) (Rosales: Ulmaceae) in

European Russia (Petrov, 2021). *Xylosandrus crassiusculus* (Motschulsky, 1866) has been recorded in Europe, Africa, Asia, and America (Atkinson et al., 2011; Gomez et al., 2018; Alonso-Zarazaga et al., 2017). This species, which is polyphagous, including coniferous and broadleaf species, was also found in *L. styraciflua* in Florida (Schedl, 1963; Wood, 1982; Pennacchio et al., 2003; Atkinson et al., 2011).

Some of the species newly recorded in Türkiye were previously detected mostly in Europe. *Hypnoidus riparius* (Fabricius, 1792) (Coleoptera: Elateridae) has been recorded in Europe and Asia (Löbl & Smetana, 2007) and is reported to be rarely seen in agricultural areas (EPPO, 2023). *Cryptophilus integer* (Heer, 1841) (Coleoptera: Languriidae) has been detected in Europe, Asia, Africa and South America (Ljubarsky, 1995; Ljubarsky, 1997; Ottó, 2004). *Anaspis* (*Silaria*) *varians* Mulsant, 1856 of the Scraptiidae family has been found in many countries in Europe and in Cyprus and Syria in Asia, while *Scraptia ferruginea* Kiesenwetter, 1861 was previously reported in Slovakia, Slovenia and Switzerland in Europe (Löbl & Smetana, 2008). *Sphindus dubius* (Gyllenhal, 1808), of the Sphindidae, has been reported from many countries in Europe, Algeria and the Canary Islands in North Africa (Löbl & Smetana, 2007). The species *Uloma ferruginea* (Piller & Mitterpacher, 1784) (Coleoptera: Tenebrionidae) has been detected in Europe; it was found in Albania, Bosnia and Herzegovina, Croatia, and Romania (de Jong et al., 2014).

Three species from the family Eucnemidae identified in the study are also among the species new for the fauna of Türkiye. These species have been reported to spread from Laos, Thailand, Japan, and India in the Eastern Palearctic Region. It was stated that these species [*Dromaeolus simplicifrons* Otto, 2016, *Brevisegmentus miyatakei* (Hisamatsu, 1955) and *Melasis balwanti* Fleutiaux, 1934 (Coleoptera: Eucnemidae)] are rare species. *Brevisegmentus miyatakei* occurs on broad-leaved trees and *M. balwanti* on *Gynocardia odorata* R. Br. (Malpighiales: Achariaceae) (Otto, 2016).

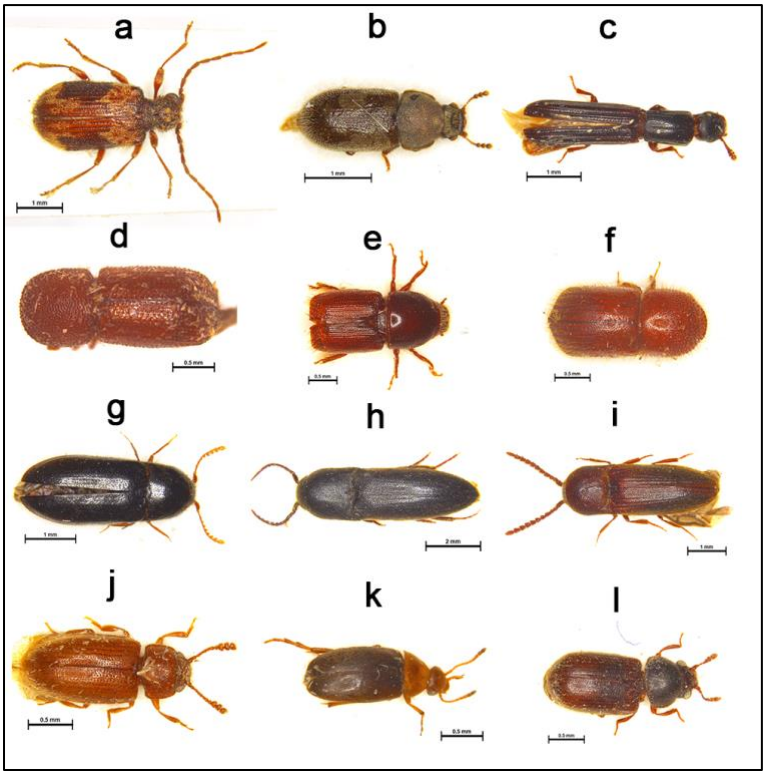

Figure 3. Examples of species of saproxylic beetle new to the fauna of Türkiye: a) *Dignomus urbanus*, b) *Cis tomentosus*, c) *Colydium filiforme*, d) *Ambrosiodmus rubricollis*, e) *Scolytus orientalis*, f) *Xylosandrus crassiusculus*, g) *Hypnoidus riparius*, h) *Dromaeolus simplicifrons*, i) *Brevisegmentus miyatakei*, j) *Cryptophilus integer*, k) *Anaspis* (*Silaria*) *varians*, l) *Sphindus dubius*.

Based on analysis of the numbers of species by family, Anobiidae was most abundant with 15 species, with Elateridae (13 species), Curculionidae and Staphylinidae (8 species each) second and equal third in abundance (Figure 4). The Curculionidae family included 555 individuals, the Anobiidae 325 individuals and the Tenebrionidae 104 individuals, the first three places most abundant in terms of individuals (Figure 5).

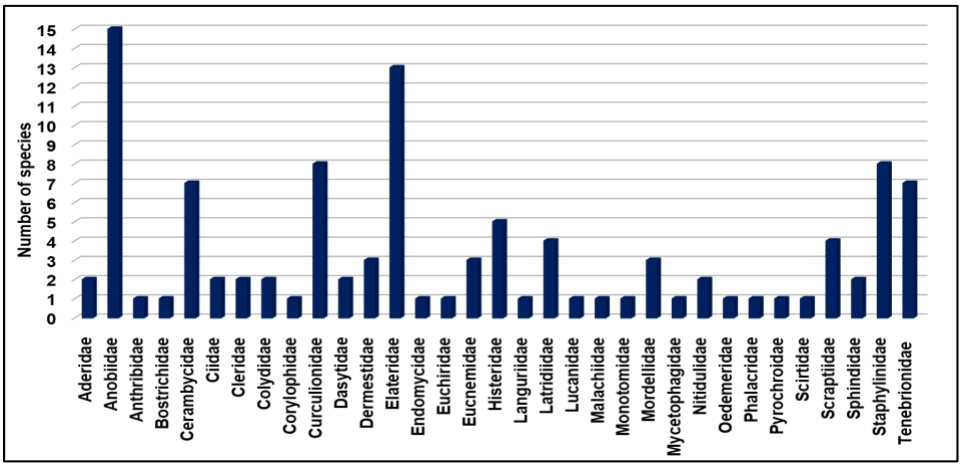

Figure 4. The number of saproxylic beetle species per family caught in studies of *Liquidambar orientalis* in Türkiye.

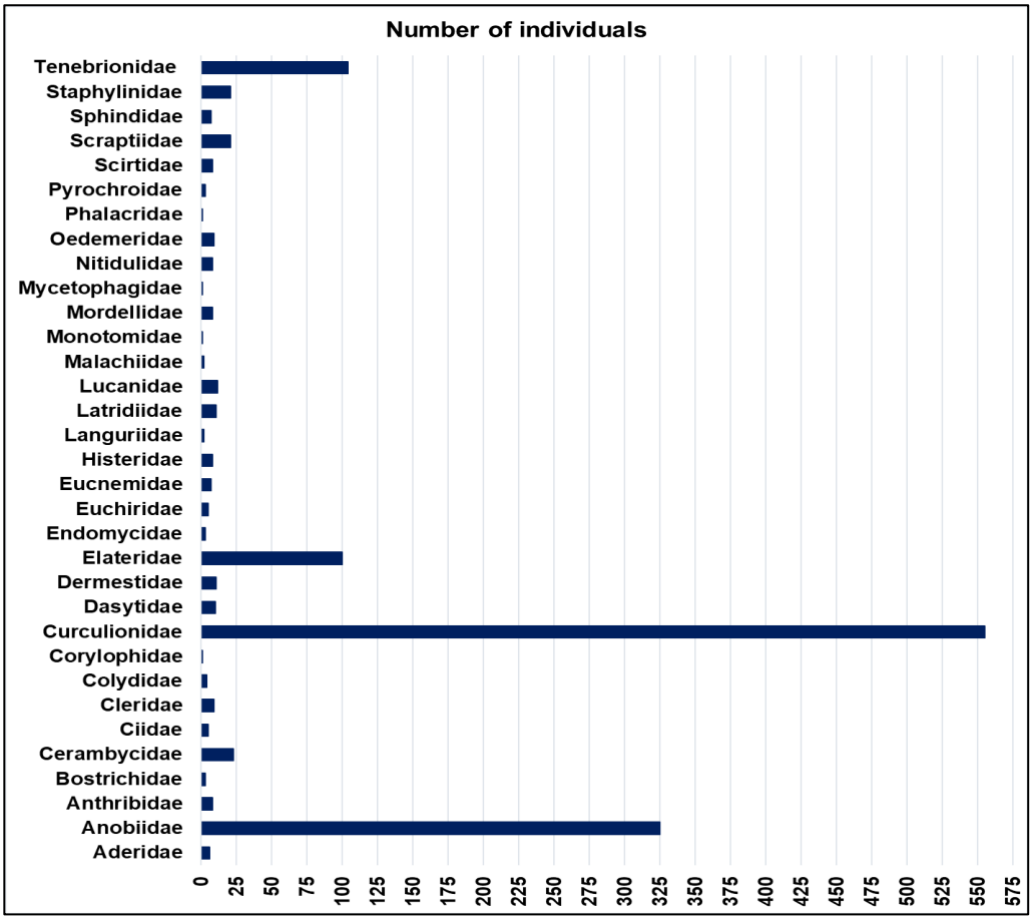

Figure 5. The number of individuals per family of saproxylic beetle caught in studies of *Liquidambar orientalis* in Türkiye.

Although the number of species in the family Buprestidae was high in saproxylic beetle studies conducted on different tree species (oak, cedar-oak, beech) in different areas (Adana-Kozan, Mersin-Gülnar, Kahramanmaraş-Andırın) in Türkiye (Gürkan, 2011; Laz, 2015; Varlı et al., 2021; Göktepe, 2022), no species in this family were detected in our study. These differences may be due to regional differences or the tree species examined (Muñoz-López et al., 2016). However, other studies and the families with high number of species in this study and other studies are similar (Gürkan, 2011; Atay et al., 2012; Laz, 2015; Varlı et al., 2021; Göktepe, 2022).

Examining numbers of species according to trap types, 1219 individuals of 97 species were obtained from window traps and 38 individuals of 19 species from pitfall traps. Of these species, 15 were found in both trap types. Four species [*Ptinus* (*Bruchoptinus*) sp.2 (Coleoptera: Anobiidae), *Stromatium unicolor* (Olivier, 1795) (Coleoptera: Cerambycidae), *Lacon punctatus* (Herbst, 1779) (Coleoptera: Elateridae) and *Tribalus anatolicus* Olexa, 1980 (Coleoptera: Histeridae)] were caught only in pitfall traps. Eighty two of 97 species were found only in window traps. Seven species [*Ptinus* (*Bruchiptinus*) sp.1 (Coleoptera: Anobiidae), *S. orientalis*, *Ectamenogonus montandoni* (Buysson, 1889) (Coleoptera: Elateridae), *Epierus comptus* Erichson, 1834 (Coleoptera: Histeridae), *Pseudepierus italicus* (Paykull, 1811) (Coleoptera: Histeridae), *Stilbus* sp. (Coleoptera: Phalacridae), *Diaperis boleti* (L., 1758) (Coleoptera: Tenebrionidae)] were collected only by light trap and hand; these species were not detected in window or pitfall traps. In places where pitfall traps were set, disturbance problems such as filling of the traps with soil and removal of the traps were encountered. The fact that some larger animal species also use the hollow trees where pitfall traps were located and that the cavities are secluded and sheltered are thought to explain the low number of individuals recovered, compared to window traps. In addition, the larger surface area of the window traps may also affect the results (Peuhu et al., 2019). In studies where these types of traps were used, it was reported that the number of species caught was lower in pitfall traps (Ranius & Jansson, 2002; Milberg et al., 2014; Peuhu et al., 2019).

A comparison of insect numbers caught in the different sampling areas showed that 72 species and 765 individuals were caught in Köyceğiz, with 71 species and 492 individuals caught in Fethiye. Although it was expected that the number of species and individuals would be higher in Fethiye due to its protected status, more individual insects were obtained in Köyceğiz. A possible reason for this effect is that the insect population is negatively affected by human tourism activities in the protected area. Since sweetgum oil is produced by peeling and wounding the bark of the trees in the area in Köyceğiz, the trees are weakened. For this reason, more individuals of Scolytinae (Curculionidae) were obtained, which includes secondary pest species.

In Fethiye, 23 saproxylic beetles belonging to 10 species of Cerambycidae [*Rhaesus serricollis* (Motschulsky, 1838), 8 individuals] and Elateridae (*Elathous rufobasalis* Wurst, 1994, 5 individuals) families were caught with light traps. In addition, 20 individuals of 7 species in Fethiye and 2 individuals of 2 species in Köyceğiz were found in fallen trees by hand collection. *Dorcus parallelipipedus* (L., 1785) (Coleoptera: Lucanidae) in the Lucanidae family was the most common species with 9 individuals.

In the study, *X. saxesenii* from the subfamily Scolytinae was the most common species found, with 492 individuals, representing approximately 38% of the total number of individuals. Of the individuals obtained from this species, 445 were from Köyceğiz and 47 from Fethiye. This situation is also possibly related to the production of sweetgum oil by peeling and injuring the bark of trees in the area in Köyceğiz. In several studies on bark and ambrosia beetle species conducted in Türkiye (Sarıkaya, 2013a, b; Sarıkaya & Sayın, 2016, Sarıkaya, 2019), *X. saxesenii* has been the most common species found. One of these studies was conducted on *L. orientalis* (Sarıkaya, 2013b). In a study conducted in the USA on *L. styraciflua*, a high number (566 individuals) of *X. saxesenii* was also detected (Ulyshen & Hanula, 2009).

Sixteen species found in this study are included in the Red List of the IUCN Europaea (Carpaneto et al., 2015). *Adelocera pygmaea* (Baudi, 1871) (Elateridae) is considered endangered (EN). *Ectamenogonus*

montandoni, *R. serricollis*, *Mycetochara quadrimaculata* (Latreille, 1804) (Coleoptera: Tenebrionidae) and *Propomacrus bimucronatus* (Pallas, 1781) (Coleoptera: Euchiridae) are classified as near threatened (NT). Of these species, 11 are classified as low risk (LC). According to IUCN Mediterranean Red List, *E. montandoni* and *P. bimucronatus* are endangered (EN) and vulnerable (VU), respectively. *Ectamenogonus montandoni* (Jansson & Coskun, 2008; Avcı et al., 2010; Atay et al., 2012) and *P. bimucronatus* (Önuçar & Ulu, 1986; Tezcan & Pehlivan, 2001; Jansson & Coskun, 2008; Göktepe, 2022) were also detected in some saproxylic beetle studies conducted in Türkiye.

The trophic category classification for 57 of the 108 species identified in the study was determined. While some species of Cerambycidae (XY: Xylophagous), Histeridae (PR: Predator), Latridiidae (MY: Mycophagous) were in one category, certain species from, for example, the Curculionidae (MY, SX: Saproxylophagous), Anobiidae (MB: Mycetophagous, SX, XY), Tenebrionidae (MB, SX) are in two or more categories. When examined at the taxon level, 15 species were classified as XY, 13 species as SX, 12 species as MY, 10 species as PR, 6 species as MB and 1 species as MM (Myrmecophilous or melittophagous). *Xyleborinus saxesenii*, with the highest number of individuals found in the study, is in the MY category. *Ectamenogonus montandoni* is also in the PR trophic category, which is categorized as endangered (EN) in the IUCN Mediterranean Red List (Carpaneto et al., 2015).

Conclusion

Understanding biodiversity has a crucial role in the conservation and management of forest areas with a sustainability approach. In particular, creatures living naturally in micro-habitats, such as tree hollows, are indicators of sustainable forest management. When the results of the study and the related literature were examined, the importance of *L. orientalis* forests to biodiversity in Türkiye and even globally becomes clear. Only 15 of the 108 insect species obtained in this study are new to the fauna of Türkiye. There is no doubt that this number will increase with further studies. The protection of this tree species, the distribution area of which is decreasing in Türkiye, will ensure the protection of the biodiversity it contains. However, tourism activities, sweetgum oil production activities, illegal cutting and clearing activities in some areas pose a risk to the future of the species. The main purpose of overall management in sweetgum oil production areas should be the protection of these trees rather than oil production. In these areas, the sustainability of the forest can be ensured by leaving old trees in the field. Moreover, oil production can be continued in these areas. In addition, the presence of old trees is very important for protecting biodiversity and ensuring the continuity of the ecosystem. In this way, it will be possible to transfer this tree species and the biodiversity it supports to future generations.

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Original article (Orijinal araştırma)

Allelopathic effect of *Origanum syriacum* var. *bevanii* (Holmes) (Lamiales: Lamiaceae) and *Rhododendron ponticum* L. (Ericales: Ericaceae) essential oils and extracts on *Meloidogyne incognita* (Kofoid & White, 1919) (Rhabditida: Meloidogynidae)[1]

Origanum syriacum var. *bevanii* (Holmes) (Lamiales: Lamiaceae) ve *Rhododendron ponticum* L. (Ericales: Ericaceae) bitkilerinin uçucu yağ ve ekstraktlarının *Meloidogyne incognita* (Kofoid & White, 1919) (Rhabditida: Meloidogynidae) üzerinde allelopatik etkisi

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Abstract

In this study, the nematicidal activity of essential oils and extracts of *Origanum syriacum* var. *bevanii* (Holmes) (Lamiales: Lamiaceae) and *Rhododendron ponticum* L. (Ericales: Ericaceae) against the second-stage juveniles (J2s) of *Meloidogyne incognita* (Kofoid & White, 1919) (Rhabditida: Meloidogynidae) were evaluated in Petri dish and pot experiments. The essential oils and extracts were applied directly to J2s at concentrations of 5 and 10% (w/v). The mortality of J2s of *M. incognita* was recorded 24, 48, and 72 hours after treatments. The results showed that a 100% mortality rate was obtained from both concentrations (5 and 10%) of *O. syriacum* var. *bevanii* essential oil application. Moreover, the pot experiment showed that *O. syriacum* var. *bevanii* essential oil (5%) (w/v) and *O. syriacum* var. *bevanii* extract (10%) was more effective against the J2s of *M. incognita* on tomato plants. Results were promising in terms of testing the effects of essential oil and extracts obtained from the determined plants in laboratory conditions against *M. incognita*.

Keywords: Antagonistic plants, biopesticides, Root-knot nematodes, susceptibility

Öz

Bu çalışmada, dağ kekiği, *Origanum syriacum* var. *bevanii* (Holmes) (Lamiales: Lamiaceae), ve ormangülü, *Rhododendron ponticum* L. (Ericales: Ericaceae) bitkilerinden elde edilen uçucu yağ ve ekstraktlarının Kök-ur nematodu, *Meloidogyne incognita* (Kofoid & White, 1919) (Rhabditida: Meloidogynidae) ikinci dönem larvaları üzerinde allelopatik etkisini, petri ve saksı denemelerinde belirlemek amaçlanmıştır. Laboratuvar denemelerinde İki farklı dozda dağ kekiği yağı (%5-10), dağ kekiği ekstraktı (%5-10), ormangülü ekstraktı (%5-10) ve bu iki bitkinin karışım ekstraktının *M. incognita* larvaları üzerine doğrudan uygulanması sonrasında 24, 48 ve 72 saat aralıklarla nematodların ölüm oranları hesaplanmıştır. Saksı denemelerinde ise dağ kekiği yağı (%5-10), dağ kekiği ekstraktı (%5-10), ormangülü ekstraktı ve dağ kekiği + orman gülü ekstraktı (%5-10) uygulamalarının bitki boyuna, köklerde urlanma seviyeleri ve yumurta paket sayıları üzerine etkisi değerlendirilmiştir. Çalışmalar sonucunda, laboratuvar denemesinde kekik yağının her iki dozunda da (%5 ve %10) %100 ölüm oranı tespit edilmiştir. Saksı denemelerinde ise %5'lik kekik yağı ve %10'luk kekik ekstraktı diğer uygulamalara göre daha etkili bulunmuştur. Bu çalışma ile dağ kekiği ve ormangülü yağ ve ekstraktlarının *M. incognita* ikinci dönem larvalarına biyolojik etkisi açısından ümitvar sonuçlar elde edilmiştir.

Anahtar sözcükler Antagonistik bitkiler, biyopestisitler, Kök-ur nematodları, duyarlılık

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Introduction

The need for safe food resources, which has increased due to the rapid increase in the world population in recent years, is directly related to the sustainability principle of agricultural production. Thus, countries are searching for innovative solutions by reviewing their agricultural policies. Root-knot nematodes (RKNs), *Meloidogyne* spp. (Rhabditida: Meloidogynidae) are organisms that live as obligate parasites on plants, with a wide distribution and host range worldwide especially in tropical and semi-tropical climate zones causing harmful results in agricultural production areas (Whitehead, 1997). Due to their complex biology, the development of plant roots slows down or is irregular and finally these plants cannot develop healthily and become stunted. Plants with extensive RKNs infestation can dry out completely (Thorne, 1961). It is common that synthetic pesticides are frequently used in the control of RKNs. This situation poses a threat to the environment and human health. Resistance develops in nematodes against pesticides and at the same time, the pesticide residues accumulate on crops and move to underground freshwater in the long term (Moens et al., 2009). For such reasons, the importance given to alternative methods that do not harm nature and can be used instead of common chemical applications has increased in recent years against RKNs. Plants are natural sources containing many active substances within their rich structure, and more than two thousand plants have been shown to have the potential to be used as bio insecticides so far (Ahmed & Grainge, 1988; Prakash & Rao, 1996; Öncüer, 2000). Furthermore, allelochemicals which are produced by plants are secondary metabolites and have an important place since they have direct or indirect effects on inducing pests and harmful organisms (Gürsoy et al., 2013). To date, there are at least 120 allelochemicals according to Fahey et al. (2001), which are known to have antagonistic interactions with bacteria, nematodes, fungi, and herbivores.

The aim of this study was to assess the effectiveness of thyme, *Origanum syriacum* var. *bevanii* (Holmes) (Lamiales: Lamiaceae) essential oil and extracts, and rhododendron, *Rhododendron ponticum* L. (Ericales: Ericaceae) extracts in vitro conditions against second-stage infective juveniles of *Meloidogyne incognita* (Kofoid & White, 1919) (Rhabditida: Meloidogynidae) both in laboratory and pot experiments.

Materials and Methods

Identification and obtaining nematodes pure culture

Root-knot nematodes were obtained from a greenhouse infested RKNs. Samples were collected at 04/07/2022 in Antalya Serik (36º55'34”N, 31º6'17”W), where tomato plants were being cultivated . Once the infested tomato plants were brought to the laboratory, egg masses were collected from infected tomato roots using forceps and were put into incubation for the hatching of second-stage juveniles (J2s). After 48 hours in a modified Baermann funnel, (using 8 cm high, 10-12 cm wide Petri dishes with a sieve inside), the emerging J2s were obtained to be used for plant infestation, with the aim of establishing a pure nematode culture. This process was repeated for 10 days during which the larvae continued to emerge from the egg masses. Initial nematodes were obtained and counted. Ten tomato plants were placed in sterilized sand in a climatic chamber set at 25±1°C, 65±5% relative humidity, and a 16:8 photoperiod, and were infested with 1000 J2s for having a pure culture of *M. incognita*. Pure *M. incognita* populations were obtained after 60 days of the plantation. Nematode-infested roots were washed in water in the laboratory and these roots were cut into 1-3 cm and observed under a light microscope. Female nematodes were once more separated and incubated using the modified Baermann funnel technique to allow J2s emergence over a period of ten days. In order to obtain nematode larvae from RKN-infested tomato plant roots. The sieve on which the egg masses were placed on the filter paper was placed in the Petri dish and was filled with sterile water, and the J2s were allowed to hatch and sink to the bottom in the water, and after 48 hours, after J2s emerged from egg masses, the water in the Petri dish was transferred to the 50 ml tubes. This process was repeated for 10 days during which the J2s continued to emerge from the egg masses. Collected J2s of *M. incognita* were left for six hours to sink to the bottom of the tube. After six hours, the

water was diluted to 15 ml. The collected nematodes were stored in the refrigerator at +4ºC. Nematode counts in 1 ml of water was made to calculate the number of larvae when the nematodes were to be used in the experiments. The nematode count was repeated three times. The average number of nematodes was calculated by multiplying the amount of water by the average number of larvae. For the Petri dish experiment, 400 *M. incognita* J2s were used in each application.

Laboratory experiment

Obtaining plant essential oils and extracts

In the study, the essential oils and extracts of Thyme (*O. syriacum var. bevanii*) and Common rhododendron (*R. ponticum* L.) were applied at two concentrations (%5-10) (w/v). The Thyme and Rhododendron plants were collected from the natural areas of Antalya province, Serik district (37.128695, 30.961959) and morphological identification was made in the Herbology Laboratory of Düzce University. Different parts of the tested plants were taken to prepare their extracts. The chosen parts were stem parts, flowers, and leaves of thyme. These plant origins were washed with distilled water to remove any dust and air-dried in direct sunlight. The dried plant materials were powdered and passed through a 60-mesh sieve. Samples of plant powders were homogenized with a laboratory blender used at 30 g from each plant material in one liter of distilled water for 10 min., and then left in dark glass bottles for 72 hr for tissue disintegration. The extracts were filtered with a Whatman filter paper to get the clear extract. The obtained extracts were dissolved as Thyme and Rhododendron at a rate of 5% and 10% (w/v) and stored in the refrigerator at +4ºC. Nematicidal activities of separate and combined applications of Thyme (*O. syriacum var. bevanii*) and Common rhododendron (*R. ponticum* L.) were tested on J2s of *M. incognita* as follows: OSEO: *O. syriacum* var. *bevanii* essential oil (%5-10) (w/v), OSE: *O. syriacum* var. *bevanii* extract (%5-10) (w/v) RPE: *R. ponticum* extract (%5-10) (w/v), OSE+RPE: *O. syriacum* var. *bevanii* and *R. ponticum* extract (%5-10) (w/v). This study was conductedin Düzce University, Herbology, and Nematology Laboratories in 2022 and 2023.

The trials were established according to the Random Plots Trial Design with 10 characters (8 treatments + 2 controls), with 3 replications. Plant essential oils and prepared plant extracts were adjusted as 10 ml of solution added to 400 *M. incognita* J2s in 1 ml of water placed in glass Petri dishes at room temperature (Oka et al., 2000). For control trials, only pure water was included into Petri dishes with J2s. The laboratory experiment was repeated twice in 2022 and 2023 at Düzce University, Faculty of Agriculture, Department of Plant Protection Laboratory.

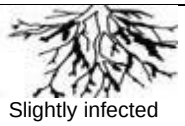
Pot experiment

The *M. incognita* J2s used in bioassays and field trials were obtained from a laboratory-reared *M. incognita* inoculum originally sourced from an infected greenhouse (04/07/2021) in Serik, Antalya, Türkiye (36º55'34”N, 31º6'17”W). The colony was purified and maintained in Düzce University, Faculty of Agriculture, Department of Agricultural Biotechnology at 6±1ºC, 60±80% relative humidity. The pot experiment was repeated twice in 2022 and 2023 at Düzce University, Faculty of Agriculture, Department of Agricultural Biotechnology Laboratory under controlled climatic conditions in the laboratory (at 25±1ºC, 60±80% relative humidity and a photoperiod of 16:8 h light: dark). Essential oil-sand mixture containing 70% sand and 30% potting essential oil was prepared and sterilized in an autoclave at 121ºC for 90 minutes.

For each application, 10 tomato plants were grown in 1-liter pots. Four-week-old BT 236 tomato plants were planted in a controlled climatic condition. Afterwards, each pot was inoculated homogeneously with 1000 *M. incognita* J2s in 30ml of distilled water, at four plots with a distance of 3-4 cm from each other near the root stem. Three replicates were applied for each experiment. After *M. incognita* inoculation to tomato plants, in order to assess the efficiency on the control of *M. incognita*, essential oil, extracts at different concentrations (OSEO: *O. syriacum* var. *bevanii* essential oil (%5-10) (w/v), OSE: *O. syriacum* var. *bevanii* extract (%5-10) (w/v) RPE:

R. ponticum extract (%5-10) (w/v), OSE+RPE: *O. syriacum* var. *bevanii* and *R. ponticum* extract (%5-10) (w/v) were applied to the pots with a volume of 30 ml of solution on the same day and compared with two control (infected and uninfected) applications. The plants were kept in climate chambers for 10 weeks at a constant temperature of 27±3ºC, 70% humidity and 16 hours of light per day. The plants were irrigated on a daily basis. Tomato plants were removed from the pots 10 weeks (70±3 days) after the application of *M. incognita* and plant essential oil and extracts. The roots of the plants were thoroughly washed with water and essential oil debris were removed gently. Egg mass counts were made under a stereo microscope with the aid of a fine needle. Then, the root-knot scale and plant height of each plant were determined according to Feldemesser & Feder (1955), Zeck (1971) and Taylor & Sasser (1978) (Table 1).

Table 1. The infection scale of *Meloidogyne incognita* in the root system (Feldemesser & Feder, 1955; Zeck, 1971)

Root development categories	0	1	2	3	4
Root side section profile					
root development index	No galling	Slightly infected	Reasonably infected	Intensely infected	Extremely infected
gall number index (%)	0	0-25	25-50	50-75	75-100

Counting and evaluation

After the laboratory experiments, live nematodes were counted with the help of an Olympos® light microscope at 24-48-72 hours after the application.

Data analysis

The data were statistically evaluated using the JMP 11 package program of the SAS Institute (SAS, 2013). The results from the laboratory experiments (*M. incognita* death/viability ratios) were subjected to a one-way ANOVA with mean percentages of death at 24-48-72 hours post application. The square root transformation was applied to the calculated median values related to the root-knot index values and the original data were given in tables. Because of the variances were homogeneous, the data obtained from two years were subjected to the analysis of variance and "LSD Multiple Comparison Test" was used for comparison of the means at a 0.05 significance level.

Results and Discussion

Laboratory experiment

As a result of the morphometric and morphological analysis of the species identification, the nematode species was determined as *M. incognita*. In the evaluations made at the 24-hour post-application counts of the laboratory trials, the minimum number of alive *M. incognita* J2s was determined in 10% (w/v) application of *O. syriacum* var. *bevanii* essential oil (Table 2).

Based on the two-year average values of laboratory experiment, it was determined that the J2s mortality rate of *M. incognita* was highest with a 10% concentration of *O. syriacum* var. *bevanii* essential oil (i) application, followed by 5% (w/v) *O. syriacum* var. *bevanii* essential oil (ii), 10% (w/v) *O. syriacum* var. *bevanii* extract (iii), 5% (w/v) *O. syriacum* var. *bevanii* + *R. ponticum* extract (iv), 10% (w/v) *O. syriacum* var. *bevanii* + *R. ponticum* extract (v), 5% (w/v) *O. syriacum* var. *bevanii* extract (vi), 10% (w/v) *R. ponticum* extract (vii), 5% (w/v) *R. ponticum* extract (viii). In the 24-hour count evaluations, the highest number of live larvae was determined in 5% *R. ponticum* extract after the control.

Table 2. Effect of treatments on *Meloidogyne incognita* J2s at laboratory condition (2022-2023 mean)*

Treatments**	% Mortality**					
	24 h		48 h		72 h	
OSEO 5%	100.0±0.1	a	100.0±0.0	a	100.0±0.0	a
OSEO 10%	100.0±0.0	a	100.0±0.0	a	100.0±0.0	a
OSE 5%	66.9±2.8	a	70.1±3.8	b	71.5±6.1	cd
OSE 10%	95.01±1.9	b	99.8±0.1	a	99.7±0.1	a
RPE 5%	25.4±9.2	d	39,4±10.5	d	51.6±9.0	e
RPE 10%	36.4±11.7	c	54,2±1.5	c	64.8±9.3	d
OSE 5% + RPE 5%	95.1±1.6	a	96.0±2.1	a	77.9±8.4	bc
OSE 10% + RPE 10%	72.5±4.4	b	76.1±7.1	b	84.7±2.6	b
F	73,3003		60,9626		50,0427	
P	<,0001		<,0001		<,0001	
DF Total	23		23		23	
DF Error	14		14		14	

* Means separation within columns using LSD comparison test at α=0.05, Data given ± are STDEV (standard deviation);
** Abbreviations: OSEO, *O. syriacum* var. *bevanii* essential oil; OSE, *O. syriacum var*. *bevanii* extract; RPE, *R. ponticum* extract.

In the evaluations made at the 48-hour counts of the laboratory experiment, the minimum number of alive J2s among the applications was *O. syriacum* var. *bevanii* essential oil at 10% (w/v) and 5% (w/v) *O. syriacum* var. *bevanii* essential oil with 100% mortality. At the trial counts of 48-hours post application, the highest efficacy was determined as follows: 10% (w/v) *O. syriacum* var. *bevanii* essential oil (i), 5% (w/v) *O. syriacum* var. *bevanii* essential oil (ii), 10% (w/v) *O. syriacum* var. *bevanii* extract (iii), 5% (w/v) *O. syriacum* var. *bevanii* + *R. ponticum* extract (iv), 10% (w/v) *O. syriacum* var. *bevanii* + *R. ponticum* extract (v), 5% (w/v) *O. syriacum* var. *bevanii* extract (vi), 10% (w/v) *R. ponticum* extract (vii) and 5% (w/v) *R. ponticum* extract (viii).

In the 48-hour counting evaluations, the highest number of alive J2s was determined in the 5% (w/v) *R. ponticum* extract after the control. In the evaluations made at the 72-hour post application, the minimum number of alive J2s among all applications was 10% (w/v) *O. syriacum* var. *bevanii* essential oil and 5% (w/v) *O. syriacum* var. *bevanii* essential oil with 100% mortality. Mortality levels were detected respectively as follows; 10% (w/v) *O. syriacum* var. *bevanii* essential oil (i), 5% (w/v) *O. syriacum* var. *bevanii* essential oil (ii), 10% (w/v) *O. syriacum* var. *bevanii* extract (iii), 10% (w/v) *O. syriacum* var. *bevanii* + *R. ponticum* extract (iv), 5% (w/v) *O. syriacum* var. *bevanii* + *R. ponticum* extract (v), 5% (w/v) *O. syriacum* var. *bevanii* extract (vi), 10% (w/v) *R. ponticum* extract (vii) and 5% (w/v) *R. ponticum* extract (viii). In the 72-hour counting evaluations, the highest number of alive J2s after the control was determined in the 5% (w/v) *R. ponticum* extract.

Pot experiment

Three characters were evaluated for each plant which were determined as follows: (i) Plant height (cm), (ii) egg package index (number of *M. incognita* eggs found on each plant), and (iii) gall number index which refers to the severity level of the infection and gall occurrence of each tomato plant. In the evaluations of plant height, egg package index, and gall number index of the pot experiment, *O. syriacum* var. *bevanii* essential oil (OSEO) (5%) and (OSEO) (10%) were statistically found to be significantly different than other applications (OSEO 5%: Plant height: 56.6±2.3 cm Egg package index: 0.51±0.56; Gall number index: 0.08±0.14), (OSEO 10%: Plant height: 53.61±4.2; Egg package index: 0.43±0.18; Gall number index: 0.08±0.14) Also, *O. syriacum* var. *bevanii* extract (OSE) of 10% was found to be close to uninfected control (OSE 10%: Plant height: 56.78±1.2; Egg package index: 2.0±0.59; Gall number index: 0.33±0.29). These

three applications of *O. syriacum* var. *bevanii* essential oil and extract were found to be the most effective applications against *M. incognita* J2s on tomato plants followed by: OSE 5%: Plant height: 54.38±1.0; Egg package index: 1.8±0.78; Gall number index: 0.33±0.29; RPE 10%: Plant height: 51.0±2.5; Egg package index: 3.3±1.03; Gall number index: 1±0.0; OSE 5% + RPE 5%: Plant height: 50.2±3.7; Egg package index: 3.65±0.95; Gall number index: 0.58±0.14; RPE 5%: Plant height: 47.31±6.9; Egg package index: 3.9±0.83b; Gall number index: 0.75±0.43; OSE 10% + RPE 10%: Plant height: 46.9±1.3; Egg package index: 3.3±0.25; Gall number index: 0.83±0.29) (Table 3-4).

Table 3. Plant height (cm), egg package index and gall number index ratio (2022-2023 mean) (Feldemesser & Feder, 1955; Zeck, 1971)

Treatments**	Plant height (cm)	Egg package index	Gall number index
OSEO 5%	56.60±2.30 ab	0.51±0.56 cd	0.08±0.14 de
OSE 5%	54.38±1.00 bc	1.80±0.78 bc	0.33±0.29 cde
RPE 5%	47.31±6.90 de	3.90±0.83 b	0.75±0.43 abc
OSE 5% + RPE 5%	50.20±3.70 cd	3.65±0.95 b	0.58±0.14 bcd
OSEO 10%	53.61±4.20 bc	0.43±0.18 cd	0.08±0.14 de
OSE 10%	56.78±1.20 ab	2.0±0.59 b	0.33±0.29 cde
RPE 10%	51.00±2.50 cd	3.30±1.03 b	1.00±0.00 ab
OSE 10% + RPE 10%	46.90±1.30 de	3.30±0.25 b	0.83±0.29 ab
Control (+)	43.30±2.00 e	6.18±1.06 a	1.25±0.25 a
Control (-)	59.20±1.30 a	0.00±0.00 d	0.00±0.00 e
F	8,3932	2,9131	2,9131
P	<,0001	0,0020	0,0020
DF Total	59	59	59
DF Error	38	38	38

* Means separation within columns using LSD comparison test at α=0.05, Data given ± are STDEV (standard deviation);
** Abbreviations: OSEO, *O. syriacum* var. *bevanii* essential oil; OSE, *O. syriacum* var. *bevanii* extract; RPE, *R. ponticum* extract.

Table 4. Plant height (cm), egg package index and gall number index (2022 and 2023) (Feldemesser & Feder, 1955; Zeck, 1971)

Treatments**	Plant height (cm)		Egg package index		Gall number index	
	2022	2023	2022	2023	2022	2023
OSEO 5%	59.97 ab*	53.23 a*	0.47 cd*	0.57 ef*	0.17 c*	0.00 e*
OSE 5%	58.90 ab*	49.87 abc*	1.97 bcd*	1.63 bc	0.33 bc*	0.33 cde*
RPE 5%	48.93 cd	45.70 cde	3.47 b	4.37 ab	0.67 abc	0.83 abc
OSE 5% + RPE 5%	55.63 bc	44.90 cde	4.03 b	3.27 bc	0.67 abc	0.5 de
OSEO 10%	55.50 bc	51.60 ab*	0.43 cd*	0.43 def*	0.00 c*	0.17 de*
OSE 10%	61.00 ab*	52.57 ab	2.90 b	1.27 cde	0.50 bc*	0.17 de*
RPE 10%	54.30 bc	47.83 bcd	2.50 bc	4.10 abc	1.00 ab	1.00 ab
OSE 10% + RPE 10%	51.00 cd	42.80 de	3.87 b	2.80 abc	1.00 ab	0.67 abcd
Control (+)	44.67 d	41.93 e	6.40 a	5.97 a	1.33 a	1.17 a
Control (-)	63.53 a	55.00 a	0.00 d	0.00 f	0.00 c	0.00 e
F	5,1474	7,1204	5,4811	7,3045	3,0949	4,4533
P	0,0011	0,0001	0,0008	0,0001	0,0164	0,0026
DF Total	29	29	29	29	29	29
DF Error	19	19	19	19	19	19

* Means separation within columns using LSD comparison test at α=0.05;
** Abbreviations: OSEO, *O. syriacum* var. *bevanii* essential oil; OSE, *O. syriacum* var. *bevanii* extract; RPE, *R. ponticum* extract.

The unconscious use of pesticides and non-compliance with the rules in practice destroy the ecological balance. At the same time, intensive use of pesticides causes the extinction of many beneficial species. RKNs are among the most important plant pests in agriculture worldwide.

Chemical origin plant protection products are used for controlling RKNs, but studies on alternative control methods have gained momentum in recent years due to the negative effects of these nematicides, most of which have systemic effects, on the environment and human health. In this process, it has been revealed that biochemical compounds obtained from plants can be used in the control of RKNs.

In this context, nematicides of plant origin can be investigated more intensively and their application areas can be expanded and they have a high potential to be used as a general control agent in suppressing the population of not only RKNs but also other plant parasitic nematodes in the essential oil. Thanks to the data obtained from these studies, an environmentally friendly control strategy against nematodes can be followed.

As a result of the laboratory trials conducted in this study, *O. syriacum* var. *bevanii* essential oil and *O. syriacum* var. *bevanii* extracts were the most effective applications with a 100% mortality rate in both concentrations (5-10%) (w/v) applied to the J2s *M. incognita* larvae. Some thyme species (*T. capitatus, O. vulgare, O. dictamnus, O. majorana*) have antagonistic relation with *Fusarium solani*, along with essential oils of lavender, rosemary, sage, and watermelon (Daferera et al., 2003). The extract obtained from the dried mint plant showed a significant nematicide effect against *M. incognita* J2s (Caboni et al., 2013).

With respect to the potential of using some plant extracts in the control of RKNs, Walker & Melin (1996) found that when the extracts of some mint cultivars were used, this caused significant reductions in root gall formation despite high nematode infestation, and that these mint cultivars were host to *M. incognita* and *M. arenaria*. In addition, they found that after growing the thyme plant in nematode-infested essential oil for a period of 12 weeks, the rate of galling was reduced by 90% in sensitive tomato roots planted, compared to tomatoes grown in the control group.

When the results from the previous studies and the current study are evaluated together, it is thought that the nematode population can be suppressed as a result of the thyme plant being grown in nematode-infested areas before the cultivated plant to be produced and mixed with the essential oil as green manure. The plant essential oils and extracts, whose effectiveness was evaluated in this study, are widely distributed in our country and can be easily obtained.

This study has yielded promising results with the potential to be an alternative to chemical nematicides used in the control of RKNs as more economical and environmentally friendly applications. It is essential to conduct comprehensive studies including pot and field trials in the future regarding the research subject.

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Original article (Orijinal araştırma)

Assessing residues of some insecticides during household processing of lemon[1]

Limonun evde işlenmesi sırasında bazı insektisitlerin kalıntılarının değerlendirilmesi

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Abstract

The goal of this study was to assess the residues of some insecticides (abamectin, buprofezin, etoxazole) applied on the lemon fruits during its cultivation and to investigate the consequence of household processing such as peeling, jam production, freezing and storage on the residues. A multi-residual analysis method based on QuEChERS (Quick, Easy, Cheap, Effective, Rugged, and Safe) procedure and liquid chromatography coupled with triple quadrupole mass spectrometry was used. Mean recovery (measure of trueness; 70-120%), precision (as repeatability and interim precision relative standard deviation <20%) and limit of quantification (0.01 mg/kg < MRLs) were in accordance with the criteria set in the international guideline. Lemon samples were purchased from Bursa markets in April 2018. The experimental studies and statistical evaluations were conducted at Bursa University Agriculture Faculty (Bursa -Türkiye) between 5 May 2018-30 July 2022. The results revealed that pesticide residues mostly dispersed on the peel, therefore, peeling step decreased the residue level by 90-100% in the pulp of the fruit. Fruit juice and jam production operations decreased the residue level by 87- 100%. Processing factors were less than 1 for fruit juice and jam processing, on the other hand, it was greater than 1 for the separation, grating, freezing and storage of the peels.

Keywords: Household food processing, insecticide residue, lemon, processing factor

Öz

Bu çalışmanın amacı, limon meyvelerinin yetiştirilmesi sırasında üzerlerine uygulanan bazı insektisit (abamectin, buprofezin ve etoxazole) kalıntılarının değerlendirilmesi ve soyma, reçel üretimi, dondurma ve depolama gibi evde yapılan işlemlerinin kalıntılar üzerindeki etkisinin araştırılmasıdır. QuEChERS (Hızlı, Kolay, Ucuz, Verimli, Sağlam ve Güvenli) prosedürüne ve üçlü kuadropol sıvı kromatografi kütle spektrometresine dayalı bir çoklu kalıntı analiz yöntemi kullanılmıştır. Ortalama geri kazanım (gerçekliğin bir ölçüsü olarak; %70-120), kesinlik (tekrarlanabilirlik ve ara kesinlik bağıl standart sapma <%20 olarak) ve yöntemin ölçüm limiti (0.01 mg/kg < MRLs) uluslararası kılavuzda belirlenen kriterlere uygun olarak elde edilmiştir. Nisan 2018'de Bursa marketlerinden limon örnekleri satın alınmıştır. Deneysel çalışmalar ve istatiksel değerlendirmeler 5 Mayıs 2018-30 Temmuz 2022 tarihleri arasında Bursa Uludağ Üniversitesi Ziraat Fakültesi'nde (Bursa-Türkiye) gerçekleştirilmiştir. Elde edilen sonuçlara göre, pestisit kalıntısının çoğunlukla meyvenin kabukları üzerinde dağıldığı, dolayısıyla soyma aşamasının meyvenin posasındaki kalıntı miktarını %90-100 oranında azalttığı gözlenmiştir. Meyve suyu ve reçel üretimini içeren işleme adımları nihai üründe kalıntı seviyesini %87-100 oranında azaltmıştır. Sonuç olarak işleme faktörleri (P_f), meyve suyu ve reçel işleme için 1'den küçük olarak, meyve kabuklarının ayrılması, rendelenmesi, dondurulması ve saklanması için ise 1'den büyük olarak elde edilmiştir.

Anahtar sözcükler: Evsel gıda işlemleri, insektisit kalıntısı, limon, işleme faktörü

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Introduction

Citrus fruits, known for their extensive nutritional value and health benefits, are grown in more than 80 countries and are among the most popular fruits consumed worldwide (Cicero et al., 2015; Calvaruso et al., 2020). The lemon fruit is the third most produced citrus fruit after oranges and tangerines (Gonzalez-Molina et al., 2010). Lemon production in Turkey was reported as 1.4 million tons in 2022. Due to the increased consumption demand, it is aimed to reach 9.5 million tons in lemon production with an increase of 4% in Turkey, America and Mexico (USDA, 2022). Lemon has health-promoting effects due to its high content of flavonoids, vitamin C, citric acid, minerals, dietary fiber, essential oils and carotenoids (Anagnostopoulou et al., 2006; Gonzalez-Molina et al., 2009; Gonzalez-Molina et al., 2010; Guimaraes et al., 2010; Lorente et al., 2014; Hassan et al., 2022). Therefore, it constitutes a very important place in a daily diet, having a role in the prevention of obesity, diabetes, cardiovascular diseases, and some of the cancer types and lowering blood lipid (Gonzalez-Molina et al., 2009). Lemon is an extremely juicy and aromatic fruit and is popular with its color and flavor. It has smooth peels with medium thickness and each fruit contains seeds up to nine (Kafa, 2015). Lemon are consumed as fresh fruit, moreover it can be processed into jam, marmalade, fruit juice and frozen form for household or industrial applications (Uysal & Polatöz, 2017; Ayyıldız, 2018). In addition, it is known that lemon is commonly used in salad dressings and pickles as a flavor enhancer and preservatives.

Various pests such as thrips, mites and aphids can attack citrus trees during the fruit growth and development (Li et al., 2020). Like other citrus, the lemon crop is prone to pests so pesticides must be applied at various stages of agricultural production (Ortelli et al., 2005; Kariathi et al., 2016; Carvalho, 2017; Elgueta et al., 2017; Rodrigues et al., 2019). Insecticides and acaricides are common type of pesticides used for citrus (Li et al., 2022). Abamectin, buprofezin and etoxazole are common active compounds of different type of commercial formulations used for citrus production as insecticides for curing pests including passer citrus rust mite *Phyllocoptruta oleivora* (Ashmead, 1879) (Acari: Eriophyidae), citrus leaf miner *Phyllocnistis citrella* (Stainton, 1856) (Lepidoptera: Gracillariidae), citrus whitefly *Dialeurodes citri* (Ashmead, 1885) (Hemiptera: Aleyrodidae), oriental yellow scale *Aonidiella citrina* (Craw, 1890) (Hemiptera: Diaspididae), citrus red scale *Aonidiella aurantii* (Maskell, 1879) (Hemiptera: Diaspididae), and citrus red mite *Panonychus citri* (McGregor, 1916) (Acari: Tetranychidae) (Anonymous, 2023a).

The application of pesticides throughout the agricultural production in the field or at the post-harvest stage dramatically increase the yield of crops. However, the misuse of pesticides can result in excess amount of residue in or on the crops which can cause health problems to the consumer and contaminates environment (Rodrigues et al., 2019; Philippe et al., 2021). Moreover, raw agricultural crops (RAC) are likely to contain pesticide residues above the maximum residue level (MRL) set at the national and international regulations in case they are harvested and consumed before the harvest period. The majority of RACs are processed before consumption (OECD, 2008). Concentration of pesticide residues in foods can be decreased by simple processing techniques such as washing, peeling, juicing, boiling, drying, fermentation or cooking (Shabeer et al., 2015; Lozowicka et al., 2016; Han et al., 2016; Acoglu et al., 2018; Catak et al., 2020; Maden & Yıldırım Kumral, 2020; Polat & Tiryaki, 2020; Yildirim Kumral et al., 2020; Acoğlu & Yolci Omeroglu, 2021; Duman et al., 2021; Balkan & Yılmaz, 2022; Polat 2021; Tiryaki & Polat, 2023; Luyinda & Yildirim-Kumral, 2023). On the other hand, toxic by-products or metabolites may also be formed at some specific food processing conditions (Han et al., 2013, 2016). The processing factor (P_f) is the ratio of the residue levels in processed products to residue level in raw agricultural crops (OECD, 2008). P_f is the main value that represents processing efficiency on the pesticide residue level. Physicochemical properties of pesticides (solubility in water, log Po/w, etc.) and application time can explain the differences in processing factor (Bonnechère et al., 2012; Scholz et al., 2017). P_f values should be taken into account for compliance of processed products (Anonymous, 2016).

There are some reported studies in literature dedicated to analysis of pesticide residue level in the lemon fruit and lemon products. Based on those studies, registered insecticides including abamectin and buprofezin and non-registered insecticide residues were reported in lemon fruits (Andrascikova & Hrouzkova, 2013; Bakirci et al., 2014; Cicero et al., 2015; Dincay & Civelek, 2017; Besil et al., 2019; Chen et al., 2021; Aslantas et al., 2023; Karaagaçlı, 2023). Moreover, the number of Rapid Alert System for Food and Feed (RASFF) notifications in 2021 for Turkey which was the most reported origin, increased from 191 to 361. Those numbers were mainly due to non-compliances for citrus fruits including lemons. Therefore, except for grapefruits, mandatory checking at the border was increased by 20% in October 2021 (EU, 2021). On the other hand, studies on the effect of household or industrial processing of lemon on pesticide residue levels are limited (Vass et al., 2015; M'hiri et al., 2018; Kowalska et al., 2022).

Pesticides having various modes of action can act in different ways after contact with the agricultural crops. Therefore, the fate of the residues depends on the physicochemical properties of the active ingredients in addition to the type of the matrix. Accordingly, P_f values for each combination should be determined separately (Han et al., 2016; Ma et al., 2019). Pesticides having systemic effect are diffused through leaves, stems or roots and are then moved within the plant by its circulatory system. Contact type of pesticides are directly applied to the outer surfaces of plants (Rodrigues et al., 2017, 2019). Therefore, the behavior of pesticides is associated not only with the processing methods, but also with their mode of action, application time, climate during plantation and physicochemical properties of the product (Lozowicka et al., 2016). The legal processing factor data base in Turkey does not provide any P_f values for abamectin and etoxazole residues in lemon products. On the other hand, the list covers only the processing factor of buprofezin for lemon juice as 0.58 (Anonymous, 2023b). To the best of our knowledge, this research was the first to determine the processing factors of those pesticides for lemon products.

The goal of this study was to assess the residues of some insecticides (abamectin, buprofezin and etoxazole) applied on the lemon fruits during cultivation and to investigate the effect of common household processing including peeling, heat treatments (blanching and boiling during jam production), freezing and storage on the fate of the residue.

Materials and Methods

Chemicals and solutions

QuEChERS extraction kits including 6000 mg anhydrous magnesium sulfate ($MgSO_4$) and 1500 mg anhydrous sodium acetate and QuEChERS clean-up kits consisting of 1200 mg $MgSO_4$ and 400 mg primary and secondary amines (PSA, 40 µm particle size) were provided from Chromabond (Germany). Solvents (acetonitrile, glacial acetic acid, methanol, formic acid), which are proper for pesticide residue analysis, were purchased from Merck (Germany) to be used in the study. Neat standards of abamectin, buprofezin and etoxazole (purity >99%) certified for pesticide residue analysis were purchased from Dr. Ehrenstorfer (Germany). Stock solutions of 1 mg/mL in acetonitrile containing 1% acetic acid were used to prepare working solutions at a concentration ranging between 20 and 800 µg /L through series of dilutions. Seven different levels of matrix matched calibration standards covering the concentrations of the target analytes in sample were diluted from working standards. Stock solutions were stored at deep-freezer (−18°C) in sealed brown glass bottles for 1 year. The other solutions were kept at 4°C for maximum 1 week. Deionized distilled water was used in the analysis (National Q purification system, Merck, Germany).

Equipment

A LC-MS-MS (Agilent 1260 II model LC-MS-MS-6470A) equipped with a 2.1 mm × 150 mm x 2.7 µm (Agilent Poroshell C18) analytical column was used for the analysis. At mass detector, heat block temperature, drying gas temperature, spray gas in ion source (N_2), drying gas in ion source (N_2), gas flow, nebulizer gas, and capillary voltage are 325°C, 400°C, 10 L/min, 11 L/min, 14 L/min, 40 psi, and 3000 V, respectively.

Positive electron spray ionization (ESI) mode was used for each pesticide. The mobile phase with 0.3 min/mL flow rate consisted of 5 mM ammonium acetate (A) and methanol in 0.1% formic acid water (B). The gradient program started with 80% A and 20% B for 0.5 min, increased linearly to 95% B in 10 minutes, hold at 95% B for 3 min. After the 13-min run time, 3-min post run followed using the initial 20% of B. The flow rate was 0.5 mL/min and the injection volume was 1 µL.

The other main equipment used in the study were homogenizator (Recht GM 200, Haan, Germany), refrigerated centrifuge (Sigma 2-16P, Osterode, Germany) top-loading balances (Shimadzu ATX224, Japan), polytetrafluoroethylene (PTFE) syringe (5mL), PTFE filter with 0.45 µm diameter, Eppendorf automatic pipettes (10, 100, 1000 µL) and LC-MS-MS vials (1.5 mL).

Sample

Approximately 30-40 kg of the lemon samples (*Citrus lamas*) were obtained from a market in Bursa in April 2018. The experimental studies and statistical evaluations were conducted at Bursa Uludağ University Agriculture Faculty (Bursa-Türkiye) between 5 May 2018 and 30 July 2022.

The samples were stored at 5-7°C and 90-95% relative humidity conditions until the further analysis. Since the mass of each lemon unit in the bulk sample ranged between 156 g and 185 g, laboratory samples not less than 1 kg and covering at least 10 units were taken from the bulk sample to comply with the criteria set in the legal legislation (EC, 2002). Except three laboratory samples separated as “non-treated control sample (C)” from the bulk sample, all laboratory samples were exposed to pesticide treatment step as explained follows.

Pesticide treatment

Active ingredients for lemon fruits were selected based on their popularity on the agricultural farming applications and their residue occurrence frequency (Andrascikova & Hrouzkova, 2013; Bakirci et al., 2014; Cicero et al., 2015; Dincay & Civelek, 2017; Besil et al., 2019; Chen et al., 2021; EU, 2021; Aslantas et al., 2023; Karaağaçlı, 2023). The commercial formulations of abamectin, buprofezin and etoxazole were selected as Asmition (18 g/L, emulsified concentrate), Korfezin (400 g/L, suspension concentrate) and Novamite (110 g/L, suspension concentrate), respectively and purchased from a local market. For calculation of processing factors, the important criteria include to have detectable level of the residues in RAC, therefore it is allowed to apply plant protection products more than the recommended dose and the RACs can be harvested before the harvest period (OECD, 2008). Based on the preliminary studies approximately one to four times of the recommended dose of the formulations were prepared (Acoglu & Yolci Omeroglu, 2021; Yolci Omeroglu et al., 2022). The laboratory samples were dipped into homogeneous solution of the formulations for 30 minutes to ensure a homogeneous distribution within and between samples and to obtain detectable residue level in the samples (Hassan et al., 2022). Accordingly, treated samples were left under sun light for 3-4 hours on polypropylene sheets allowing drying of fruit outer surface. Samples were kept at +4°C for 1 day till the further step.

Household processing

After treating samples with commercial formulations, three laboratory samples were kept as control samples (TC) without exposing any household processing (Figure 1). The experimental details were shown in Figure 2 and explained as follows. Each processing steps were repeated three times with three different treated laboratory samples. Prior to processing, each fruit unit in the laboratory samples were gently washed under tap water for 2-3 minutes.

Processing into lemon peels (LP) and pulp (LPu):

The peels were removed from the fruits with a knife. It has been determined that the mass of peel to pulp ranged between 26% and 32% in lemons (Figure 1b, c).

Processing into lemon juice (LJ):

Lemon samples were gently divided into two equal parts with a kitchen knife and lemon juice was produced by a kitchen processor (Arzum, Turkey). The mean pH of the juices was determined as 2.2±0.05 (Mettler Toledo Seven compact pH/Ion pHmeter, Canada) (Figure 1d).

Processing into lemon zest (LZ) and storage at frozen conditions:

Lemon peels were zested and stored at -20°C for three months. Analytical samples were taken monthly throughout the storage period (Figure 1f).

The lemon peels, pulps, juice and zest were stored in polypropylene sample vessels at -20°C till further analysis.

Processing into lemon jam (LJ):

The recipe described by Yolci Omeroglu et al. (2022) was applied for jam production. The average pH of the jams obtained was 3.45 ±0.06 and the water-soluble dry matter (Brix) was 72.65±0.64 g/100 g (RA-500 Model Kyoto Electronics Manufacturing Co. Ltd., Japan) (Figure 1e). Lemon jam was stored at room temperature till further analysis.

Figure 1. Household processing of lemon; a) Pesticide treatment, b) Lemon peel (LP), c) Lemon pulp (LPu), d) Lemon juice (LJ), e) Lemon jam (LJ), f) Lemon zest (LZ).

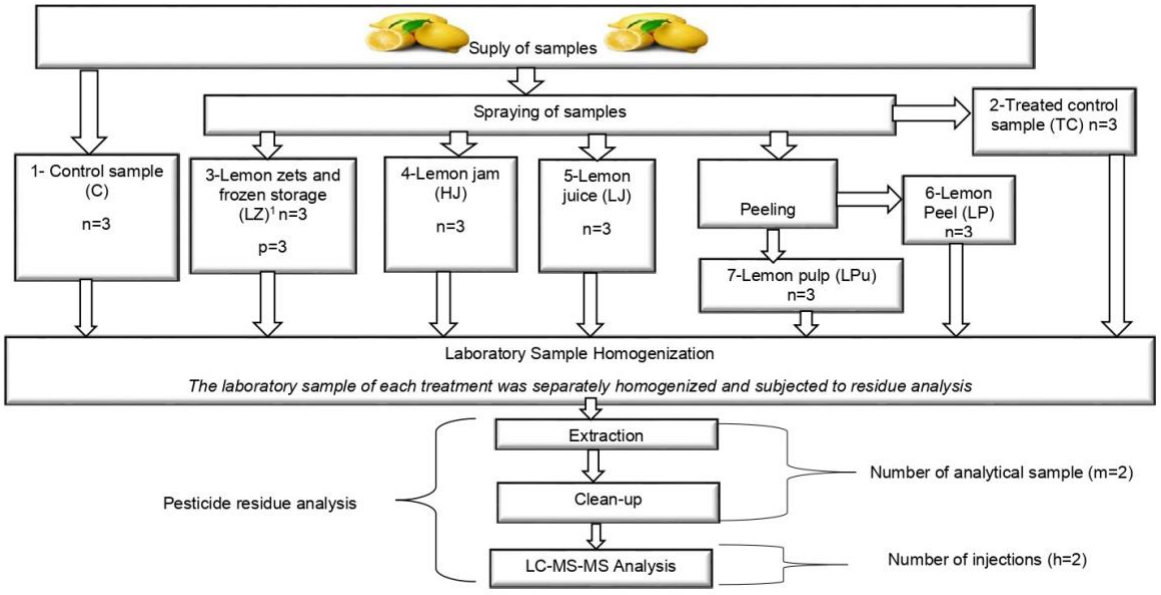

Figure 2. Experimental pattern ("n" represents the replicate number of the processing and the number of the laboratory samples"; "p" represents number of storage period" ([1] Samples were taken each month during three months of frozen storage period).

Pesticide residue analysis

Each laboratory samples were handled separately throughout the pesticide residue analysis explained below.

Lemon jams (LJ) were homogenized thoroughly with a food chopper (RechtGM 200, Haan, Germany). Lemon pulp (LPu), lemon peel (LP) and control samples (C, TC) were homogenized with the chopper till obtaining particle size of 2-3 mm. Lemon juice and lemon zest did not go through a sample processing step. Analytical samples taken from the laboratory sample were kept in PTFE sample vessels at -20°C for till extraction and cleanup step as explained in Figure 2.

Pesticide residue analysis including extraction, clean-up and LC-MS-MS steps was based on a validated standard multi-residue method, namely QuEChERS (AOAC, 2007). The details of the method were provided in Figure 2 and Figure 3. The information on LC-MS-MS identifications are given in Table 1.

Table 1. LC-MS-MS identification details

Analyte	Molecular formula	Mode of Action	MRL (mg/kg)	LOQ[a] (mg/kg)	Retention time (min)	Precursor ion (m/z)	Product ions (m/z)	Cone Voltage (V)	Collusion Energy (V)
Abamectin	$C_{18}H_{72}O_{14}$	Semi-Systemic	0.04	0.01	12.822	895.9	751.4	100	-48
						895.9	327.2		-8
Buprofezin	$C_{16}H_{23}N_3OS$	Contact	0.01	0.01	11.815	306.2	201.1	150	-6
						306.2	116.1		-9
Etoxazole	$C_{21}H_{23}F_2NO_2$	Contact	0.1	0.01	12.147	360.3	141.1	140	-15
						360.3	340.2		-30

[a] LOQ represents limit of quantification.

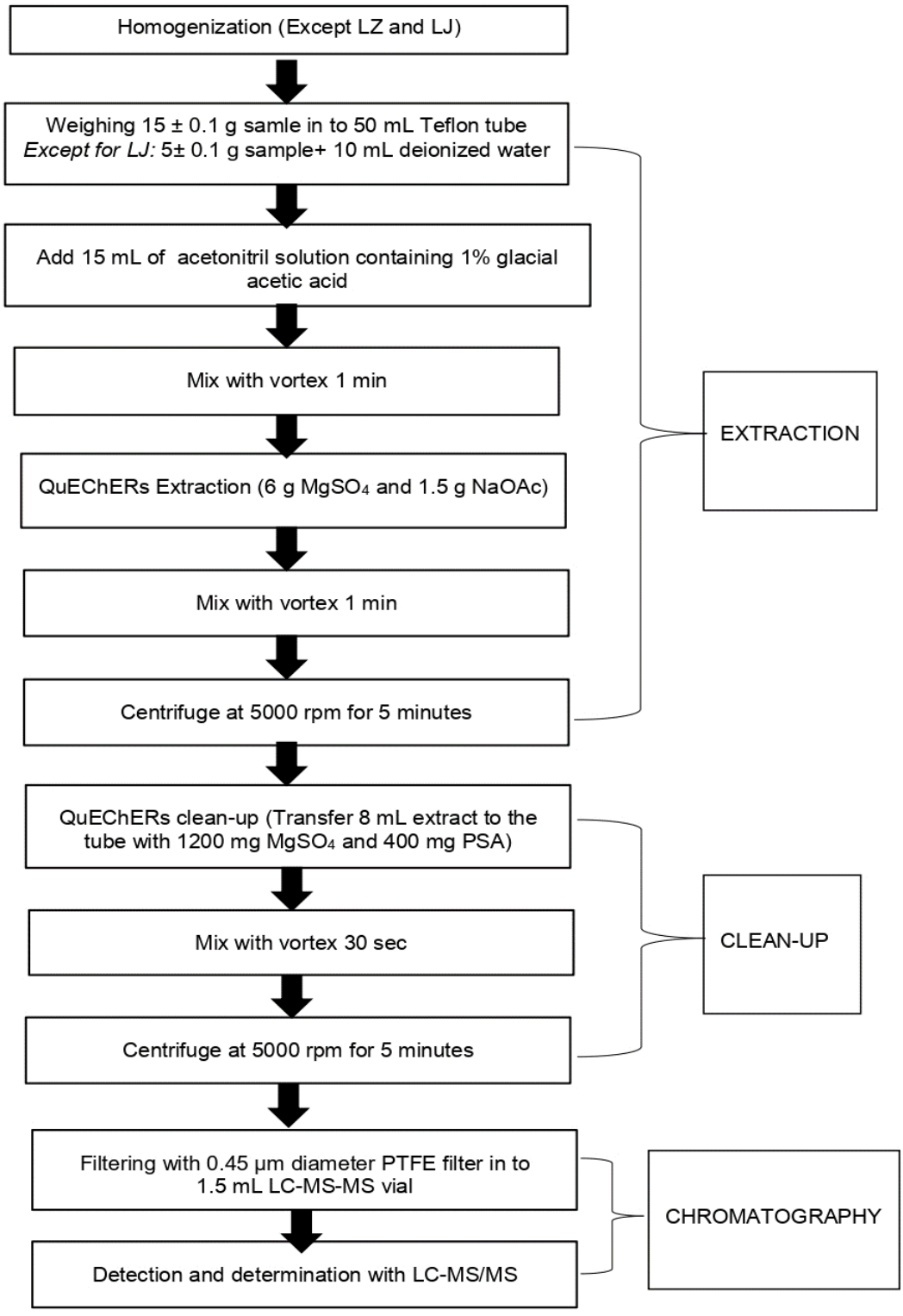

Figure 3. Pesticide residue analysis method based on QuEChERS extraction (AOAC, 2007).

Method verification study was conducted in our laboratory prior to application of the analytical method for the analysis of the sample, and quality control studies during each batch of analysis were performed according to principles recommended by European SANTE/11312/2021 Guidance Document (SANTE, 2021) and EURACHEM guidelines (EURACHEM, 2012, 2014).

Processing factor (P_F)

The processing factor (P_f) is the ratio of the pesticide amount in the processed product (PP) to raw agricultural crops (RAC) (OECD, 2008; Claeys et al., 2011; Scholz et al., 2017). A factor less than or greater than 1 indicates decrease or concentration, respectively. The equation of the processing factor is given in equation 1.

$$P_F = \frac{PP}{RAC} \qquad (1)$$

Where PP refers to the residue level in the processed lemon samples (GP, HJ, LJ, LP, and LPu). RAC refers to residue level in raw agricultural crops (TC). PP in Equation 1 was replaced with LOQ of the method if its nominal value was lower than LOQ. Accordingly, P_f value was expressed with an asterisk "<".

Experimental design and statistical analysis

Experimental design was illustrated at Figure 2. Each process was repeated three times with three different laboratory samples (n=3). Two analytical portions from each laboratory sample was taken for further pesticide residue analyses (m=2). Subsequently, from each analytical portion's duplicate measurements with LC-MS-MS (h=2) were performed. At Table 2 and Table 3, results were expressed as mean ± standard deviation (n=3). One-way analysis of variance (ANOVA) followed by a Tukey post hoc test was used to compare significance differences among household process in terms of pesticide residue levels and processing factors. The SPSS software (version 20.0; SPSS Inc., Chicago, IL, USA) was used for statistical analysis, and $p < 0.05$ was considered as statistically significant.

Results and Discussion

Method verification and quality control studies

Prior to application of a validated method into a routine analysis laboratory, method verification study should be performed to provide evidence that the validated method can be applied by the laboratory. In this context, the reliability of the method was successfully proved at our laboratory conditions and reported previously by Yolci Omeroglu et al. (2022) for orange matrix. Based on the results, mean recovery (as a measure of trueness; 70-120%), precision (as repeatability and interim precision relative standard deviation-RSD_r and RSD_{wR} <20%) and LOQ of the method (0.01 mg/kg< MRL) were in accordance with the criteria set European SANTE/11312/2021 Guidance Document (SANTE, 2021). Moreover, measurement uncertainty complied with the maximum default relative expanded measurement uncertainty set as 50%. To specify the linearity of the method and to determine the residue level in each of the sample, seven level matrix -matched calibration curve covering the concentrations of sample ranging between 10 µg/kg and 1500 µg/kg was constructed at each batch of analysis. Matrix matched calibration curve was prepared from the extract of control lemon samples (C) with a residue level lower than LOQ of the method. A weighted linear calibration function (R^2) > 0.9990) was obtained to determine the concentrations of the residues in the samples as µg/kg (Yolci Omeroglu et al., 2018).

Since both orange and lemon fruits are typical representative commodities for citrus fruits which are categorized under the commodity group with high acid content and high water content, the method verification study reported previously by Yolci Omeroglu et al. (2022) for orange matrix was considered as appropriate for lemon matrix (SANTE, 2021). On the other hand, during routine analysis of each of analytical batch, quality control studies including deviation of calculated concentration, recovery and duplicate samples were also conducted. Deviation of calculated concentration of the calibration standards by the calibration function from the true concentrations was calculated at each batch of the analysis for quality

control purposes. It changed between -7.30% and 19.87%, which complied with the range (±20) specified at the SANTE/11312/2021 Guidance Document (SANTE, 2021). Moreover, recoveries of all analytes within the scope of the study measured at each batch of analysis were determined by spiking the blank lemon samples at LOQ level of the method. Individual recoveries ranged between 83% and 103% (n=7) and were compatible with the practical default range of 60-140% stated in the guideline (SANTE, 2021). The difference between the duplicate measurements of each analytical sample were less than the repeatability limit (r) at 95% confidence level (EURACHEM, 2014), which was based on the average RSD_r value (3%, n=72) obtained during method verification study.

Assessing residue of insecticides during household processing of lemon

Average concentration of abamectin, buprofezin, and etoxazole residues in pesticide treated control lemon samples (TC) were obtained as 0.011, 0.246, and 0.246 mg/kg, respectively. After processing of TC samples into LP, LPu, LJ, LZ, the levels of aforementioned pesticide residues changed from <LOQ to 0.043 mg/kg, 0.020 to 1.060 mg/kg, <LOQ to 0.593 mg/kg, respectively. Based on the statistical results shown in Table 2, different type of household processing changed the residue levels significantly ($p < 0.05$) from the levels found in TC samples.

Processing into lemon peels (LP) and pulp (LPu):

Peeling process increased the residue concentrations in lemon peels significantly ($p < 0.05$) compared to control samples (TC). The increase in concentration changed from 3.3 to 8.5 fold. Pesticide residues were reduced as a result of the separation of the lemon peels from the fruit pulp. Peeling process eliminated totally abamectin residue in the pulp, while it reduced buprofezin concentration by 90%. In line with our findings, a study reported by Li et al. (2012) revealed that residues of imidacloprid, carbendazim, abamectin and cypermethrin in orange decreased by peeling process. Liu et al. (2016) concluded that spirotetramate, and its metabolites B-enol, B-glu and B-keto accumulated on the peel of citrus. Moreover, Calvaruso et al. (2020) reported that 43% of the fenhexamid residue in lemon peel was transferred from the peel to albedo, and 18% from albedo to the pulp resulting a low penetration through the pulp.

Similarly, other studies in the literature revealed that peeling substantially reduced pesticide residues in fruits (Boulaid et al., 2012; Reiler et al., 2015; Yolci-Omeroglu et al., 2015). For instance, it was reported that the reduction of pesticide residues by peeling of tomatoes accounted as 70% for pyridaben and 100% for pyrifenox and tralomethrin (Cengiz et al., 2007). Liu et al. (2014) prevailed a decrease in thiophanate-methyl by 84.2% with the peeling of tomatoes, while its metabolite carbendazim decreased by 87.3%. Naman et al. (2022) reported that mancozeb residue decreased by 72.59% by the peeling of apple. Likewise, Peng et al. (2014) reported that imidacloprid, pyraclostrobin, azoxystrobin and fipronil residues in jujube fruits decreased in the pulp of the fruit and the residues remained on the peel. In the same manner, residues of chlorpyrifos-methyl, phenitrothion and procymidone in peach (Balinova et al., 2006), thiophanate-methyl and carbendazim in tomato (Liu et al., 2014), chlorothalonil, difenoconazole and azoxystrobin residues in tomatoes (Rodrigues et al., 2017) remained on the peel after peeling process.

Peeling is an important step in the processing of many fruits and vegetables. Since the majority of pesticides are applied directly to crops, peeling is one of the most effective ways to reduce pesticide residues penetrated into the cuticle layer (Dorđević & Durović-Pejčev, 2016; Chung, 2018). The increase in residue concentration can be related to the physical and chemical properties of pesticides especially to the octanol-water coefficient (log Po/w) in addition to their mode of action. Abamectin has a semi-systemic effect while buprofezin and etoxazole have a contact effect. Abamectin, buprofezin and etoxazole have low water solubility but high log Po/w values (4.4, 3.8 and 5.6, respectively). Therefore, they tend to adhere to the cuticular waxes or deeper layers rather than diffusing through the pulp of the fruit (Holland et al., 1994; Kaushik et al., 2009). Kimbara et al. (2012) reported that cutin and waxes on the outer surface of the citrus have important functions on the protection of pesticide residues physically. Liu et al. (2016) reported that spirotetramate residue in citrus peels increased compared to the initial concentration. In a study reported

by Peng et al. (2014), it was observed that residues of imidacloprid, pyraclostrobin, azoxystrobin and fipronil deposited on the peels of jujube fruits rapidly penetrated into the epicuticular waxes and the cuticle, so that the concentration of pesticide residues on the peels increased compared to the pulp of the fruit. Yolci Omeroglu et al. (2022) concluded the same findings for abamectin, buprofezin and etoxazole in orange. They attributed to these findings to the cuticular waxes which thought to be acted as a transport barrier to prevent forming residual deposits in the citrus pulp.

Table 2. The effect of different processing techniques on pesticide residue in lemon samples (n=3)[1]

		Pesticides (mg/kg, *average±standard deviation*)					
No	Process	Abamectin[2,3]		Buprofezin[2,4]		Etoxazole[2, 3]	
1	Treated control lemon sample (TC)	0.011±0.001	c	0.246±0.030	d	0.040±0.003	e
2	Lemon peel (LP)	0.036±0.003	a	0.660±0.017	c	0.343±0.032	d
3	Lemon pulp (LPu)	<LOQ		0.024±0.001	e	<LOQ	
4	Lemon juice (LJ)	<LOQ		0.035±0.001	e	<LOQ	
5	Homemade jam (HJ)	<LOQ		0.020±0.001	e	<LOQ	
6	Frozen lemon zest (1st month of storage) (LZ_1)	0.043±0.005	a	1.060±0.034	a	0.593±0.011	a
7	Frozen lemon zest (2nd month of storage) (LZ_2)	0.042±0.009	a	0.910±0.020	b	0.530±0.010	b
8	Frozen lemon zest (3rd month of storage) (LZ_3)	0.019±0.002	b	0.650±0.036	c	0.490±0.010	c

[1] "n" represents the replicate number of the laboratory samples"; "m" refers to the number of analytical samples taken from each laboratory sample; "h" refers to the number of injections made for each analytical sample;
[2] There is a difference between the averages indicated by different lowercase letters in the same column (P <0.05);
[3] Since the abamectin and etoxazole concentrations of pulp, juice and jam <LOQ of the method, they were excluded from the statistical analyses. Therefore, degrees of freedom (df) between group = 5-1= 4; df within group = (5 x3)-5=10;
[4] Degrees of freedom (df) between group = 7-1= 6; df within group =(7 x3)-7=14.

Processing into lemon juice (LJ):

Consumption of fruit juice is a very convenient way to consume more fruits (Lozowicka et al., 2016). During the extraction of juice from the plant tissues, the diffusion of the residues throughout the fruit juice is based on the distribution behavior of the residue between the peel and pulp in addition to their physicochemical properties (Dordevic & Durovic-Pejcev, 2016). In this study, for non-polar compounds (log Po/w ≥ 5.6), complete disappearance of abamectin and etoxazole residues were observed in lemon juice, while a reduction of 86% of buprofezin residue was prevailed (Table 1). Since any heat treatment process for sterilization/pasteurization of homemade juice was not included in the production step, the reduction of the pesticide residues in juice can only be attributed to deposition of the residues on the wax and cuticular section of the outer surface, which is related with their higher octanol-water partition coefficient (log Po/w). Yolci Omeroglu et al. (2022) observed the same findings for orange juice. Furthermore, Tang et al. (2023) stated that degradation or dissipation of five pesticides including etoxazole occurred at a rate of at least 37.6 % in sterilized citrus juice compared with the residual levels in raw citrus. On the other hand, for concentrated citrus juice due to the heat concentration, the degradation rate was diverse compared to sterilized citrus juice.

In line with our findings, Athanasopoulos & Papas (2000) reported that azinphos-methyl residues totally disappeared in lemon juice during processing step. In another study, it was found that abamectin, residue decreased by 46.0% during fruit juice production (Li et al., 2012). Naman et al. (2022) determined that chlorpyrifos residue reduced by 100% in pear juice. Likewise, mancozeb residue in apple juice was reduced by 100%. The findings reported by Hendawi et al. (2013) prevailed that reduction of imidacloprid residue in strawberry juice was related with its lower water solubility (514 µg/mL) and higher octanol-water coefficient (2.7). Moreover, the other studies in the literature supported the relation between the fate of the pesticide during fruit juice production and the physicochemical properties of pesticide (Rasmusssen et al., 2003; Martin et al., 2013; Kwon et al., 2015; Li et al., 2015; Lozowicka et al., 2016; Hassan et al., 2022).

Processing into lemon zest (LZ) and storage at frozen conditions:

Lemon peels are processed into zest form and commonly used for baking purposes to provide pleasant aroma. Lemon zest can be stored in frozen forms to prolong the shelf life. Freezing, as one of the

most widely used food preservation methods, provides better preservation of taste, texture and nutritional value in foods than other methods (Kaushik et al., 2009). In Table 1, the changes of pesticide residues during production of lemon zest and throughout the storage period at -20°C were shown. After processing into lemon zest, concentration of abamectin, buprofezin and etoxazole residues were found to be significantly more compared to their concentration in control lemon samples (TC) ($P < 0.05$). This observation can be attributed to the accumulation of the pesticide on the exocarp of the lemon fruit. Higher oil solubility affinity of the pesticides analyzed within the scope of the study lead to absorption of their residues by waxy outer surface of the lemon. The findings reported by Yolci Omeroglu et al. (2022) supported our conclusions. According to results shown in Table 1, throughout the frozen storage period for three months, pesticide residue level decreased significantly ($P<0.05$) with a ratio ranged between 17% and 55%. Similarly, it has been reported in literature for different type of pesticide and matrix combinations, even though storage of agricultural crops at frozen conditions, pesticide residue level decreased throughout the increasing storage period (Abou-Arab, 1999; Hamilton et al., 2004; Chauhan et al., 2012; Öğüt et al., 2014; Bouzari et al., 2015). On the other hand, study reported by Oliva et al. (2017) revealed that after freezing and during storage of the zucchini, residue levels of trifloxystrobin and myclobutanil decreased less than 1%. In the same study, the losses in imidaclorpid and diethofencarb were observed much greater (31.7 and 9.8%, respectively), with no significant variations observed between the storage period of 15 days and 30 days.

Processing into lemon jam (LJ):

Jam is a product obtained by cooking the fruit to a certain consistency using sucrose and other additives. The recipe used in the scope of the study included the removing of the outer layer of the fruit gently by grating followed by the boiling of pulps in water 3 times for 15 min. To overwhelm the bitter taste of the outer layer, each time water was replaced with the fresh one. The other steps in the production were similar to common jam processing including cooking step at 95°C for 30 min (Yolci Omeroglu et al., 2022). In the scope of the study, abamectin and etoxazole residues was not detected in the final product, while 92% reduction in buprofezin residue was revealed. In line with our findings, Liu et al. (2016) reported that one of the metabolite forms of spirotetramat, namely B-keto, in marmalade was reduced by 68% compared to the initial concentration, while the other metabolites (B-enol, B-glu and B-mono) were completely removed. The more recent study by Naman et al. (2022) determined that the mancozeb residue decreased by 100% in apple jam. Those findings were mainly due to the steps included in the jam production as explained above. Moreover, it can be attributed to their chemical and thermal degradation during heat treatment process in addition to their water solubility (Kaushik et al., 2009; Bajwa & Sandhu, 2014; Dordevic & Durovic-Pejcev, 2016; Lozowicka et al., 2016).

Processing factor

Food processing may reduce or increase the level of the residues in final products compared to their initial level in raw agricultural crops depending on the physicochemical properties of the pesticide, type of the matrix and the operations included in the processing (Shabeer et al., 2015; Oliva et al., 2017). The effects of different type of household processing on the processing factors were summarized in Table 3.

Table 3. The effect of different processing techniques on average processing factors (n=3)[1,2]

		Average processing factor (Pf) ± std deviation					
No	Process	Abamectin		Buprofezin		Etoxazole	
1	Fruit peel separation (P)	3.307±0.448	Ba	2.701±0.327	Cb	8.598±0.646	Ab
2	Fruit pulp separation (LPu)	<0.050±0.001	Bc	0.101±0.017	Ac	<0.050±0.001	Bc
3	Fruit juice processing (FJ)	<0.862±0.080	Abc	0.146±0.010	Bc	<0.251±0.023	Bc
4	Homemade jam production (HJ)	<0.862±0.070	Abc	0.081±0.010	Bc	<0.251±0.023	Bc
5	Frozen storage of lemon zest (1st month) (GP_1)	3.752±0.740	Ba	4.329±0.399	Ba	14.921±1.530	Aa
6	Frozen storage of lemon zest (2nd month) (GP_2)	3.624±0.775	Ba	3.732±0.526	Ba	13.333±1.448	Aa
7	Frozen storage of lemon zest (3rd month) (GP_3)	1.669±0.184	Cb	2.649±0.177	Bb	12.315±1.122	Aa

[1] There is a difference between the means indicated by different lowercase letters in the same column ($P<0.05$); degrees of freedom (df) between group= 7-1= 6; df within group= (7 x3)-7=14);

[2] There is a difference between the means shown with different capital letters in the same row ($P<0.05$); degrees of freedom (df) between group=3-1= 2; df within group= (3 x3)-3=6.

Since concentration of pesticide residue in TC samples ranged from 0.011 mg/kg to 0.246 mg/kg (Table 2) was higher than the LOQ of the analytical method, calculation of processing factors met the criteria set in the OECD guideline (OECD, 2008).

It has been observed that there was a remarkable effect on the removal of pesticide residues by processing of lemon fruit into pulp, juice and jam. Therefore, P_f values for those steps for all of the three pesticides were determined to be less than 1. P_f values were found to be more than 1 due to the increase in the concentration of pesticide residues in the peel part and frozen grated peel part. These results were attributed to log Po/w values of pesticides. The highest processing factors were obtained for etoxazole and in addition, in some studies in the literature, processing factors have been calculated and it has been reported that the processing factor is bound on the type of the active ingredient, its physicochemical properties in addition to type of the carriers used in the formulation (Li et al., 2012; Han et al., 2013; Tiryaki & Özel, 2019; Polat & Tiryaki, 2020; Tiryaki & Polat, 2023). It can be indicated that the results of those studies are compatible with our findings.

Conclusions

Pesticides as plant protection products constitute an important place in boosting agricultural production. Even though pesticides can increase yield of the agricultural products at a limited extent, an abuse use may cause health risk both to human being and the environment. Therefore, to control products on the market with legal limits, extensive studies should be conducted to reveal the effects of processing on pesticide residue and to calculate related processing factors. In the light of needs, the current work was conducted to investigate effect of some representative household processing on insecticide residues in lemon. In the study, the required method performance criteria were met. The QuEChERS method was successfully applied for analysis of abamectin, buprofezin and etoxazole residue in lemon and its products. It was concluded that fate of pesticide residues depended on the type of treatment to be applied to the food, the physicochemical structure of pesticides and nature of the product. It was determined that the pesticide residue levels in lemon pulp, juice and jam obtained from lemon fruits were significantly reduced. It was observed that the insecticide residues were commonly distributed on the lemon peel due to the physicochemical properties of the pesticides and nature of the crop. Processing factors vary according to the physicochemical properties of pesticides and processing methods; therefore, future studies should be conducted for different combinations of pesticide, matrix and processing methods. Moreover, effect of pre-harvest interval on the fate of the pesticide should be examined in future risk assessment studies. Additionally, if possible, field treated samples should be used for the estimation of processing factor accurately.

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Original article (Orijinal araştırma)

The diversity and host interactions of aphids (Hemiptera: Aphididae) on different plant communities in an urban ecosystem[1]

Kentsel bir ekosistemde farklı bitki komüniteleri üzerindeki afitlerin (Hemiptera: Aphididae) çeşitliliği ve konukçu bitki etkileşimleri

Berna YILMAZ[2] **Şahin KÖK[3,4*]**

Abstract

The aim of this study was to reveal the diversity and host interactions of aphids on different plant communities in an urban ecosystem in the northwest of Türkiye between April and October from 2021 to 2022. From the sampling, 55 aphids from 26 genera in the family Aphididae (Hemiptera) on 65 host plant of 26 families were determined. From the aphids, *Capitophorus archangelskii* Nevsky, 1928 and *Uroleucon leontodontis* (Hille Ris Lambers, 1939) are found to be new to the aphid fauna of Türkiye. In the urban ecosystem, 108 interactions between aphids and hosts, including the new records of the interactions for Türkiye were identified on different plant communities. Also, we revealed the biodiversity of aphids and hosts interactions in various plant communities in the urban ecosystem. Our results showed that the species richness and abundance of aphids were significantly higher on the herbaceous plants compared to other communities. Also, interactions between aphids and their hosts in the herbaceous plants were more diverse than the trees and shrubs. Accordingly, the results of our study revealed that biodiversity of interactions between aphids and their hosts was higher on the herbaceous plants compared to other plant communities in the urban ecosystem.

Keywords: Aphid, diversity, host plant community, interaction, urban ecosystem

Öz

Bu çalışmada 2021 ve 2022 yıllarında Nisan ve Ekim ayları arasında kuzeybatı Türkiye'de bir kentsel ekosistemde farklı bitki komüniteleri üzerindeki afitlerin çeşitliliği ve konukçu etkileşimlerinin ortaya çıkarılması amaçlanmıştır. Örneklemelerin sonucunda 26 familyaya bağlı 65 konukçu bitki üzerinde Aphididae (Hemiptera) familyasından 26 cinse bağlı 55 afit tespit edilmiştir. Afitlerden, *Capitophorus archangelskii* Nevsky, 1928 ve *Uroleucon leontodontis* (Hille Ris Lambers, 1939) Türkiye afit faunası için yeni kayıtlardır. Kentsel ekosistemde, farklı konukçu bitki komüniteleri üzerinden Türkiye için yeni etkileşim kayıtlarını da içeren afitler ve konukçu bitkileri arasında 108 etkileşim tespit edilmiştir. Ayrıca, kentsel bir ekosistemdeki farklı bitki komüniteleri üzerindeki hem afitlerin hem de afit-konukçu etkileşimlerinin biyoçeşitlilik değerleri de ortaya çıkarılmıştır. Sonuçlarımız, afitlerin tür zenginliği ve bolluğunun yabancı otlar üzerinde diğer bitki komünitelerine kıyasla önemli ölçüde daha yüksek olduğunu göstermiştir. Benzer şekilde, yabancıotlar üzerindeki afit-konukçu etkileşimleri de ağaçlar ve çalılar üzerinde olduğundan daha fazla çeşitlilik göstermiştir. Bu doğrultuda, çalışmamızın sonuçları kentsel bir ekosistemde yabancıotlar üzerindeki afitlere ve konukçuları arasındaki etkileşimlerin biyoçeşitliliğinin diğer bitki kominitelerine göre daha yüksek olduğunu ortaya koymuştur.

Anahtar sözcükler: Afit, çeşitlilik, konukçu bitki komünitesi, etkileşim, kentsel ekosistem

[1] This study is a part of the MSc thesis of the first author.

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Introduction

Urban areas including parks, landscaped areas, green spaces, roadsides, green roofs, and the gardens of homes and buildings in the world are important biodiversity hotspots for many species. These areas play an important role in the conservation and sustainability of plant and animal biodiversity (Threlfall et al., 2016; Durà et al., 2023). Also, park ecosystems in urban areas can be home to many rare species, and support the population development of vulnerable species. It is widely known that the abundance and species richness of certain arthropod species change in urban ecosystems compared to their surrounding natural habitats. Plant diversity in urban ecosystems can be higher, and even more diverse than in adjacent natural habitats (Hope et al., 2003; Smith et al., 2006). Variations in host plant communities in urban ecosystems may affect the diversity of herbivorous arthropods and their abundance, species richness, host plant preference and natural enemies (Kareiva, 1983; Shrewsbury & Raupp, 2006; Bennewicz & Barczak, 2016). The diversity of plant communities is known to have a significant positive correlation with the species richness of pest insects. In addition, many studies have demonstrated that due to their richer vegetational diversity or complexity, urban ecosystems support the greater abundance or richness of natural enemies, especially predators and parasitoids with a wide variety of prey (Tooker & Hanks, 2000; Frank & Shrewsbury, 2004; Shrewsbury et al., 2004; Tomanović et al., 2006, 2009; Kavallieratos et al., 2013, 2016).

Some species described as urbanophiles show considerable success in urban ecosystems (Shochat et al., 2010). Aphids (Hemiptera Aphididae), one of the most important examples of these arthropod urbanophile species, are one of the most destructive pest insect groups in both agricultural and urban ecosystems. The common presence of aphids in urban ecosystems is supported by their cyclical parthenogenesis (Simon et al., 2002), as well as different levels of urbanisation and land cover (Barczak et al., 2021). Also, water availability gradient and vegetation diversity in urban ecosystems positively affects the increase in the abundance and breeding of aphids (Andrade et al., 2017).

It is evident that host plant communities have largely influenced the diversity of aphid species. Approximately 40% of known aphid species live on trees, with the other 55% preferring to feed on host herbaceous plants and shrubs (the remaining 5% live on unknown hosts). Some aphids, about 10% of them, have a heteroecious life cycle. In this cycle, aphids migrate to secondary hosts consisting of flowering herbaceous hosts in the summer after spending all the seasons except summer on primary hosts (Blackman & Eastop, 2006). Therefore, investigating the preferences of aphids for different host plant communities within an ecosystem is important both in terms of obtaining data on the host plant selection of aphids and in gaining a better understanding of their biology, life cycles, and management. Some studies have been carried out on aphid-host interactions on all plant communities in different areas in urban ecosystems (Borowiak-Sobkowiak & Wilkaniec, 2010; Bennewicz & Barczak, 2014; Barczak et al., 2021). However, it is clear that the data on the biodiversity of aphid-host plant interactions on different plant communities such as trees, shrubs, and herbaceous plant, needs to be collected and studied separately.

As can be understood from the above, numerous studies investigating aphid-host plant interactions in urban ecosystems have commonly focused on plant communities such as trees and shrubs in parks and landscaped areas. Based on the fact that herbaceous host plants represent an important stage in the life cycles of many aphid species, we were interested in how the biodiversity of aphid-host interactions on different host plant communities would change in all urban ecosystems including parks, landscaped areas, roadsides, and the gardens of homes and buildings. In this context, we aimed to reveal the diversity of aphid species and their host plant interactions on different plant communities such as trees, shrubs and herbaceous plants in an urban ecosystem in northwest Türkiye.

Materials and Methods

Sampling site

Our sampling area consists of the city centre of the Çanakkale Province including the central district of Kepez (Figure 1). Approximately 198,000 people live here, in an area of 12 km^2. There are also many urban areas in the Çanakkale Province, including park-landscaped areas such as Halkbahçesi Park, Sarıçay Park, the Terzioğlu Campus, Esenler Özgürlük Park, the Dardanos Campus, Osnabrück Park, street medians and roadsides, as well as the gardens of homes and buildings, which contain numerous different plant communities.

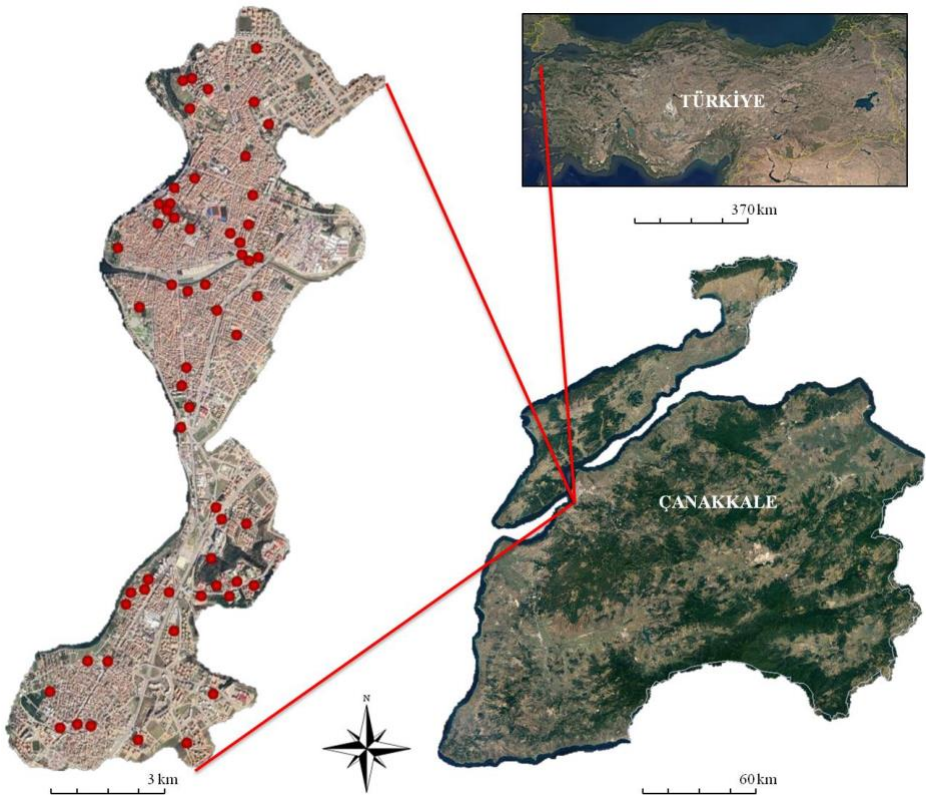

Figure 1. Map showing our sampling area in the Çanakkale Province of northwest Türkiye.

The sampling method and identification of the aphids and their hosts

In order to determine the diversity and interactions of aphids on different plant communities in the urban ecosystem, aphid sampling was conducted from the host plants such as trees, shrubs and herbaceous plants in parks, landscaped areas, roadsides, and gardens of homes and buildings in the city centre of Çanakkale located in the northwest of Türkiye. For the homogeneous sampling, all the areas here were visited once a week or sometimes more between April and October from 2021 to 2022. To determine the presence of aphid colonies, different parts of all the plant species were checked. The individuals of the aphid colonies on the host plants were put in cryotubes filled with 70% ethanol. Afterward, these specimens were clarified and prepared according to the protocol proposed by Hille Ris Lambers (1950). The identification of sampling specimens was carried out by the second author based on the keys of Blackman & Eastop (2006, 2023) using a LEICA DM 2500 microscope with LAS software and an HD camera. The scientific names of the identified aphids were provided and checked from Favret (2023). For the identification of host plants, trees or shrubs were photographed during the aphid sampling, and herbaceous plants were brought to the laboratory for the herbarium. The slide materials of the identified aphid species were kept in the Plant Protection Department of Agricultural Faculty in Çanakkale Onsekiz Mart University.

Data analysis

For visualization of the network of aphids-hosts interactions on different plant communities such as trees, shrubs and herbaceous plants in the urban ecosystem, the interaction graphs were constituted by using the "plotweb" function of the Bipartite software based on the relative abundance data of aphids and their hosts. For the calculation of the biodiversity values of the aphids-hosts interactions on different plant communities, i.e. Shannon's diversity index (*H'*), interaction evenness (*E*), *H2*, linkage density, links per species and connectance, were used for the "networklevel" function of the Bipartite software. Also, the modularity *(M)* and nestedness *(N)* values of the aphids-hosts interaction networks were calculated using the functions of "metaComputeModules" and "nested" (Beckett, 2016) in the Bipartite (Dormann et al., 2021). In addition, the "diversityresult" function in the BiodiversityR of R software (3.6.1) (Kindt & Kindt, 2019; R Core Team, 2023) was used to calculate the biodiversity values such as the richness *(S)* and abundance *(N)* of the aphids on the trees, shrubs and herbaceous plants.

Results and Discussion

The diversity of the aphids in the urban ecosystem

This study has revealed the diversity of the aphids and the interactions of aphid-host plants in different plant communities in a specific urban area, and determined 55 aphid species from 26 genera in the family Aphididae (Hemiptera) on 65 host plant species belonging to 26 plant families. Of these aphids, *Capitophorus archangelskii* (A23) and *Uroleucon leontodontis* (A54) are new to the aphid fauna of Türkiye. Also, *Brachycaudus tragopogonis setosus* (A21), *Cinara neubergi* (A29) and *Lipaphis lepidi* (A36), which are only reported in a few regions, are rare aphid species recorded in Türkiye. In addition to these species, *Aphis cytisorum* (A4), *Chaetosiphon tetrarhodum* (A24), *Chaitophorus populeti* (A26), *Rhopalosiphum nymphaeae* (A46) and *Uroleucon cichorii* (A52) were recorded for the first time in the Çanakkale Province, where this study was conducted. In terms of the genera diversity of the aphids, the most aphid species were identified in the genera *Aphis* with fifteen species, followed by the genera *Uroleucon* with five species. On the other hand, only one species from the genera *Brevicoryne*, *Capitophorus*, *Chaetosiphon*, *Eucallipterus*, *Hyperomyzus*, *Liosomaphis*, *Lipaphis*, *Macrosiphoniella*, *Myzus*, *Panaphis*, *Phorodon*, *Rhodobium*, *Sarucallis*, *Sitobion*, *Tinocallis* and *Trama* were identified. The aphid species identified in this study are presented in Table 1.

Table 1 The aphid species determined in the urban ecosystem

Code	Aphid Species	Code	Aphid Species	Code	Aphid Species
A1	*Acyrthosiphon gossypii* Mordvilko,1914	A20	*Brachycaudus* sp.	A39	*Macrosiphum rosae* (Linnaeus, 1758)
A2	*Acyrthosiphon lactucae* (Passerini, 1860)	A21	*Brachycaudus tragopogonis setosus* (Hille Ris Lambers, 1948)	A40	*Macrosiphum* sp.
A3	*Aphis craccivora* Koch, 1854	A22	*Brevicoryne brassicae* (Linnaeus, 1758)	A41	*Myzus persicae* (Sulzer, 1776)
A4	*Aphis cytisorum* Hartig, 1841	A23	*Capitophorus archangelskii* Nevsky, 1928	A42	*Panaphis juglandis* (Goeze, 1778)
A5	*Aphis fabae* Scopoli, 1763	A24	*Chaetosiphon tetrarhodum* (Walker, 1849)	A43	*Phorodon humuli* (Schrank, 1801)
A6	*Aphis gossypii* Glover, 1877	A25	*Chaitophorus leucomelas* Koch, 1854	A44	*Rhodobium porosum* (Sanderson, 1900)
A7	*Aphis hederae* Kaltenbach, 1843	A26	*Chaitophorus populeti* (Panzer, 1801)	A45	*Rhopalosiphum maidis* (Fitch, 1856)
A8	*Aphis nasturtii* Kaltenbach, 1843	A27	*Cinara cedri* Mimeur, 1936	A46	*Rhopalosiphum nymphaeae* (Linnaeus, 1761)
A9	*Aphis nerii* Boyer de Fonscolombe, 1841	A28	*Cinara fresai* Blanchard, 1939	A47	*Sarucallis kahawaluokalani* (Kirkaldy, 1907)
A10	*Aphis pomi* De Geer, 1773	A29	*Cinara neubergi* (Arnhart, 1930)	A48	*Sitobion avenae* (Fabricius, 1775)
A11	*Aphis punicae* Passerini, 1863	A30	*Cinara tujafilina* (Del Guercio, 1909)	A49	*Tinocallis saltans* (Nevsky, 1929)
A12	*Aphis ruborum* (Börner & Schilder, 1931)	A31	*Eucallipterus tiliae* (Linnaeus, 1758)	A50	*Trama caudata* Del Guercio, 1909
A13	*Aphis rumicis* Linnaeus, 1758	A32	*Hyalopterus amygdali* (Blanchard, 1840)	A51	*Uroleucon aeneum* (Hille Ris Lambers, 1939)
A14	*Aphis solanella* Theobald, 1914	A33	*Hyalopterus pruni* (Geoffroy, 1762)	A52	*Uroleucon cichorii* (Koch, 1855)
A15	*Aphis* sp.	A34	*Hyperomyzus lactucae* (Linnaeus, 1758)	A53	*Uroleucon jaceae* (Linnaeus, 1758)
A16	*Aphis spiraecola* Patch, 1914	A35	*Liosomaphis berberidis* (Kaltenbach, 1843)	A54	*Uroleucon leontodontis* (Hille Ris Lambers, 1939)
A17	*Aphis umbrella* (Börner, 1950)	A36	*Lipaphis lepidi* (Nevsky, 1929)	A55	*Uroleucon sonchi* (Linnaeus, 1767)
A18	*Brachycaudus cardui* (Linnaeus, 1758)	A37	*Macrosiphoniella sanborni* (Gillette, 1908)		
A19	*Brachycaudus helichrysi* (Kaltenbach, 1843)	A38	*Macrosiphum euphorbiae* (Thomas, 1878)		

Of the aphids new for Türkiye, *C. archangelskii* (A23), which feeds on the undersides of the leaves of *Elaeagnus* spp. (Elaeagnaceae), is distributed in Afghanistan, the Caucasus, India, Iran, Kazakhstan, Pakistan, and Uzbekistan. Another new species, *U. leontodontis* (A54), is distributed on *Leontodon* spp. in Europe (Blackman & Eastop 2023). In this study, *C. archangelskii* (A23) was identified from *Elaeagnus angustifolia* (Elaeagnaceae) and *U. leontodontis* (A54) from *Leontodon* sp. (Asteraceae).

Detailed descriptions and slides of the new aphid species for Türkiye are provided below:

***Capitophorus archangelskii* Nevsky, 1928**

Specimens examined. Türkiye: 4 apterous viviparous ♀, Çanakkale, 07.VI.2022, on *E. angustifolia*.

Color of body of living apterous viviparous female is light green, oval shaped, about 1.725 mm. Body parts are densely bearing long and thick capitate hairs: 5 on antenna segment I, 4 on antenna segment II, 8 on antenna segment III, 16 on dorsal each abdominal segment 1-4 (Figure 2a, e). Apterous viviparous female specimens on the slide; whole antenna is pale (Figure 2b), 1.548 mm length, and about 0.898 x body length. Processus terminalis of antenna segment VI 5.579 x base part of antenna segment VI (Figure 2d). Antenna segment III about two times shorter than segment VI, antenna segment IV and V are close in length. Length of antenna segments (I-VI) 0.098-0.060-0.305-0.245-0.230-0.608 mm. Maximum hair length on antenna segment III about 0.933 x basal diameter of same segment III (Figure 2c). Width of head about 0.372 mm, and pale. Rostrum is pale (Figure 2g), the length of ultimate rostral segment (RIV+V) 0.189 mm and has only two hairs, RIV+V 2.039 x hind tarsus segment II. Whole segments of legs are pale. Femur with long capitate hairs (Figure 2h), hind tarsus segments I and II are 0.023 mm and 0.092 mm (Figure 2ı). Siphinculi is 0. 625 mm, pale, cylindrical, not swollen, distinctly imbricated and not reticulated zone (Figure 2f). Siphinculi 4.092 x cauda, 0.362 x body length, 2.052 x length of antenna segment III. Cauda with an average of six hairs is pale, broadly and very shorter than siphinculi (Figure 2f). Lenght of cauda is 0.153 mm, about 0.813 x RIV+V, 0.504 x length of antenna segment III, 1.276 x width of cauda.

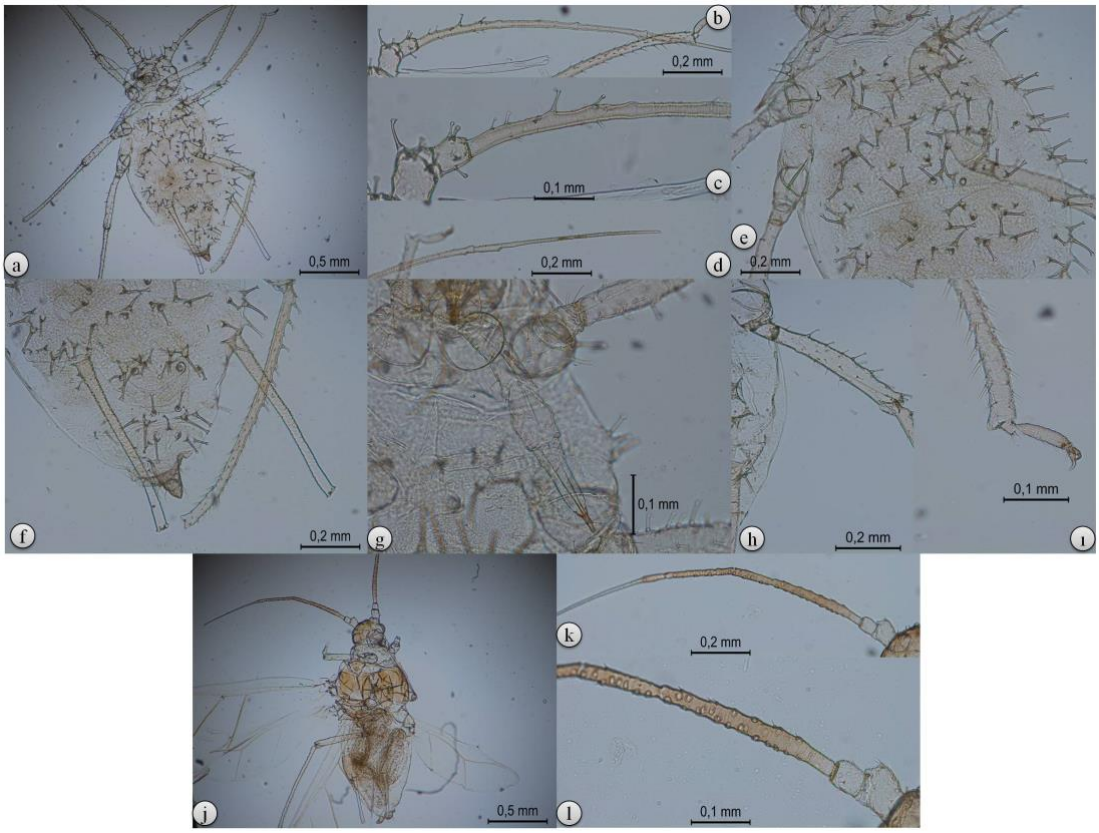

Figure 2. *Capitophorus archangelskii*: a) the body of an apterous viviparous female; b) whole antenna; c) hair on the antenna segment III; d) antenna segment VI (base + processus terminalis); e) capitate hairs on the dorsal abdominal segments; f) siphinculi and cauda; g) rostrum and ultimate rostral segment; h) capitate hairs on the femur; ı) hind tarsus segments I and II; j) the body of an alatae viviparous female, k,l) secondary rhinaria on the antennal segment III, IV, V.

Specimens examined. Türkiye: 1 alatae viviparous ♀, Çanakkale, 07.VI.2022, on *E. angustifolia*.

Color of body of living alatae viviparous female is light green. Alatae viviparous female specimens on the slide; body is 1.686 mm (Figure 2j). Antenna is dark, 1.396 mm length, and about 0.827 x body length. Processus terminalis of antenna segment VI 5.891 x base part of antenna segment VI. Antenna segment III longer than IV, and shorter than VI. Length of antenna segments (I-VI) 0.077-0.056-0.314-0.200-0.177-0.572 mm. Secondary rhinaria of antenna segments: 24 on segment III, 11 on segment IV, 3 on segment V (Figure 2k, l). Width of head

about 0.304 mm, and dark. Length of ultimate rostral segment (RIV+V) 0.165 mm and has only two hairs, RIV+V 1.918 x hind tarsus segment II. Mesothorax is deep brown or dark. Abdomen has a largely square dark green patch in front of siphinculi. Siphinculi is 0.398 mm, cylindrical and not swollen. Siphinculi 3.790 x cauda, 0.236 x body length, 1.267 x length of antenna segment III. Cauda with an average of six hairs is bluntly pointed, 0.105 mm, about 0.636 x RIV+V, 0.334 x length of antenna segment III, 1.500 x width of cauda.

***Uroleucon leontodontis* (Hille Ris Lambers, 1939)**

Specimens examined. Türkiye: 8 apterous viviparous ♀, Çanakkale, 26.V.2021 and 24.VI.2021 on *Leontodon* sp.

Color of body of living apterous viviparous female is dark brown-shiny, and body length is 3.068 mm. Apterous viviparous female specimens on the slide; whole antenna is dark (Figure 3c) and about 1.328 x body length (Figure 3a, b), processus terminalis of antenna segment VI 5.868 x base part of the same segment (Figure 3e), length of antenna segments (I-VI) 0.193-0.119-1.265-0.685-0.591-1.269 mm. Antenna segment III has average 46 secondary rhinaria (Figure 3d), maximum hair length on antenna segment III about 0.756 x basal diameter of the ame segment. Width of head about 0.619 mm, and dark. Antennal tubercle well developed (Figure 3a). Rostrum is dark, the length of ultimate rostral segment (RIV+V) 0.250 mm and has 7-9 hairs (Figure 3ı), RIV+V 1.384 x hind tarsus segment II. Dorsal abdomen has distinctive dark markings mostly with hairs (Figure 3b, f). Segments of legs; coxa dark, trochanter and basal part of femur pale, apical part of femur and whole tibia dark. Segments I and II of hind tarsus are 0.046 and 0.180 mm, and dark (Figure 3g). First tarsal segment of legs has 5-5-5 hair number (Figure 3j). Siphinculi is 0.991 mm, wholly dark and with reticulated zone (Figure 3b, h). Siphinculi 1.507 x cauda, 0.321 x body length, 0.779 x length of antenna segment III. Cauda with an average of 16 hairs is tongue-shaped, and paler than siphinculi (Figure 3b, h). Lenght of cauda is 0.656 mm, about 2.614 x RIV+V, 0.518 x length of antenna segment III, 2.701 x width of cauda.

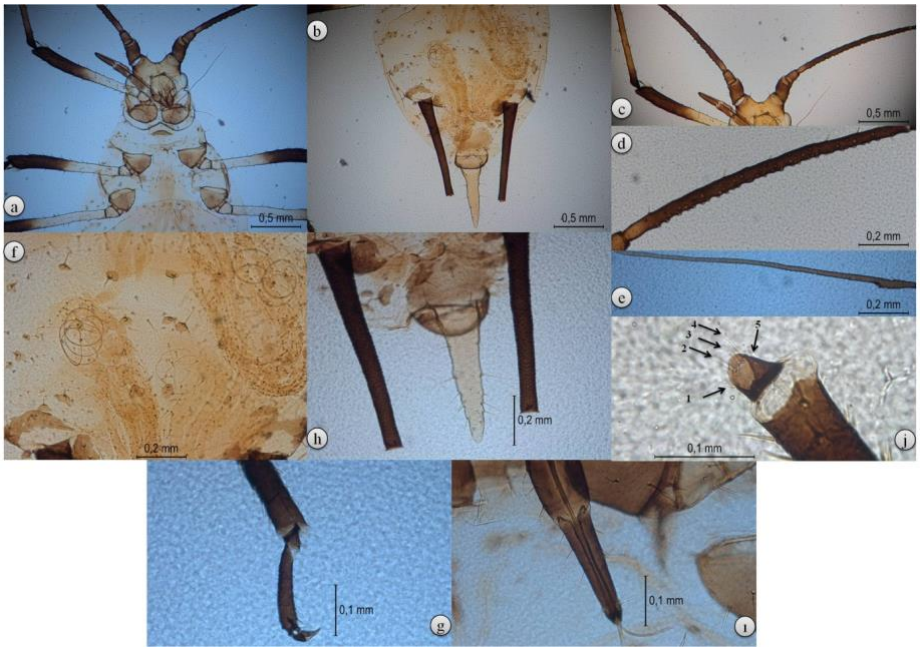

Figure 3. *Uroleucon leontodontis*: a,b) the body of an apterous viviparous female; c)whole antenna; d) secondary rhinaria on the antennal segment III; e) antennal segment VI (processus terminalis and the basal part of the antennal segment VI); f) dark markings on the abdomen; g) hind tarsus segments; h) siphinculi and cauda; ı) ultimate rostral segment; j) first tarsal segment.

The interactions of the aphids and host plants in the urban ecosystem

A total of 108 aphid-host plant interactions, including new interaction records were revealed in the urban ecosystem in the northwest Türkiye. From the different plant communities in the urban ecosystem, the highest aphid-host plant interactions were determined on the herbaceous plants with 52 interactions, followed by the trees with 32 interactions and the shrubs with 24 interactions. Among these, the interactions of *Aphis cytisorum* (A4) - *Spartium junceum* (Leguminosae) (H54), *Aphis spiraecola* (A16) - *Cercis siliquastrum* (Leguminosae) (H9) and *Kerria japonica* (Rosaceae) (H19), *Aphis solanella* (A14) - *Mirabilis jalapa* (Nyctaginaceae) (H29), *C. archangelskii*

(A23) - *E. angustifolia* (H13), *C. neubergi* (A29) - *Pinus pinea* (Pinaceae) (H34), and *U. leontodontis* (A54) - *Leontodon* sp. (H22) were recorded for the first time in Türkiye. The host plant species of the aphids identified in this study are presented in Table 2.

Table 2 The host plant species of the aphids determined in the urban ecosystem

Code	Host Plant	Code	Host Plant	Code	Host Plant
H1	Asteraceae	H23	*Lepidium draba* L. (Brassicaceae)	H45	*Robinia pseudoacacia* L. (Leguminosae)
H2	*Berberis thunbergii* DC. (Berberidaceae)	H24	*Malus floribunda* Siebold ex Van Houtte (Rosaceae)	H46	*Rosa* sp. (Rosaceae)
H3	*Brassica* sp. (Brassicaceae)	H25	*Malva sylvestris* L. (Malvaceae)	H47	*Rubus* sp. (Rosaceae)
H4	*Capsella rubella* Reut. (Brassicaceae)	H26	*Malva vulgaris* Fr. (Malvaceae)	H48	*Rumex conglomeratus* Murray (Polygonaceae)
H5	*Capsicum annuum* L. (Solanaceae)	H27	*Medicago sativa* L. (Leguminosae)	H49	*Rumex crispus* L. (Polygonaceae)
H6	*Carduus pycnocephalus* L. (Asteraceae)	H28	*Medicago* sp. (Leguminosae)	H50	*Rumex patientia* L. (Polygonaceae)
H7	*Cedrus deodora* (Roxb. ex D.Don) G.Don (Pinaceae)	H29	*Mirabilis jalapa* L. (Nyctaginaceae)	H51	*Rumex* sp. (Polygonaceae)
H8	*Centaurea* sp. (Asteraceae)	H30	*Nerium oleander* L. (Apocynaceae)	H52	*Silybum marianum* (L.) Gaertn. (Asteraceae)
H9	*Cercis siliquastrum* L. (Leguminosae)	H31	*Oenothera biennis* L. (Onagraceae)	H53	*Sonchus* sp. (Asteraceae)
H10	*Chrysanthemum* sp. (Asteraceae)	H32	*Photinia serrulata* Siebold & Zucc. (Rosaceae)	H54	*Spartium junceum* L. (Leguminosae)
H11	*Citrus* sp. (Rutaceae)	H33	*Phragmites australis* (Cav.) Trin. ex Steud. (Poaceae)	H55	*Spiraea* × *vanhouttei* (Briot) Zabel (Rosaceae)
H12	*Dasypyrum villosum* (L.) Borbás (Poaceae)	H34	*Pinus pinea* L. (Pinaceae)	H56	*Tanacetum* sp. (Asteraceae)
H13	*Elaeagnus angustifolia* L. (Elaeagnaceae)	H35	*Pittosporum tobira* (Thunb.) W.T. Aiton (Pittosporaceae)	H57	*Tilia cordata* Mill. (Malvaceae)
H14	*Euonymus japonicas* Thunb. (Celastraceae)	H36	*Platycladus orientalis* (L.) Franco (Cupressaceae)	H58	*Tragopogon porrifolius* L. (Asteraceae)
H15	*Hedera helix* L. (Araliaceae)	H37	*Populus alba* L. (Salicaceae)	H59	*Tribulus terrestris* L. (Zygophyllaceae)
H16	*Hibiscus syriacus* L. (Malvaceae)	H38	*Portulaca oleracea* L. (Portulacaceae)	H60	*Ulmus minor* Mill. (Ulmaceae)
H17	*Juglans regia* L. (Juglandaceae)	H39	*Prunus cerasifera* Ehrh. (Rosaceae)	H61	*Viburnum tinus* L. (Adoxaceae)
H18	*Juniperus Sabina* L. (Cupressaceae)	H40	*Prunus domestica* L. (Rosaceae)	H62	*Vicia faba* L. (Leguminosae)
H19	*Kerria japonica* (L.) DC. (Rosaceae)	H41	*Prunus persica* (L.) Batsch (Rosaceae)	H63	*Vicia villosa* Roth (Leguminosae)
H20	*Lactuca viminea* (L.) J. Presl & C. Presl (Asteraceae)	H42	*Prunus* sp. (Rosaceae)	H64	*Wisteria sinensis* (Sims) Sweet (Leguminosae)
H21	*Lagerstroemia indica* L. (Lythraceae)	H43	*Punica granatum* L. (Lythraceae)	H65	*Zea mays* L. (Poaceae)
H22	*Leontodon* sp. (Asteraceae)	H44	*Pyracantha coccinea* M. Roem. (Rosaceae)		

Considering the aphid-host interactions on trees in the urban ecosystem, *Hyalopterus pruni* (A33) fed on four tree species and was the most common aphid. Also, *Aphis craccivora* (A3), *A. spiraecola* (A16) and *Hyalopterus amygdali* (A32) preferred three tree species for feeding. On the other hand, the remaining aphids were mostly determined on only one tree species. As for the host trees, *Prunus domestica* (Rosaceae) (H40) and *Prunus* sp. (Rosaceae) (H42) visited by four aphid species were the most preferred host trees. These were followed by *Citrus* sp. (Rutaceae) (H11) and *Prunus persica* (Rosaceae) (H41), each preferred by three aphid species (Figure 4). When taking results for the shrubs into consideration, it becomes clear that diversity of the aphids, host plants and their interactions are significantly less than in the other plant communities. *Aphis spiraecola* (A16), collected from eight host shrubs species, was the most common aphid, and it was followed by *Aphis gossypii* (A6), collected from five host shrubs. As for the host shrubs, *Rosa* sp. (Rosaceae) (H46) was the most visited shrub species preferred by five aphids (Figure 5). Among all the plant communities, greatest diversity of aphids, host plants and their interactions were found for the herbaceous plants. In terms of aphids, *A. craccivora* (A3) and *Aphis fabae* (A5) fed on eight host herbaceous plants and were the most common. In this interaction network, each of the 19 aphid species was determined on only one host herbaceous plant. As for the host herbaceous plants, *Sonchus* sp. (Asteraceae) (H53) hosted the most aphids with five species (Figure 6).

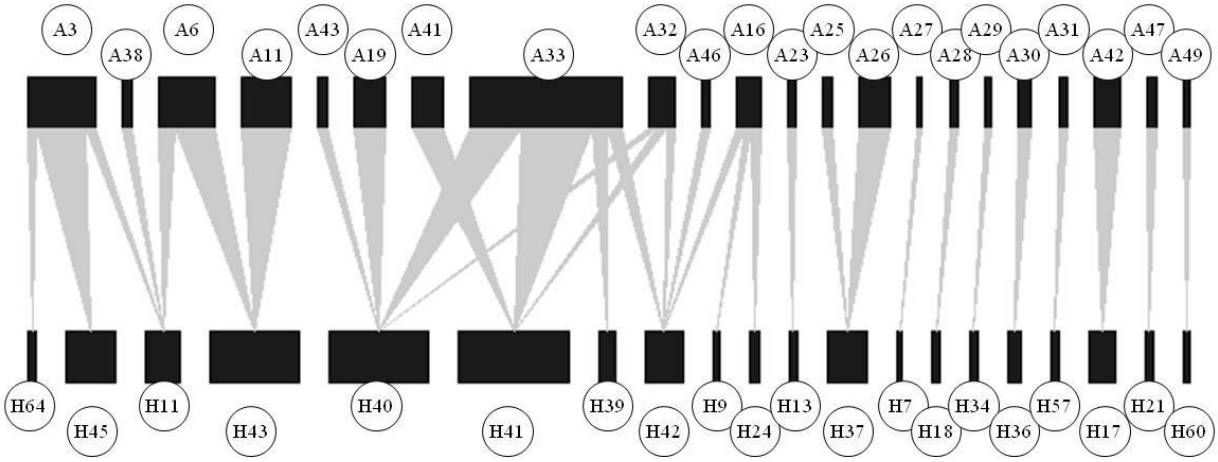

Figure 4. The graph showing the network of the aphids (upper part) - host trees (lower part) interactions in the urban ecosystem. The black bars and the grey bars show the abundance and interactions of the species, respectively.

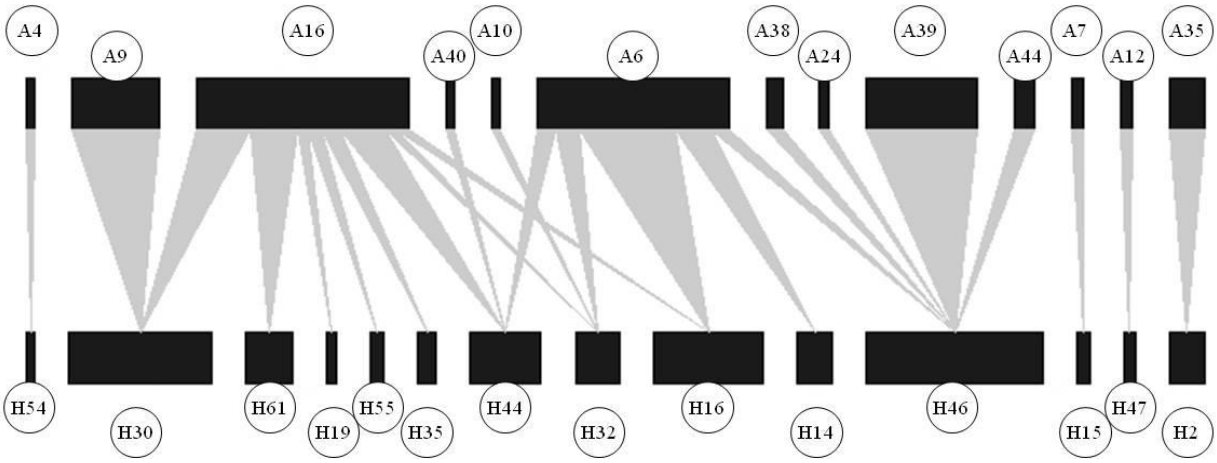

Figure 5. The graph showing the network of the aphids (upper part) - host shrubs (lower part) interactions in the urban ecosystem. The black bars and the grey bars show the abundance and interactions of the species, respectively.

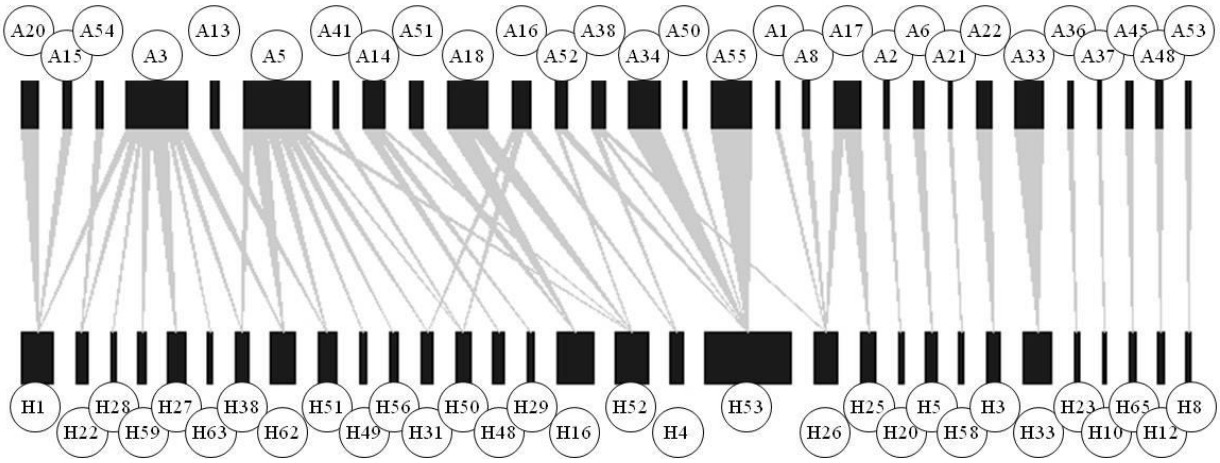

Figure 6. The graph showing the network of the aphids (upper part) - host herbaceous (lower part) interactions in the urban ecosystem. The black bars and the grey bars show the abundance and interactions of the species, respectively.

In our study of the species richness of aphids in different plant communities in an urban ecosystem, the results showed that the aphids on the host herbaceous plants have higher species richness than those on the host trees and shrubs (*S*= 29 on HH, *S*=22 on HT, *S*= 13 on HS). Similarly, the abundance of aphids on the host herbaceous plants was also higher than in the other plant communities (*N*= 2170 on HH, *N*=1502 on HT, *N*= 1674 on HS) (Table 3). To reveal more detailed information on the aphid-host plant interactions on the different plant communities in the urban ecosystem, we also obtained data on the biodiversity of these interactions. The connectance value, i.e. the realised proportion of possible links, was higher in the host shrubs than the other plant communities. Similarly, while the highest value of links per species was recorded for the host shrubs, the lowest value was found for the host trees. Also, the results of the analysis of the nestedness and modularity values of the interactions in different plant communities clearly showed that the network of interactions in the host trees was more nested than those on the host shrubs and herbaceous (*N*= 17.412 on HT, *N*= 13.712 on HS, *N*= 8.492 on HH). But, the network of interactions on the host herbaceous was more modular than those on the host trees and shrubs (*M*= 0.777 on HH, *M*= 0.717 on HT, *M*= 0.628 on HS). The biodiversity of the aphid-host plant interactions on the different plant communities in the urban ecosystem clearly showed that the interactions on the host herbaceous plants were more diverse than those on the host trees and shrubs (*H'*= 3.720 on the HH, *H'*= 3.150 on the HT, *H'*= 2.821 on the HS). Concordantly, the interaction evenness value for the host herbaceous plants was more balanced compared to the other plant communities (*E*= 0.547 on HH, *E*= 0.542 on HS, *E*= 0.517 on HT). The values of H2, which is defined as a network-level measure of specialisation, revealed that the specialisation in the network of aphid-host trees and aphid-host herbaceous plants was higher than in that of aphid-host shrubs (Table 3). The results thus clearly showed that in the urban ecosystem, diversity of the network of aphid-host herbaceous plants was higher than that in the host trees and shrubs.

Table 3 The biodiversity values of aphids and their host interactions on different plant communities in the urban ecosystem

Networks	Biodiversity of aphids		Biodiversity of interactions of aphids - different plant communities							
	Richness *(S)*	Abundance *(N)*	Connectance	Links per species	Linkage density	Nestedness *(N)*	Modularity *(M)*	Shannon diversity of interactions *(H')*	Interaction evenness *(E)*	H2
Network of aphids - host trees (HT)	22	1502	0.073	0.762	2.023	17.412	0.717	3.150	0.517	0.859
Network of aphids - host shrubs (HS)	13	1674	0.131	0.889	2.683	13.712	0.628	2.821	0.542	0.717
Network of aphids - host herbaceous plants (HH)	29	2170	0.057	0.866	2.557	8.472	0.777	3.720	0.547	0.841

Discussion

Urban areas are home to many ecosystems such as parks, landscaped areas, roadsides, and the gardens of homes and buildings with different plant communities. Furthermore, urbanisation can support biodiversity thanks to rich habitat diversity, providing new shelter and food sources for many invertebrates (Weller & Ganzhorn, 2004; Breuste et al., 2008; Bennewicz & Barczak, 2014). Urban ecosystems are known to affect populations of aphids in certain plant communities (Jaśkiewicz, 2005). In addition, aphids, which are one of the important groups of sucking insects, can provide an important food source for parasitoids, predators and other animals in both crop and non-crop habitats. Therefore, any increase or decrease in aphid populations can affect the presence and numbers of these organisms in urban ecosystems (Kamiński et al., 2016; Tena et al., 2016). In this regard, detailed data on the presence, species richness, relative abundance, host plant communities' preferences and biodiversity of aphids in urban ecosystems will contribute to a better understanding of the aphid-host plant interactions in these areas.

In their study on aphids in urban ecosystems, Bennewicz & Barczak (2016) investigated the diversity of aphids in two different plant communities, i.e. the so-called southern slope and downtown in the city of Bydgoszcz in Poland. As a result, they revealed a total of 39 aphid species with 32 aphids on 31 hosts in the southern neighbourhood and 24 aphid species on 23 hosts in the other area. Six aphid species were also determined on the genera of *Prunus*, i.e. *Prunus cerasifera* (Rosaceae) and *P. domestica*, in the sampling area. Similar results were obtained in our study, where seven aphid species, namely *A. spiraecola*, *Brachycaudus helichrysi*, *H. pruni*, *Myzus persicae*, *Phorodon humuli* and *R. nymphaeae* were identified on host trees belonging to the genera

Prunus. Hence, it may be interpreted that some plants from host trees in the genera *Prunus* are very attractive to aphids in both urban and crop ecosystems since urban ecosystems comprise not only ornamental plants in parks and landscaped areas, but also crop trees such as cherry, peach and plum in areas including roadsides and the gardens of homes and buildings. In another study, Borowiak-Sobkowiak & Wilkaniec (2010) identified 67 aphids on 56 host shrubs and host trees in the Park of Cytadela in the city of Poznan in Poland, which included one of the host plant ecosystems we focused on in our study. In our study, 32 aphid species were determined on 34 trees and shrubs in areas containing all urban ecosystems. However, it should be noted that in addition to aphid species on the trees and shrubs in urban ecosystems, our study also focused on the host herbaceous plants in these areas. From this perspective, the result of the determination of 29 aphid species on 31 host herbaceous plants in our study showed that aphid diversity is quite high on herbaceous plants as well as trees and shrubs in urban areas. Also, Borowiak-Sobkowiak & Wilkaniec (2010) emphasised that certain aphid species reduced the decorative value of ornamental plants by causing damage such as the discoloration, leaf curling, and drying of plant parts. In parallel with this, our study yielded some similar observations, although our data is not quantitative. Our observations showed that *Aphis nerii* and *A. spiraecola*, *A. craccivora*, *Liosomaphis berberidis* and *Macrosiphum rosae* caused serious decorative damage to the stems, leaves and flowers of *Nerium oleander* (Apocynaceae), *Robinia pseudoacacia* (Leguminosae), *Berberis thunbergii* (Berberidaceae) and *Rosa* sp., respectively. In another study, which investigated plant communities and associated aphid communities in different urban park ecosystems, 66 aphid species were identified on 75 plant species (Barczak et al., 2021). The results of the study emphasised that the differences between the aphid assemblages were closely related to the plant diversity in urban park plantations. The results from our study, revealing 52 aphid-host interactions on herbaceous plants, 32 aphid-host interactions on trees and 24 aphid-host interactions on shrubs, strongly support these data.

The studies presented above as well as the results of our study clearly demonstrate that urban ecosystems harbour rich aphid biodiversity and aphid-host plant interactions. Although many studies have been carried out on aphid interactions on all host plant communities in urban areas, the lack of data on the biodiversity of aphid-host plant interactions on different plant communities such as trees, shrubs, and herbaceous plants separately was considered an important gap. Considering that some aphid species have a heteroecious life cycle (Blackman & Eastop, 2023), it is clear that discovering more about these pests and their interactions on different plant communities is necessary in order to gain a better understanding of the biology and control strategies of aphids. In this regard, our results supporting this phenomenon showed that *A. gossypii* and *Macrosiphum euphorbiae*, which are important polyphagous aphid species, were determined on all three plant communities, as well as *A. craccivora*, *H. pruni*, and *M. persicae* on both the host trees and herbaceous plants in the urban ecosystem in in the northwest of Türkiye. Furthermore, the fact that the aphid species mentioned here are important ornamental plant pests in landscaped areas supports the need for a more detailed investigation of aphid - host plant interactions on different plant communities in urban ecosystems. Such a detailed investigation of aphid diversity and their host plant interactions in urban ecosystems will contribute not only to determining the control strategies of pest aphids after their infestation of plants, but also to the selection of trees and shrubs with high resistance to aphid damage thus enhancing the pest control programmes of ornamental plants. Additionally, different host plant communities in urban ecosystems can host the interactions of aphids' natural enemies, especially parasitoids. Numerous studies on this subject have shown that some host plants such as *B. thunbergii*, *Euonymus* sp. (Celastraceae), *Hibiscus syriacus* (Malvaceae), *N. oleander*, *Rosa* sp., *Salix alba* (Salicaceae), *Tamarix chinensis* (Tamaricaceae), and *Viburnum* sp. (Adoxaceae) are reservoirs for numerous parasitoid-aphid interactions (Lumbierres et al., 2005; Tomanović et al., 2006, 2009; Kavallieratos et al., 2013, 2016). These reserve hosts can contribute significantly to the biocontrol of aphid pests in urban areas where the use of chemicals is undesirable due to the density of human populations.

For the reasons presented here, it may be concluded that investigation of aphid-host interactions in different plant communities in urban ecosystems will contribute to closing an important gap. The results we present in this study show that urban ecosystems host a very rich aphid diversity, and these areas have significant potential to reveal new aphid species and aphid-host interaction records for cities and countries. Since not only certain landscape and ornamental plants, but also many herbaceous or cultivated plants such as tree species are commonly distributed in urban ecosystems, it is believed that the results of the host plant preference of aphids among the different plant communities in the urban ecosystems in our study have the potential to serve as an important guide in the design of landscape plants, pest control management and biological control of pest aphids in these areas.

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Original article (Orijinal araştırma)

Efficiency of temperature and storage duration on some morphological measurements and reproductive capacity of the entomopathogenic nematode *Heterorhabditis bacteriophora* Poinar, 1976 (Rhabditida: Heterorhabditidae)'s Turkish HBH hybrid strain[1]

Entomopatojen nematod *Heterorhabditis bacteriophora* Poinar, 1976 (Rhabditida: Heterorhabditidae)'nın Türk HBH hibrit ırkının bazı morfolojik özellikleri ve üreme kapasitesi üzerinde sıcaklığın ve depolama süresinin etkisi

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Abstract

Entomopathogenic nematodes (EPNs) are successfully used in the biological control of agricultural insect pests. This study aims to determine the body length of hermaphrodite individuals, egg diameter and reproductive capacity obtained from Infective Juveniles (IJs) stored at different temperatures and durations. *Heterorhabditis bacteriophora* Poinar, 1976 (Rhabditida: Heterorhabditidae)'s Hybrid Strain HBH was used in the study. IJs stored at 15, 25 and 35°C for 7, 14 and 21 days were inoculated onto *Galleria mellonella* L., 1758 (Lepidoptera: Pyralidae) last instar larvae at a dose of 100 IJs. On the 2nd day of infection, hermaphrodite individuals and eggs were obtained by dissecting the larvae. The reproductive capacity was determined 10-12 days after infection. The study was conducted in Bursa Uludağ University, Faculty of Agriculture, Plant Protection Department, Nematology Laboratory in 2023. In conclusion, the longest hermaphrodite individuals and egg diameter were obtained as 6207.22 µm and 55.65 µm, respectively from the IJs stored for 7 days at 15°C. The highest reproductive capacity was also observed as 167.500 IJs per *G. mellonella* larva in IJs stored under the same conditions with respect to temperature and time. This study is important for assessing the morphological effects of different temperature values and storage durations on EPNs.

Keywords: Body length, egg diameter, hermaphrodite, *Heterorhabditis bacteriophora,* reproductive capacity

Öz

Entomopatojen nematodlar (EPN), tarımsal zararlıların biyolojik mücadelesinde başarıyla kullanılmaktadır. Bu çalışmanın amacı farklı gün ve sıcaklıklarda depolanmış olan Infektif Juvenillerden (IJ) elde edilen hermafrodit bireylerin vücut uzunluğunun, yumurta çapının ve üreme gücünün belirlenmesidir. Bu çalışmada *Heterorhabditis bacteriophora* Poinar, 1976 (Rhabditida: Heterorhabditidae)'nın Hibrit Irkı HBH kullanılmıştır. 15, 25 ve 35°C'de 7,14 ve 21 gün depolanmış olan IJ'ler 100 IJ dozunda *Galleria mellonella* L., 1758 (Lepidoptera: Pyralidae)'nın son dönem larvası üzerine inoküle edilmiştir. Enfeksiyonun gerçekleştiği 2. gün sonunda larvalar disekte edilmiş, hermafrodit bireyler ve yumurtalar elde edilmiştir. Üreme gücü ise enfeksiyon gerçekleştikten 10-12 gün sonra belirlenmiştir. Bu çalışma 2023 yılında Bursa Uludağ Üniversitesi, Ziraat Fakültesi, Bitki Koruma Bölümü, Nematoloji Laboratuvarı'nda yürütülmüştür. Sonuç olarak, en uzun hermafrodit bireyler ve yumurta çapı 15°C'de 7 gün muhafaza edilmiş olan IJ'lerden elde edilmiştir. Bu değerler sırasıyla 6207.22 µm ve 55.65 µm olarak belirlenmiştir. En yüksek üreme gücüde aynı sıcaklık ve günde tutulmuş olan IJ'lerde görülmüştür. Bu değer 167.500 IJs/*G. mellonella* larva olarak belirlenmiştir. Bu çalışma farklı sıcaklık değerlerinin ve depolama süresinin EPN'lerin üzerindeki morfolojik etkilerin belirlenmesini içeren önemli bir çalışmadır.

Anahtar sözcükler: Vücut uzunluğu, yumurta çapı, hermafrodit, *Heterorhabditis bacteriophora*, üreme gücü

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Introduction

Entomopathogenic nematodes (EPNs) are highly effective biological control agents against insect pests. These organisms are natural enemies of many economically important insect species, and in recent years, restrictions on pesticide usage have increased the importance of these organisms in the control of pests (Ehlers, 1996; Shapiro-Ilan et al., 2006; Susurluk & Ehlers, 2008; Dede et al., 2022).

Entomopathogenic nematodes which belong to the families Heterorhabditidae and Steinernematidae, are organisms that predominantly spend their life cycles searching for hosts in the soil (Boemare et al., 1996). The life cycle of individuals belonging to the Heterorhabditidae family is expressed as egg, juvenile 1, juvenile 2, juvenile 3 (Infective Juvenile), juvenile 4, and adult stages. In the first generations of this family, the adults are composed of hermaphroditic individuals (Johnigk & Ehlers, 1999). EPNs, in their Infective Juveniles (IJs) form, possess the ability to search for hosts for months without feeding (Susurluk & Ehlers, 2008). The IJs penetrate the host tissue through natural openings such as the mouth, spiracles, anus, or through wounds formed on their bodies. After entering the host tissue, the IJs release the symbiotic bacteria with which they live in a symbiotic relationship into the host tissue, leading to host septicaemia and eventual death within approximately 36-48 hours (Kaya & Gaugler, 1993; Ehlers, 2001; Ehlers & Shapiro-Ilan, 2005; Ulu & Susurluk, 2014). The EPNs kill their hosts with the help of gram-negative bacteria belonging to the Enterobacteriaceae family, with whom they have a symbiotic relationship within their bodies, and they are able to multiply within the host (Ehlers, 2001; Lewis et al., 2006). The IJs of the Heterorhabditidae family carry scattered gram-negative bacteria belonging to the species *Photorhabdus* spp. in their hemolymph, whereas the IJs of the Steinernematidae family carry *Xenorhabdus* spp. within a specialized vesicle inside their bodies (Boemare et al., 1996; Forst & Nealson, 1996; Susurluk, 2008).

Environmental factors significantly affect the lives and activities of EPNs. Additionally, they determine the distribution, mobility, infection potential and population dynamics of these organisms. Among these environmental factors, temperature is one of the most crucial factors for EPNs (Kahel-Raifer & Glazer, 2000; Shapiro-Ilan et al., 2006; Ulu & Susurluk, 2014). The increase in temperature can enhance the metabolic rate and infective abilities of EPNs while reducing their developmental periods. However, excessively high temperatures can have a detrimental effect on the activity and survival capabilities of these organisms. Similarly, low temperatures can also reduce the activity and slow down the development of EPNs (Bilgrami & Gaugler, 2007; Shaurub et al., 2015; Lillis et al., 2023). Temperature tolerance can vary among each EPN species, often being associated with their geographic distribution. While some species are more easily adapted to hot climate regions, others are better suited to cooler areas. This adaptability allows EPNs to achieve more successful results on hosts in their natural habitats and in agricultural applications (Kahel-Raifer & Glazer, 2000; Stuart el al., 2006; Vashisth et al., 2013; Lillis et al., 2023).

The main objective of this study is to determine the length of hermaphrodite individuals of *H. bacteriophora* HBH hybrid strain after its IJs are stored at different temperatures (15, 25, and 35°C) and specific time intervals (7, 14, and 21 days). Additionally, the aim is to determine the length of egg diameter and reproductive capacity.

Materials and Methods

Entomopathogenic nematode species

In this study, a single species of EPN was employed. The species under investigation was *Heterorhabditis bacteriophora*, specifically the HBH hybrid strain, which was developed and patented (TPMK Patent No: TR 2013 06141 B) at the Nematology Laboratory, Department of Plant Protection, Faculty of Agriculture, Bursa Uludağ University. HBH hybrid strain was harvested on *Galleria mellonella* L., 1758 (Lepidoptera: Pyralidae) last instar larvae and subsequently stored at a temperature of 4°C (Kaya & Stock, 1997; Ulu & Susurluk, 2014) until further utilization. For this study, three-day-old isolates were utilized. The HBH hybrid strain is described as a specially adapted strain to the climatic conditions of Türkiye (Ulu & Susurluk, 2014, 2021; Şahin et al., 2018). IJs of the HBH hybrid strain were incubated at temperatures of 15, 25, and 35°C for durations of 7, 14, and 21 days. Subsequently, their reproductive capacities were determined. Additionally, the body lengths and egg diameters of the hermaphroditic individuals derived from the incubated IJs were measured. A temperature of 4°C was designated as the control group.

Experimental design

In the experiments, the hybrid strain HBH was preserved in 60 ml of Ringer's solution (Ringer, 1882) within a 250 ml culture flask with a filter cap, capable of accommodating approximately 1000±20 IJs. The study encompassed different temperature conditions, namely 4, 15, 25 and 35°C, with storage durations of 7, 14 and 21 days for each temperature setting. Specifically, designated days were assigned for the storage periods at each temperature. Typically, EPNs are stored at 4°C due to their ability to maintain viability over an extended period (Ehlers, 2001). Hence, 4°C was utilized as the control temperature in this study to establish a baseline reference. The reproductive capacity, hermaphrodite length, and determination of egg diameter were assessed using last instar (6^{th} stage) larvae of *G. mellonella*, which were employed to be hosts during the inoculation stage. The larvae were placed in 24-well tissue culture plates, with each well measuring 1.5 cm in diameter and 3 cm in depth. The plates were covered with 10% moist alluvial soil, and the inoculation process was conducted.

Measurement of hermaphrodite length and egg diameter

Heterorhabditis bacteriophora HBH hybrid strain was kept in incubators with the specified temperatures and selected days then applied to 24-well tissue culture plates containing *G. mellonella* larvae. The plates were covered with 10% moist alluvial soil, and the inoculation process was conducted. Three days after this treatment, insect larvae were transferred from 24-well tissue culture plates to white traps and two days later, the infected larvae were dissected to obtain the hermaphrodites contained within. The lengths of hermaphrodites were measured. The sizes of the eggs were also measured. The identification of obtained hermaphrodite individuals and eggs was conducted using the Leica DM500® Binocular microscope. The images captured from the microscope were instantly transferred to a computer using the integrated Leica DFC295® Digital Color Camera. Subsequently, the analyses performed on the real-time images were conducted using the Leica Application Suite Version 3.6® (LAS V3.6®) software.

Determining the reproductive capacity of HBH hybrid strain

Heterorhabditis bacteriophora HBH hybrid strain was kept at the specified temperatures (15, 25, and 35°C) for the indicated days (7, 14, and 21 days) and the reproductive capacity of these strains on *G. mellonella* was determined. This phase of the study was generally conducted as follows. Firstly, infection was performed on the last larval stage of *G. mellonella* and IJs obtained as a result of infection were stored at 4°C for 3 days. Subsequently, these IJs were removed from the storage condition and kept in an incubator at 15°C for 7 days, after which infection was performed by applying 100 IJs on *G. mellonella*. After 10-12 days, the reproductive capacity was determined on white trap. At other temperature values, these strains were kept in incubators for the specified days and experiments were implemented in the same way. The reproductive capacity was assessed using last instar *G. mellonella* larvae, which had an average weight of approximately 300±10 mg and a length of approximately 2 cm. The quantity of emerging IJs was determined as the total number obtained from the larvae with these characteristics.

Statistical analyses

JMP® Pro 16 software was used to perform analysis of variance on hermaphrodite body length, eggs diameters and reproductive capacity. Furthermore, the least significant difference test ($p < 0.05$) was used to determine the difference between means. All assessments were performed four times, with five measurements taken at each repetition.

Results

The length of hermaphrodite and eggs diameters of the HBH hybrid strain

According to the results of the study, the longest body length value observed in hermaphrodite individuals of *H. bacteriophora* HBH hybrid strain was found in hermaphrodite individuals derived from IJs incubated for 7 days at 15°C. This length value was determined to be 6207.22 µm. On the 14^{th} and 21^{st} days at 15°C, these values were obtained to be 6199.29 µm and 5637.46 µm, respectively. When the body lengths of hermaphrodite individuals derived from IJs incubated for 7, 14, and 21 days at 25°C in the incubator were examined, these values were found to be 5336.15 µm, 5335.98 µm, and 5433.51 µm, respectively. The body lengths of hermaphrodite

individuals derived from IJs incubated at 35°C for the specified days were examined, and the longest length value was observed in hermaphrodite individuals derived from IJs kept at 35°C for 7 days, and this value was determined to be 5268.51 µm (Figure 1). The body lengths of hermaphrodite individuals derived from IJs incubated for 14 and 21 days at 35°C were found to be 5213.63 µm and 4898.82 µm, respectively. Finally, the body lengths of hermaphrodite individuals derived from IJs stored at 4°C as a control were determined as a reference. This value was obtained to be 5253.41 µm. Based on all the obtained data, a statistically significant difference was observed among the values (F= 12.79; df= 9,190; p <0.0001) (Table 1).

Table 1. The lengths of hermaphrodite individuals were obtained from the incubation of IJs at the specified days and temperatures (Mean±S.E.). There is no statistically significant difference between the values represented by the same letters

EPN	Temperatures (°C)	Time (day)	Hermaphrodite Length (µm)±SE		F (df); p
Heterorhabditis bacteriophora HBH Hybrid Strain	4	7	5253.41±44.32	c	F (9,190)= 12,79; p <0.0001
	15		6207.22±164.20	a	
	25		5336.15±71.11	bc	
	35		5268.51±97.51	c	
	15	14	6199.29±225.04	a	
	25		5335.98±71.10	bc	
	35		5213.63±111.51	cd	
	15	21	5637.46±103.33	b	
	25		5433.51±94.43	bc	
	35		4898.82±97.04	d	

The diameters of the eggs within the hermaphrodite individuals derived from the stored IJs at the specified days and temperatures were also determined. According to the obtained results, the longest egg diameter was found within the hermaphrodite individuals derived from IJs kept for 7 days at 15°C. This diameter value was determined as 55.65 µm. When all the specified days and temperatures were examined, a statistically significant difference was only observed between the 7th day at 15°C and the 21st days of all temperature values used in the experiment. No statistically significant difference was found among the other temperature values and days (F= 1.71; df= 9,190; p=0.089).

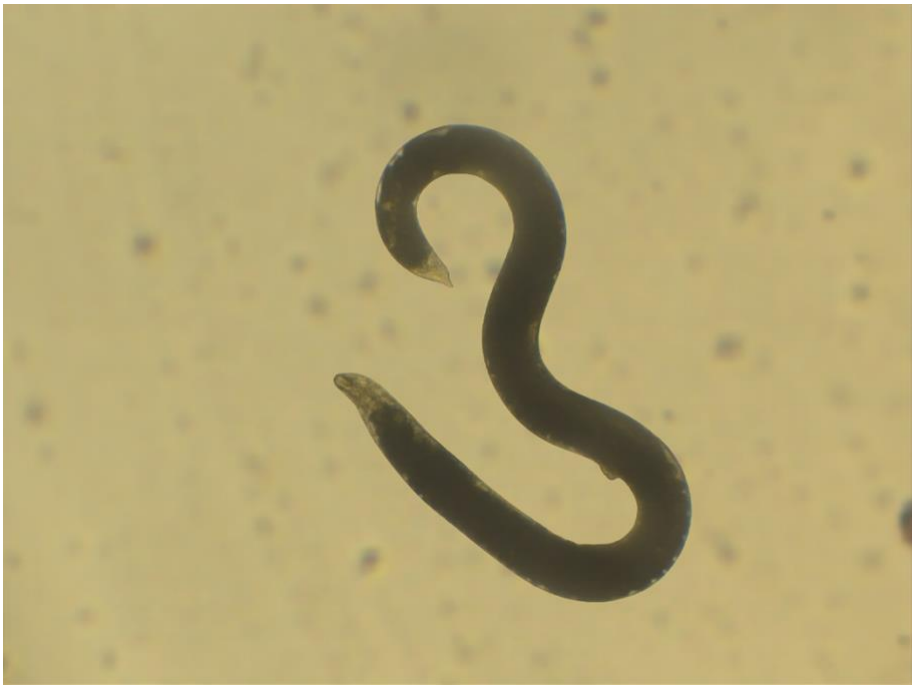

Figure 1. The microscopic image of a hermaphroditic individual derived from IJs (infective juveniles) stored for 21 days at 35°C.

The reproductive capacity of the HBH hybrid strain

The IJs of HBH hybrid strain were kept at the specified temperatures for the indicated days and subsequently, the reproductive capacity of these individuals was determined on the last instar larvae of *G. mellonella*. According to the results, the reproductive capacity was found to be higher in IJs stored for 7 days at 15°C. This value was determined to be 167.500 IJs per *G. mellonella* larva. At 15°C, on the 14th and 21st days, these values were obtained to be 157.750 and 149.500 IJs, respectively. The highest reproductive capacity at 25°C was observed in individuals kept in the incubator for 7 days, with a value of 144.500 IJs. The amounts of IJs obtained on the 14th and 21st days, at 25 ºC were found to be 139.000 and 129.000 IJs, respectively. In this study, the lowest reproductive capacity was observed in individuals kept for 21 days at 35°C. This value was determined to be 103.750 IJs. For individuals incubated at this temperature for 7 and 14 days, these values were obtained to be 133.500 and 118.750 IJs, respectively. Finally, when examining the reproductive capacity of IJs stored at 4°C as a control, this value was found to be 119.250 IJs. Statistically significant difference was found between the obtained values (F= 57.78; df= 9,190; p < 0.0001) (Table 2).

Table 2. The reproductive capacity of *H. bacteriophora* HBH Hybrid Strain IJs were obtained from the incubation of IJs at the specified days and temperatures (Mean±S.E.). There is no statistically significant difference between the values represented by the same letters

EPN	Temperatures (°C)	Time (day)	Reproductive Capacity IJ±S.E.		F (df); p
Heterorhabditis bacteriophora HBH Hybrid Strain	4		119.250±2839.50	g	F (9,190)= 12,79; p <0.0001
	15	7	167.500±3213.86	a	
	25		144.500±2111.99	cd	
	35		133.500±2812.09	ef	
	15	14	157.750±2129.83	b	
	25		139.000±2164.30	de	
	35		118.750±2711.45	g	
	15	21	149.500±1810.06	c	
	25		129.000±2039.09	f	
	35		103.750±3262.12	h	

Discussion

Entomopathogenic nematodes are commonly used in agricultural fields for the purpose of pest control through biological control (Gaugler, 1988; Gaugler et al., 1997; Shapiro-Ilan et al., 2006; Campos-Herrera et al., 2012). However, due to their physiological and morphological characteristics, EPNs are highly susceptible to extreme temperature and humidity conditions (Kung et al., 1991; Grant & Villani, 2003; Lillis et al., 2022; Lillis et al., 2023). Such environmental factors can significantly impact their various attributes, including their efficacy on the host, thereby weakening their overall effectiveness (Shapiro-Ilan et al., 2011; Ulu & Susurluk, 2014; Zhang et al., 2019). Temperature, being one of the most prominent environmental factors, plays a crucial role during the storage and transportation of EPNs. These conditions can greatly influence the survival and quality of the nematodes; therefore, temperature is recognized as one of the most influential environmental factors affecting EPNs (Susurluk & Ehlers, 2008; Ulu et al., 2016; Dede et al., 2022; Dzięgielewska et al., 2023).

Recent studies have mainly focused on the effects of temperature on the efficacy of EPNs on the host, their survival abilities under different temperature conditions, optimal temperature ranges, and longevity at different temperatures (Půža & Mráček, 2007; El-Lakwah & Yousef, 2013; Ulu & Susurluk, 2014; Lephoto & Gray, 2020; Ulu et al., 2021; Nouh, 2022). However, there is limited research on the effects of long-term exposure to different temperatures on their reproductive capacity, the characteristics of hermaphroditic individuals, and eggs (Griffin, 1996; Boff et al., 2000; Mejia-Torres & Saenz, 2013).

Similarly, in a study conducted by Boff et al. (2000) changes in the characteristics (including activity, reproductive capacity, and body length) of IJs belonging to a specific strain of *Heterorhabditis megidis* (Rhabditida: Heterorhabditidae), stored at different temperature values for nearly 70 days, were determined at biweekly intervals. The results revealed that individuals kept at 10 and 15°C exhibited the highest activity, reproductive capacity, and body length. Mejia-Torres & Saenz (2013) conducted a study in which IJs derived from a specific

isolate of the Heterorhabditidae family were incubated at different temperatures for up to 16 weeks. Subsequently, the reproductive capacity, viability, and efficacy of these IJs were determined. It was determined that the optimal temperature range for this isolate was between 20 and 25°C. This finding appears to be in line with the results of the present study.

In the study conducted by Fitters et al. (2001), a specific isolate belonging to the species *H. megidis* was incubated at different temperatures for up to three weeks. This study focused on the efficacy and reproductive capacity of IJs on *Otiorhynchus sulcatus* (Coleoptera: Curculionidae). The findings of this study revealed that species stored below 20°C exhibited higher efficacy and reproductive capacity compared to those stored at or above 20°C. Similarly, in a study conducted by Wang & Grewal (2002), a specific isolate of *H. bacteriophora* was exposed to stress factors such as temperature and drought. Subsequently, the reproductive capacity and viability of the IJs in stock were examined. Based on the obtained data, it was determined that *H. bacteriophora* is sensitive to environmental conditions such as high temperature and drought. A decrease in the reproductive capacity of this species was revealed when exposed to prolonged periods of high temperatures. The results obtained in the present study are in accordance with these findings. The study conducted by Bütüner et al. (2023) the effect of high temperature and storage duration on *H. bacteriophora*, *Steinernema carpocapsae*, and *S. feltiae* (Rhabditida: Steinernematidae) isolates was examined. The results of the study revealed that prolonged storage at high temperatures led to a decrease in the efficacy of these species on their hosts. Additionally, it was observed that the mortality rates in the IJs (infective juveniles) increased proportionally with the duration of exposure to high temperatures. Particularly, the IJs of *H. bacteriophora* were significantly negatively affected by high temperatures and extended storage durations. The results obtained in the present study align with these findings.

According to these results, it has been observed that high temperatures and long-term storage have a negative effect on the body length of hermaphrodite individuals and egg diameters obtained from IJs preserved at different temperatures and days. However, it has been determined that the body lengths of hermaphrodite individuals and egg diameter values obtained at certain temperature values and days are longer than those values in the control group. While there have been studies examining the impact of high temperatures and long-term storage on the reproductive capacity of IJs, no study has been encountered to date regarding the effects of high temperatures and long-term storage on hermaphrodite individuals and eggs. In this respect, the present study has provided valuable data for future studies.

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Original article (Orijinal araştırma)

Acute and chronic exposure risks of insecticide residues in fresh commodities collected from Bursa (Türkiye) province markets during winter season[1]

Kış sezonunda Bursa ili (Türkiye) satış noktalarından toplanan farklı taze tüketim ürünlerindeki insektisit kalıntılarının akut ve kronik risk değerlendirmesi

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Abstract

This study shows the findings about pesticide residues and the associated acute and chronic exposure risks of different fresh commodities collected from different markets located in Bursa province (Türkiye) during 2023 winter season. For this purpose, pesticide residue levels of the collected samples were analysed with LC-MS/MS. Highest levels of insecticide and acaricide residues were detected in some lettuce, parsley, dill, carrot, pear, mandarin and banana samples and they were exceeded the maximum residue limit (MRL). The acute and the chronic exposures to pesticides were assessed by using the highest and the average redidue levels of each pesticide respectively. Highest acute exposure was calculated as acute reference dose (ARfD) exceedance rate and it was 104.27% for indoxacarb in apples, 107.06% and 137.11% for lambda-cyhalothrin in pears and mandarins, and 158.2% for phosmet in pears. For all commodity types, none of the pesticide residues displayed chronic hazard. When the cumulative long-term exposure evaluated, none of the insecticides was found to be risky for adults. The findings showed that the levels of insecticide residues on lettuce, parsley, dill, carrot, apple, pear, mandarin, orange and banana samples collected from Bursa markets in winter 2023 could not be considered as an important public health risk.

Keywords: Acute, chronic, insecticide residues, risk assessment

Öz

Bu çalışma, 2023 yılı kış sezonunda Bursa ili (Türkiye) yerel satış noktalarından toplanan farklı taze tüketim ürünleri üzerindeki pestisit kalıntıları ve bunların tüketiciler üzerine olan akut ve kronik maruziyet risklerine ait bulguları rapor etmektedir. Bu amaçla toplanan örneklerin LC-MS/MS kullanılarak kalıntı düzeyleri tespit edilmiştir. Bulgulara göre, toplanan bazı marul, maydanoz, dereotu, havuç, armut, mandalina ve muz örneklerinde tespit edilen en yüksek insektisit ve akarisit kalıntıları maksimum kalıntı limitlerini (MRL) aşmıştır. Akut ve kronik maruziyetler, pestisitlerin ortalama ve en yüksek kalıntı konsantrasyonları kullanılarak değerlendirilmiştir. En yüksek akut tehlike, akut referans doz aşımı (ARfD) olarak hesaplanmıştır ve bu değer indoxacarb için elmada %104.27, lambda-cyhalothrin için armut ve mandalinada sırasıyla %107.06 ve %137.11 ve phosmet için armutta %158.2 olarak bulunmuştur. Tüm ürünlerde her bir pestisit kalıntısı için kronik tehlike gözlenmemiştir. Kümülatif uzun süreli maruz kalma değerlendirildiğinde, yetişkinler için hiçbir insektisitin risk oluşturmadığı tespit edilmiştir. Bulgular, 2023 yılında Bursa pazarlarından toplanan marul, maydanoz, dereotu, havuç, elma, armut, mandalina, portakal ve muz örneklerinde insektisit kalıntılarının görülmesinin büyük bir halk sağlığı riski olarak değerlendirilemeyeceğini göstermektedir.

Anahtar sözcükler: Akut, kronik, insektisit kalıntıları, risk değerlendirmesi

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Introduction

Türkiye is one of the largest fruit and vegetable producer, following China, India, Brazil and the USA (FAO, 2021). According to the data from Turkish Statistical Institute, 19.5 million tons of fruits and 25.6 million tons of vegetables were produced in 2021 in Türkiye. Previous studies have showed that some vegetables and fruits have protective impacts against the development of serious human diseases such as cardiovascular problems, diabetes, obesity and cancer (Ferretti et al., 2010). Their protective roles could be originated from the various nutrients which contain fiber, vitamins and phytonutrients (Prior, 2003). For these reasons, health authorities encourage that consumers eat at least five portions of fresh fruit and vegetables daily (TÜBER, 2019). Besides health benefits of fruit and vegatables, the agricultural chemicals which are widely used to control pests during their cultivations may lead to health problems for consumers (Baldi et al., 2001; Lozowicka, 2015). Some insecticides have been related with a wide range of human health hazards, ranging from acute to chronic impacts (Calvert et al., 2001; Bhanti & Taneja, 2007). Chronic health effects (such as, various types of cancers, disorders in the endocrine, reproductive system, and embryonic development) may occur years after even minimal exposure to pesticides in the environment, food and water (Berrada et al., 2010; Yousefi et al., 2022). The long-term health problems are particularly serious when these commodities are consumed continuously as fresh and processed foods (Solecki et al., 2005).

In order to protect public health, regular monitoring of insecticide residues and dietary risk assessment are important tasks for human health. For this reason, based on the maximum residue limits (MRL) for each insecticide and commodity, their residues are regularly monitored in fresh foods as they are eaten raw (Ambrus et al., 2023). Nevertheless, the insecticide residues above their MRL may be detected on fresh fruits and vegetables. The reasons for the residues are (1) paying insufficient attention to pre-harvest Interval (PHI), (2) the use of very high dose of pesticides due to development of resistance in pests, (3) the use of pesticide mixtures in order to provide broad spectrum protection against several pests, (4) the application mistakes during pesticide spraying (Waichman et al., 2007; Darko & Akoto, 2008). Recently, European markets are requesting particular specifications such as application of pesticide residues below MRL as well as limitations for multi-residues and indexes for acute and chronic risk assessments. Although the establishment of MRLs is based on good agricultural practices (GAP) data on fresh foods derived from commodities, these are not toxicological limits (Blasco et al., 2006). Nevertheless, exceedance of MRLs is significant violations of GAP, and MRLs can not be considered as reliable tools for the assessment of the acute and chronic risks alone. Therefore, dietary risk assessment of insecticides has recently gained a great attention (Nasreddine & Parent-Massin, 2002; Gebara et al., 2011; Marete et al., 2020; Chen et al., 2011; Balkan & Yılmaz, 2022b). The long term (chronic) dietary risk assessments are made based on daily food consumption and detected pesticide residue data on each commodity. Then, the estimated chronic dietary exposure is compared with the acceptable daily intake (ADI) value which gives the concentration of a chemical that can be consumed over a long period without adverse health effects. For the short-term (acute) dietary risk assessment, the Acute Reference Dose (ARfD) is used to identify possible consumer health risks. The ARfD gives the concentration of a chemical that can be ingested over a short period of time (one meal, one day) without significant risks. For acute assessments one should focus on the edible portion of food commodities on the market, whereas for chronic assessments one should focus on raw agricultural commodities (Brancato et al., 2018).

Some commodities, namely carrot, lettuce, parsley, dill, apple, banana, pear, mandarin and orange are commonly consumed as main fruits and vegetables for Turkish consumers during the winter season. Therefore, assessing the risk of pesticide residues in these commodities intended for human consumption is necessary. One of the significant parameters in the evaluation of acute or chronic dietary risks is the frequency of exposure. The more the consumer is exposed to the chemical, the faced risk is higher. For this reason, in this study, it is desired to focus on the fruits and vegetables that people living in Bursa province consume frequently during the winter period. For this purpose, 223 people were asked about their consumption preferences in the winter period before the study. According to the results of the survey, the most commonly consumed items among Bursa consumers are 5 fruits (apple, pear, banana, mandarin and orange) and 4 vegetables (lettuce, parsley, dill and carrot), which were accepted as the research material. This study,

which analyzes the exposure of consumers during the winter period, has a unique value in this respect. The aims of the current study were to investigate pesticide residues in widely consumed seasonal fruit and vegetable samples collected from the Bursa markets and to conduct acute and chronic health risk assessments for human, based on exposure to the detected residue concentrations determined in 5 fruits and 4 vegetable commodities.

Materials and Methods

Chemicals and reagents

Insecticide standarts (Dr. Ehrenstorfer GmbH, Wesel, Germany) and other solvents and reagents used are of analytical grade. Chemical and toxicological properties of acaricides and insecticides are shown in Table S1 (PPDB, 2023; EU Pesticide Database, 2023). Quick Easy Cheap Effective Rugged Safe (QuEChERS) extraction kits [6 g anhydrous magnesium sulfate ($MgSO_4$) + 1 g anhydrous sodium acetate (NaOAC)] and clean-up kits [1.2 g $MgSO_4$, 0.4 g primary and secondary amines (PSA, 40 µm particle size) + 0.4 g C_{18}] were used.

Instruments and LC-MS/MS conditions

LC-MS/MS device was used for chromatographic analyses (Agilent 1260 Infinity II HPLC System and Agilent 6470 Triple Quadrupole Liquid-Mass Spectrometry). The device is connected with Agilent Poroshell SB-C_{18} (3 mm x 100 mm x 2.7 µm) column. Flow rate, injection volume and total run time were 0.5 mL/min, 1 µL and 15 minutes, respectively. Two mobile phases were used namely A (0.1% formic acid+1mM ammonium fomat in water and B (Metanol). Following gradient program is used: 0-0.05 min. 70% A; 8 min. 5%; 8-12.5 min. 5% A; 12.6 min. 70% A; 12.6-15 min 70% A. Retention times (tR), precursor ion and fragment ions of each acaricides and insecticides are given in Table 1. The other instruments used in the current study are blender (Retsch, GM 300), precise balance (Ohaous, AV812), centrifuge (OHAOUS, FC5706), orbital shaker (Biosan, PSU-10İ), vortex (FAITHFUL, MX-S), micropipets (Eppendorf, K49321I, L17301I, M32978I), and ultra pure water machine (MX-S).

Verification of the analysis

Verification studies were performed in an accredidated analysis laboratory based on the criteria of Analytical Quality Control and Method Validation Procedures for Pesticide Residues Analysis in Food and Feed SANTE 11312/2021, such as linearity, recovery, precision and limit of quantification (LOQ). Calibration (matrix match standards) was performed on blank tomato representing fresh vegetables and fruits (CAC, 2003; SANTE, 2021). Blank tomato samples of 1 kg were homogenized with a blender. For recovery tests, 15 g blank samples were spiked with 100 µL of insecticide spike solutions (in MeCN). Tests were conducted in five replicates (five replicate analytical portions). Linearity was evaluated using six levels ranging from 5 µg to 250 µg L^{-1} prepared with MeCN. Matrix matched calibration curve was used to quantify insecticides. Recovery and precision parameters were determined for two spiking concentrations (10 and 50 µg kg^{-1}) across five different time points and by two different analysts. Calibration analysis results, retention times (tR) and selected ion groups of the analyzed insecticides were given in Table 1. Matrix-matched calibration curves of the 38 insecticides were linear (R^2 = 0.998-0.999). The retention times (tR) ranged between 0.99-10.83 min. The regression equations of the matrix-matched calibration curves were used for quantification of the insecticides. Trueness and precision were assessed based on recovery, repeatability and reproducibility parameters (Tiryaki, 2016; SANTE, 2021). Detection limits (LODs), LOQs, recovery rates (%) and relative standart deviations for repeatability and reproducibility (RSD_r and RSD_{wr} %) of all insecticides were found compatible with SANTE 2021 criteria. The LOQ values were quite lower than the MRLs of each insectides (Table 5). The recovery rates of the insecticides for two spike levels were calculated between 90.46-117.41 and 96.16-115.55, respectively. The highest RSD_r and RSD_{wr} were 12.64 and 17.56 for 10 µg kg^{-1} and 7.50 and 8.30 for 50 µg kg^{-1} respectively. All vertification parameters were compatible with SANTE 11312/2021 criteria (SANTE, 2021).

Table 1. Calibration analysis results, retention times (tR) and selected ion groups and their collision energies of the analyzed pesticides

Pesticide	tR* min	Calibration equation y=a+bx	Determination co-efficient, R^2	Precursor ion, m/z (CE**)	Fragment ion, m/z (CE)
Acetamiprid	2.67	y=18094.5x+15269.9	0.9999	223.1	126.1 (17), 56.2 (11)
Abamectin	9.95	y=466.678172x+480.11	0.9993	895.2	327.3 (50), 449.3 (48)
Bifenazate	7.45	y=21034.5x+9728.6	0.9996	301.2	170.0 (20), 198.0 (5)
Bifenthrin	10.25	y=2251.4x+2674.5	0.9992	440.2	166.1 (20), 181.1 (7)
Chlorantraniliprole	5.73	y=1256.6x+1590.5	0.9994	484.0	283.9 (21), 285.9 (21)
Chlorfenvinphos	8.21	y=853.35x+6375.48	0.9996	359.1	155.1 (7), 99.1 (29)
Chlorpyrifos	9.26	y=4163.3x+827.61	0.9997	351.9	199.9 (15), 197.9 (15)
Chlorpyrifos methyl	8.58	y=1450.84x-511.92	0.9980	321.9	125 (17), 289.9 (11)
Clofentezine	8.39	y=7331.31x-3939.88	0.9988	303.1	102.1 (37), 138.1 (9)
Clothianidin	2.71	y=1586.6x-1078.8	0.9993	250.1	132 (15), 169.1 (13)
Cypermethrin	8.77	y=805.29x-670.28	0.9994	433.0	126.8 (34), 191.0 (12)
Cyromazine	0.99	y=12507.8x-1505.7	0.9994	167.3	85.2 (17), 125.2 (15)
Deltamethrin	8.76	y=436.67x-846.63	0.9996	522.8	280.6 (12), 505.8 (6)
Diflubenzuron	7.81	y=1993.8x+2330.67	0.9997	310.9	141 (15), 158 (6)
Emamectin B1a	8.81	y=18167.36x-2108.51	0.9991	886.5	126.0 (40), 158.0 (40)
Ethoprophos	7.68	y=13696.7x+12063.0	0.9998	243.0	130.9 (20), 172.9 (10)
Etoxazole	9.37	y=12903.5x+8505.09	0.9996	360.0	113.0 (23), 141.0 (15)
Fenbutatin oxide	10.83	y= -3261.8x-4249.4	0.9987	519.3	197 (55), 351.1 (35)
Fenvalerate	9.67	y=252.2x+37.99	0.9996	439.0	167 (14), 169 (10)
Flubendiamide	7.91	y=2762.65x+3704.33	0.9994	681.0	253.9 (40), 273.9 (24)
Imidacloprid	2.54	y=2626.98x+2617.6	0.9994	256.1	175.0 (12), 209.0 (10)
Indoxacarb	8.55	y=708.68x+67.82	0.9990	528.1	150.0 (16), 203.0 (36)
Lambda cyhalothrin	7.88	y=425.78x-65.61	0.9993	467.1	225.0 (14), 450.0 (6)
Malathion	6.33	y=6662.9x-116.79	0.9995	330.9	127.0 (4), 285.0 (38)
Metaflumizone	8.90	y=9023.9x+25059.9	0.9998	505.0	117.0 (48), 302.0 (10)
Methoxyfenozide	7.23	y=8824.6x-2323.0	0.9993	369.1	133.1 (28), 149 (14)
Novaluron	8.65	y=1028.11x-1218.79	0.9975	492.7	140.7 (46), 158.0 (12)
Phosmet	6.64	y=950.85x+292.58	0.9998	317.9	133 (28), 160 (21)
Primicarb	4.88	y=21360.3x-20650.8	0.9995	239.2	72.1 (15), 182.1 (11)
Pirimiphos methyl	8.40	y=45364.5x-2259.3	0.9981	360.2	108.1 (31), 164.1 (19)
Pyridaben	9.75	y=34981.4x+19361.8	0.9988	365.2	147.1 (23), 309.1 (7)
Pyriproxyfen	9.18	y=50164.4x+21561.5	0.9992	322.2	96.1 (11), 185.0 (19)
Spinosad	7.32	y=3266.3x-3304.6	0.9989	732.5	98.2 (55), 142.1 (35)
Spirodiclofen	8.66	y= 5572x-8635.00	0.9985	411.0	71.0 (16), 313.0 (11)
Spirotetramat	7.54	y= 4124.6x-1872.5	0.9992	374.1	302.1 (23), 330.1 (21)
Quinalphos	8.49	y= 5142.1x-3133.1	0.9997	146.1	91 (24), 118 (10)
Tau fluvalinate	8.92	y=2080.2x+3139.7	0.9997	503.1	181.1 (25), 208.1 (15)
Thiacloprid	3.09	y=18017.3x-13317.1	0.9994	253.0	90.0 (35), 126.0 (16)

*tR, retention time (min); ** CE, Collision Energy (V)

Consumer surveys

Both online and face to face questionnaire surveys applied in Bursa province between November 2022 and February 2023. For online surveys, the google form link was shared via mails and various social media networks. The survey consisted of 223 respondents. The respondents consisted of 68% females and 32% males (age 16 to 70) and the largest proportion (74%) was comprised of middle-aged respondents (age 23 to 45). Mean body weight of female and male respondents determined as 64.86 kg and 81.16 kg respectively (female and male mean body weight 70.05 kg). The survey questions were provided in the Table 2.

Table 2. Questions in consumer questionnaire surveys

Questions		Answers	
Do you consume X commodity?		Yes: ……..	No:…….
If yes; What is your consumption frequency?	everyday day:…	per week:…	days per month: ...
Specify your individual daily consumption amount for X commodity in portions:		…….. portions*	
Specify the maximum amount of X commodity that you can consume at one time		…….. portions	

* The following data were used in the grammatical translation of the survey results (TÜBER 2019):
2 cups or 2 fists or 1 large bowl = 1 standard portion=75 g of dill; 2 cups or 2 punches or 1 large bowl = 1 standard portion= 75 g of lettuce/or parsley; 1 medium size or 1 cup or 1 punch = 1 standard portion= 150gr of carrots; 1 medium size; 7 cm in diameter or 1 fist size = 1 standard portion= 150gr apples/oranges; 1 small size or 5 pieces = 1 standard portion= 150gr pears; 2 medium size-6 cm diameter = 1 standard portion= 150gr mandarins; 1 hand length or sliced 2/3 small bowl= 100g of banana.

Collecting samples

The agricultural commodity samples, namely carrot, lettuce, parsley, dill, apple, banana, pear, mandarin and orange, were collected from different local open markets and supermarkets of Bursa province for 4 weeks during February 2023. Each commodity sample (totally 99) of about 1 kg were homogenized and 15 g analytical portions (in triplicates) were obtained for the analysis. Extraction and cleaning procedures are shown in Figure 1 (Lehotay, 2007). Spiked and collected samples were analysed in LC-MS/MS system.

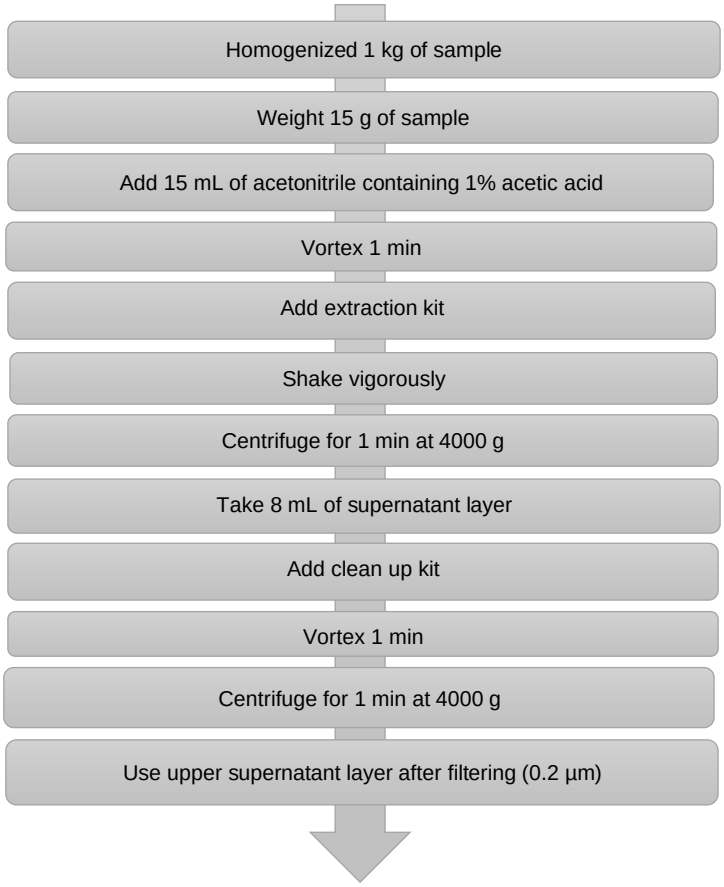

Figure 1. Analytical steps for extraction and cleaning (QuEChERS-AOAC Official Method 2007.01).

Methodology for assessing dietary intake of insecticides

Estimation of acute and chronic risks to consumer health were performed based on the previous studies (Chen et al., 2011; Kazar Soydan et al., 2021). The dietary exposure to insecticides has been calculated in order to assess the acute consumer health risk for adults.

The following input values are required to calculate the actual acute exposure:

- Maximum residue level of each insecticide obtained from analysis of the above-mentioned samples of 5 fruits and 4 vegetables in 2023
- Annual fruit/vegetable consumptions per person (97.5th percentile of eaters) were determined based on the survey results of present study (Table 3).
- The average body weight of an adult is taken as 70 kg based on our survey and TUIK data (2022).

The estimated short-term intake (ESTI) was calculated based on the following formula:

$$ESTI = \frac{LP\ x\ MRL\ x\ CF\ x\ PF\ x\ VF}{BW} \quad (1)$$

Where, *LP*, Large portion reported (kg day^{-1}) (97.5th percentile of eaters); *MR*L, Maximum residue level for each commodity (mg kg^{-1}); *CF,* Conversion factor residue definition enforcement to residue definition risk assessment; *PF*, Processing factor or peeling factor; *VF*, Variability factor was used as 7 for orange, mandarin, apple, pear, banana; 5 for carrot and lettuce; 1 for parsley and dill according to Brancato et al. (2018); *BW*, mean body weight for the subgroup of the population related to mean consumption (kg).

An estimate of pesticide intake in the diet was compared to the ARfD of each insecticide (Table S1). The acute hazard index (*aHI*) was calculated as follows:

$$aHI = \frac{ESTI}{ARfD} \quad (2)$$

aHI ≤ 100% indicates that adverse effects are not likely to occur and thus can be considered to have negligible hazard.

The dietary exposure to pesticides has been calculated in order to assess the chronic consumer health risk for the adults indicated in EFSA PRIMo revision 3 (Brancato et al., 2018).

The estimated daily intake (*EDI*) of pesticide residues was calculated with the following formulas:

$$Cp,f = \frac{Cavg.pos.p.f\ x\ Npos.p.f}{Np,f} \quad (3)$$

$$EDI = Cp_{,f}\ .\ MCf \quad (4)$$

Where: $C_{p,f}$, the average content (mg kg^{-1}) of pesticide p in commodity f; $C_{avg,pos,p,f,}$ the average content (mg kg^{-1}) of pesticide p in commodity f with detected residues; $N_{pos,p,f,}$ the number of samples with detected residues; $N_{p,f,}$ the number of commodities analyzed for the pesticide *EDI*: the estimated daily intake (mg kg^{-1} bw day^{-1}) for each combination of pesticide p and commodity f; MC_f, is the average consumption rate of that commodity (g^{-1} bw day^{-1}) from obtained present study survey results.

The chronic risk assessment of intakes compared to pesticide toxicological data was performed by calculating the Chronic Hazard Quotient (cHQ) by dividing EDI by the relevant acceptable daily intake (ADI):

$$cHQ = \frac{EDI}{ADI}\ 100\% \quad (5)$$

The level of concern for cHQ value was set as 1. Therefore, c*HQ ≤ 1* indicates that adverse effects are not likely to occur and thus can be considered to have negligible hazard.

Results and Discussion

Consumer survey

The survey results on vegetable and fruit consumption behaviour of Bursa community were given in Table 3. This survey was conducted due to the lack of food consumption data for Turkish citizen and the obtained data was used in the assesment of chronic and acute dietary risks. Two significant data were provided from this survey for chronic and acute dietary risk assesments, respectively: (1) Daily consumption data (gr/person/day) and (2) Maximum consumption amount in a single meal (g/person). When daily consumption rates for each commodity were compared with the Turkish Statistical Institute (TUIK) database (TUIK, 2021), the values for carrot, pear and apple were similar, where as lettuce, orange, mandarin and banana values were higher. When the results were compared with the EU community, consumption rates for lettuce, parsley, orange and banana were similar but carrot, apple and pear consumption of Bursa community was lower (Brancato et al., 2018). Maximum consumption in single meal values could not be compared with the TUIK database since there is no data regarding the Turkish community. However, maximum consumption results for all commodites were found lower compared with those consumed by EU communities (Table 3). The discrepancy of consumption data from EU commodity, could be due to the differences in factors like consumption habits, geographical origin and availability of the product, nutrition regimes, enjoyment of food (Kapoor & Kar, 2022), and also sociodemographic characteristics such as age, gender, education and income (Mata et al., 2023). Since there is limited data about the comsumption habits of the Turkish community, a simple questionnaire like the one used in this research has upgraded the accuracy and reliability of the acute and chronic risk assessment for the community.

Table 3. Consumer preferences for fruits and vegetables in Bursa province

	Daily consumption (g/person/day)			Daily consumption (g/bw/day)*	Maximum consumption in single meal (g/person)		Maximum consumption in single meal** (kg/day)
Commodity	Bursa community	EU community (Brancato et al., 2018)	Turkish community (TUIK, 2021)	Bursa community	Bursa community	EU community (Brancato et al., 2018)	Bursa community
Lettuce	25.03	36.69	14.79	0.357	93.35	159.80	0.09
Parsley	15.48	2.54	unknown	0.221	57.82	79.90	0.03
Dill	5.76	37.13	unknown	0.082	33.96	unknown	0.24
Carrot	52.93	29.93	14.25	0.756	238.61	259.40	0.06
Apple	61.60	202.18	85.45	0.879	217.22	664.00	0.22
Pear	13.65	44.26	13.15	0.195	121.66	781.70	0.12
Orange	67.57	65.124	26.85	0.965	263.68	996.50	0.26
Mandarin	75.28	10.64	21.09	1.075	283.30	720.94	0.28
Banana	48.83	54.78	24.93	0.697	184.53	611.00	0.18

*MCf, is the average consumption rate of that commodity; **LP, Large portion.

Residues in the different commodities

The co-occurrence of insecticide residues is given in detail in Table 4. Among fruit samples, highest rate of samples with insecticide residues were calculated in apple (100%), pear (90.91%) and lettuce (90.91%). Except banana samples, residues of two or more insecticides were found in all other commodities. The five commodities, namely parsley, dill, apple, pear and mandarin, contained 4 and more insecticide residues with the ratios of 18.18, 36.36, 36.36, 36.36 and 27.27%, respectively. Three commodities, such as, dill, pear and mandarin, were contaminated with seven pesticide residues (with 9.1, 18.2 and 9.1%, respectively). Similarly, survey studies conducted in other countries reported presence of multiple pesticide residues (four or more) in different commodities such as pear, parsley, mandarin, orange, banana, apple (Chen, 2011; Ersoy et al., 2011; Esturk et al., 2014; Al-Shamary et al., 2016, El Hawari et al., 2019; Al-Nasir et al., 2020; Kazar Soydan et al., 2021; Kottadiyil et al., 2023). In accordance with previous studies,

the most frequent combinations of pesticides detected in the same sample were acetamiprid, cypermethrin, deltamethrin and imidacloprid (Chen, 2011; Ersoy et al., 2011; Jallow et al., 2017; El Hawari et al., 2019; Kumari, 2019; Kazar Soydan et al., 2021; Kottadiyil et al., 2023).

Table 4. Number of samples with multiple insecticide residues for each commodity

Commodity	Rate of samples with multiple residues (%)								
	0	1	2	3	4	5	6	7	Total (%)
Lettuce	9.09	63.64	27.27	-	-	-	-	-	90.99
Parsley	54.55	9.09	-	18.18	9.09	9.09	-	-	45.45
Dill	36.36	9.09	9.09	9.09	18.18	9.09	-	9.09	63.64
Carrot	63.64	27.27	9.09	-	-	-	-	-	36.36
Apple	-	-	36.36	27.27	18.18	9.09	9.09		100.00
Pear	9.09	18.18	9.09	27.27	9.09	9.09	-	18.18	90.99
Orange	72.73	9.09	18.18	-	-	-	-	-	27.27
Mandarin	45.45	18.18	-	9.09	18.18	-	-	9.09	54.55
Banana	54.55	45.45	-	-	-	-	-	-	45.45

MRL levels of 38 insecticides for each commodity and MRL exceedance rate (fold) were given in Table 5. The most of the MRL levels were provided from Turkish Food Codex (TGK, 2021). Since some insecticides used in certain commodities in Türkiye are not registrated, their MRL levels were obtained from the EU authorities (EU Pesticide Database, 2023). In the present study, insecticide residues in some of samples exceeded their MRL levels. In our study, 16.2% of the samples exceeded the approved MRL levels of detected insecticide and acaricides. Considering the highest residue concentrations detected in the current study, fenbutatin oxide and imidacloprid residues in lettuce exceeded their MRLs 2.6 and 2.8 folds, respectively. Imidacloprid MRL exceedance was also reported in nectarin samples (Serbes & Tiryaki, 2023). In parsely, chlorpyrifos and pirimiphos methyl residues was detected above 1.30 and 5.75 folds of their MRLs, respectively. The highest MRL exceedance was observed in dill with cypermethrin (1.26 folds), ethoprophos (4.85 folds), imidacloprid (1.26 folds), malathion (5.85 folds) and spirotetramat (1.08 folds). In carrot, one of the two insecticides exceeded MRL level (Imidacloprid 2.80 folds). In fruits, there were relatively fewer instances of insecticides exceeding their MRL levels: diflubenzuron (4.50 folds) in pear; chlorpyrifos (9.50 folds) and fenvalerate (1.35 folds) in mandarin and tau-fluvalinate (2.90 folds) in banana (Table 6). Previous studies reported that 8.4-22% of fruit and vegetable samples contained pesticide residues above the approved MRL levels (Chen et al., 2011, EL-Saeid & Selim, 2013; Jallow et al., 2017; Mebdoua et al., 2017; Algharibeh & Al Fararjeh, 2019; Gondo et al., 2021; Balkan & Kara, 2022; Wang et al., 2022). Similarly, Estürk et al. (2014) and Balkan & Yılmaz (2022a) also reported MRL exceedance in some pesticides detected in lettuce, parsley and various leafy vegetables.

Table 5. MRLs of insecticides

Pesticide	LOQ (µg kg $^{-1}$)	MRL (mg kg^{-1})*								
		L	PA	D	C	A	PE	O	M	B
Acetamiprid	5.55	1.5	3.0	0.05	-	0.8	0.4	0.9	0.9	-
Abamectin	4.37	-	-	0.05	-	-	0.03	-	-	-
Bifenazate	6.50	-	-	-	-	0.7	-	-	-	-
Bifenthrin	6.86	-	-	-	-	-	-	-	0.05	-
Chlorantraniliprole	7.97	-	-	-	-	0.5	0.5	-	-	-
Chlorfenvinphos	9.79	-	-	-	-	-	-	-	0.01	-
Chlorpyrifos	6.97	-	0.01	-	-	-	-	-	0.01	-
Chlorpyrifos methyl	9.03	-	-	0.01	0.04	-	-	-	-	-
Clofentezine	8.03	-	-		-	0.5	-	-	-	-
Clothianidin	6.57	-	-	0.2	-	-	-	-	-	-
Cypermethrin	6.63	-	-	0.1	-	1	1.0	-	2.0	-
Cyromazine	5.58	0.01	-		-	-	-	-	-	-
Deltamethrin	7.39	0.5	2.0	0.1	-	0.2	0.1	-	-	-
Diflubenzuron	9.68	-		-	-	5	0.01	-	-	-
Emamectin B1a	6.63	-	0.2	-	-	-	-	-	-	-
Ethoprophos	6.75	-	-	0.02	-	-	-	-	-	-

Table 5. Continued

Pesticide	LOQ (µg kg $^{-1}$)	MRL (mg kg^{-1})*								
		L	PA	D	C	A	PE	O	M	B
Etoxazole	7.63	-	-	-	-	-	-	-	0.1	-
Fenbutatin oxide	7.08	0.01	0.02	-	-	-	-	-	-	-
Fenvalerate	9.04	-	-	-	-	0.05	-	-	0.02	-
Flubendiamide	6.93	-	-	-	-	0.8	-	-	-	-
Imidacloprid	5.68	0.01	0.05	0.05	0.01	-	0.5	-	-	-
Indoxacarb	9.95	-	-			0.5	-	-	-	-
Lambda cyhalothrin	7.90	-	-	0.3	-	-	0.08	0.2	-	-
Malathion	8.69	-	-	0.02	-	-	-	-	2.0	-
Metaflumizone	9.24	-	-	0.1	-	-	-	-	-	-
Methoxyfenozide	6.63	-	-	-	-	2	-	-	-	-
Novaluron	7.70	-	-	-	-	0.01	0.01	-	-	-
Phosmet	9.78	-	-	-	-		0.5	-	-	-
Pirimicarb	5.70	-	3.0	5.0		0.5	-	-	-	-
Pirimiphos methyl	5.96	-	0.02	3.0	-	-	-	-	-	-
Pyridaben	5.94	-	0.02			0.9	-	-	0.3	-
Pyriproxyfen	5.98	-	-	-	-	-	0.2	-	0.6	0.7
Spinosad	6.84	10	-	-	-	-	-	-		-
Spirodiclofen	6.20	-	-	-	-	0.8	0.8	-	0.4	-
Spirotetramat	7.91	-	4.0	0.1		-	-	1.0	-	-
Quinalphos	7.36	-	-	-	-	-	-	0.01	-	0.01
Tau fluvalinate	8.58	-	-	-	-	0.3		0.4	0.4	-
Thiacloprid	5.53	-	-	-	-	0.3	0.3	-	-	-

*MRL levels were obtained from TGK or from EU database: -: not detected in this commodity, L: lettuce, PA: parsely, D: dill, C: carrot, A: apple, PE: pear, O: orange, M: mandarin, B: banana.

Table 6. MRL exceedance rate of the highest insecticide residues

Pesticide	MRL exceedance rate (fold)								
	L	PA	D	C	A	PE	O	M	B
Acetamiprid	0.11	0.03	5.44	-	0.02	0.04	0.01	0.01	-
Abamectin	-	-	0.32	-	-	0.17	-	-	-
Bifenazate	-	-	-	-	0.02	-	-	-	-
Bifenthrin	-	-	-	-	-	-	-	0.88	-
Chlorantraniliprole	-	-	-	-	0.04	0.02	-	-	-
Chlorfenvinphos	-	-	-	-	-	-	-	0.90	-
Chlorpyrifos	-	1.30	-	-	-	-	-	9.50	-
Chlorpyrifos methyl	-	-	4.10	0.53	-	-	-	-	-
Clofentezine	-	-		-	0.09	-	-	-	-
Clothianidin	-	-	0.04	-	-	-	-	-	-
Cypermethrin	-	-	1.26	-	0.02	0.01	-	0.01	-
Cyromazine	0.40	-	-	-	-	-	-	-	-
Deltamethrin	0.13	0.01	0.21	-	0.03	0.05	-	-	-
Diflubenzuron	-	-	-	-	0.01	4.50	-	-	-
Emamectin B1a	-	0.22	-	-	-	-	-	-	-
Ethoprophos	-	-	4.85	-	-	-	-	-	-
Etoxazole	-	-	-	-	-	-	-	0.05	-
Fenbutatin oxide	2.60	0.35	-	-	-	-	-	-	-
Fenvalerate	-	-	-	-	0.08	-	-	1.35	-
Flubendiamide	-	-	-	-	0.01	-	-	-	-
Imidacloprid	2.80	0.18	1.26	2.80	-	0.09	-	-	-
Indoxacarb	-	-	-	-	0.05	-	-	-	-
Lambda cyhalothrin	-	-	0.17	-	-	0.55	0.13	-	-
Malathion	-	-	5.85	-	-	-	-	0.08	-
Metaflumizone	-	-	0.26	-	-	-	-	-	-
Methoxyfenozide	-	-	-	-	0.01	-	-	-	-
Novaluron	-	-	-	-	1.00	0.70	-	-	-
Phosmet	-	-	-	-	-	0.03	-	-	-
Pirimicarb	-	0.01	0.08	-	0.02	-	-	-	-
Pirimiphos methyl	-	5.75	0.17	-	-	-	-	-	-
Pyridaben	-	0.75	-	-	0.02	-	-	0.06	-
Pyriproxyfen	-	-	-	-	-	0.33	-	0.01	0.01
Spinosad	0.05	-	-	-	-	-	-	-	-
Spirodiclofen	-	-	-	-	0.01	0.07	-	0.01	-
Spirotetramat	-	0.01	1.08	-	-	-	0.01	-	-
Quinalphos	-	-	-	-	-	-	0.40	-	2.90
Tau fluvalinate	-	-	-	-	0.01	-	0.02	0.03	-
Thiacloprid	-	-	-	-	0.09	0.15	-	-	-

-, not detected in this commodity: L: Lettuce, PA: parsely, D: dill, C: carrot, A: apple, PE: pear, O: orange, M: mandarin, B: banana.

Chronic and acute dietary risk assessments in different commodities

For the risk assessment of insecticide and acaricide residues in each commodity, Cpf (the average content of pesticide p in commodity f) and HR (highest residue) for fruits and vegetables were given in Tables 7 and 8 respectively. The ARfD and ADI values for each pesticide were previously given in Table S1. The other important parametres, daily consumption (MCf) and maximum consumption in single meal (LP) were also shown in Table 3. Using all these parameters, the estimated daily intake (EDI) for chronic risk and the estimated short-term intake (ESTI) for acute risk were calculated. Thus, the chronic hazard quotient (cHQ) and acute hazard index (aHI) for adults were listed in Tables S2 and S3. According to the findings of the current study, the chronic hazard was not observed for any of the insecticides in all commodities. The cHQ of many pesticides were close to zero or <0.010. The highest cHQ values were 0.1286 for emamectin B1a in parsley, 0.1813 for ethoprophos in dill and 0.1368 in chlorfenvinphos for mandarin. Moreover, when the cumulative long-term exposure (total cHQ) was evaluated, none of the insecticides was found risky for adults.

Table 7. Mean and highest insecticide residue levels detected in fruits

Pesticide	Commodity									
	Apple		Pear		Orange		Mandarin		Banana	
	Cp.f	HR	Cp.f	HR	Cp.f	HR	Cp.f	HR	Cp.f	HR
Acetamiprid	0.001	0.019	0.001	0.017	0.001	0.012	0.001	0.005	-	-
Abamectin	-	-	0.001	0.005	-	-	-	-	-	-
Bifenazate	0.001	0.011	-	-	-	-	-	-	-	-
Bifenthrin	-	-	-	-	-	-	0.004	0.044	-	-
Chlorantraniliprole	0.001	0.019	0.001	0.009	-	-	-	-	-	-
Chlorfenvinphos	-	-	-	-	-	-	0.001	0.009	-	-
Chlorpyrifos	-	-	-	-	-	-	0.009	0.095	-	-
Chlorpyrifos methyl	-	-	-	-	-	-	-	-	-	-
Clofentezine	0.004	0.044	-	-	-	-	-	-	-	-
Clothianidin	-	-	-	-	-	-	-	-	-	-
Cypermethrin	0.001	0.017	0.001	0.008	-	-	0.001	0.008	-	-
Cyromazine	-	-	-	-	-	-	-	-	-	-
Deltamethrin	0.001	0.006	0.001	0.005	-	-	-	-	-	-
Diflubenzuron	0.005	0.057	0.005	0.045		-	-	-	-	-
Emamectin B1a	-	-	-	-	-	-	-	-	-	-
Ethoprophos	-	-	-	-	-	-	-	-	-	-
Etoxazole	-	-	-		-	-	0.001	0.005	-	-
Fenbutatin oxide	-	-	-	-	-	-	-	-	-	-
Fenvalerate	0.001	0.004	-	-	-	-	0.003	0.027	-	-
Flubendiamide	0.001	0.011	-	-	-	-	-	-	-	-
Imidacloprid	-	-	0.003	0.047	-	-	-	-	-	-
Indoxacarb	0.001	0.024	-	-	-	-	-	-	-	-
Lambda cyhalothrin	-	-	0.002	0.044	0.002	0.026	-	-	-	-
Malathion	-	-	-	-	-	-	0.006	0.155	-	-
Metaflumizone	-	-	-	-	-	-	-	-	-	-
Methoxyfenozide	0.001	0.015	-	-	-	-	-	-	-	-
Novaluron	0.001	0.01	0.001	0.007	-	-	-	-	-	-
Phosmet	-	-	0.001	0.013	-	-	-	-	-	-
Pirimicarb	0.001	0.009	-	-	-	-	-	-	-	-
Pirimiphos methyl	-	-	-	-	-	-	-	-	-	-
Pyridaben	0.001	0.011	-	-	-	-	0.002	0.018	-	-
Pyriproxyfen	-	-	0.003	0.065	-	-	0.001	0.006	0.001	0.005
Spinosad	-	-	-	-	-	-	-	-	-	-
Spirodiclofen	0.001	0.008	0.003	0.052	-	-	0.001	0.005	-	-
Spirotetramat	-	-	-	-	0.001	0.010	-	-	-	-
Quinalphos	-	-	-	-	0.001	0.004	-	-	0.002	0.029
Tau fluvalinate	0.001	0.004	-	-	0.001	0.006	0.001	0.013	-	-
Thiacloprid	0.002	0.029	0.003	0.044	-	-	-	-	-	-

Table 8. Mean and highest insecticide residue levels detected in vegetables

Pesticide	Commodity							
	Lettuce		Parsley		Dill		Carrot	
	Cp.f	HR	Cp.f	HR	Cp.f	HR	Cp.f	HR
Acetamiprid	0.009	0.171	0.007	0.103	0.014	0.272	-	-
Abamectin	-	-	-	-	0.002	0.016	-	-
Bifenazate	-	-	-	-	-	-	-	-
Bifenthrin	-	-	-	-	-	-	-	-
Chlorantraniliprole	-	-	-	-	-	-	-	-
Chlorfenvinphos	-	-	-	-	-	-	-	-
Chlorpyrifos	-	-	0.001	0.013	-	-	-	-
Chlorpyrifos methyl	-	-	-	-	0.002	0.041	0.002	0.021
Clofentezine	-	-	-	-	-	-	-	-
Clothianidin	-	-	-	-	0.001	0.008	-	-
Cypermethrin	-	-	-	-	0.012	0.126	-	-
Cyromazine	0.001	0.004	-	-	-	-	-	-
Deltamethrin	0.002	0.066	0.002	0.024	0.001	0.021	-	-
Diflubenzuron	-	-	-	-	-	-	-	-
Emamectin B1a	-	-	0.003	0.043	-	-	-	-
Ethoprophos	-	-	-	-	0.009	0.097	-	-
Etoxazole	-	-	-	-	-	-	-	-
Fenbutatin oxide	0.0024	0.026	0.001	0.007	-	-	-	-
Fenvalerate	-	-	-		-	-	-	-
Flubendiamide	-	-	-		-	-	-	-
Imidacloprid	0.003	0.028	0.001	0.009	0.006	0.063	0.002	0.028
Indoxacarb	-	-	-		-	-	-	-
Lambda cyhalothrin	-	-	-	-	0.004	0.05	-	-
Malathion	-	-	-		0.006	0.117	-	-
Metaflumizone	-	-	-		0.002	0.026	-	-
Methoxyfenozide	-	-	-		-	-	-	-
Novaluron	-	-	-		-	-	-	-
Phosmet	-	-	-		-	-	-	-
Pirimicarb	-	-	0.003	0.029	0.021	0.396	-	-
Pirimiphos methyl	-	-	0.006	0.115	0.045	0.496	-	-
Pyridaben	-	-	0.001	0.015	-	-	-	-
Pyriproxyfen	-	-	-		-	-	-	-
Spinosad	0.0424	0.466	-		-	-	-	-
Spirodiclofen	-	-	-		-	-	-	-
Spirotetramat	-	-	0.002	0.025	0.009	0.108	-	-
Quinalphos	-	-	-		-	-	-	-
Tau fluvalinate	-	-	-		-	-	-	-
Thiacloprid	-	-	-		-	-	-	-

The highest total cHQ values were observed for chlorfenvinphos (0.1368), chlorpyrifos (0.1189), emamectin B1a (0.1286), ethoprophos (0.1813), lambda cyhalothrin (0.1233), pirimiphos methyl (0.1268) when all the commodities were considered together. Among these insecticides, chlorfenvinphos and chlorpyrifos were banned in Türkiye in 2010 and 2020, respectively (BKU, 2023). Similarly, the chronic risks for the detected residues of these insecticides were also found negligible for human health with the previous studies conducted with peach, apple, pepper, tomato and cucumber by different reserchers (Mebdoua et al., 2017; El Hawari et al., 2019; Camara et al., 2020; Catak & Tiryaki 2020; Dulger & Tiryaki 2021; Zhang et al., 2021). The highest acute hazard index values obtained with this study exceeded ARfD for adults and calculated as 104.27% for indoxacarb in apples, 158.2% for phosmet in pears and 107.06% and 137.11% for lambda cyhalothrin in pears and mandarin, respectively. Acute toxicity risks of indoxacarb, phosmet and lambda-cyhalothrin were also reported by different previous studies (Mebdoua et al., 2017; El Hawari et al., 2019). Based on the WHO hazard classification, indoxacarb, lambda-cyhalothrin and phosmet are moderately hazardous insecticides (Classs II, Table S1). Although the highest residues of some insecticides, namely chlorpyrifos, cypermethrin, diflubenzuron, ethoprophos, fenbutatin oxide, fenvalerate, imidacloprid,

tau-fluvalinate, malathion, pirimiphos methyl and spirotetramat exceeded their MRL levels, the risk assessment in the present study showed that there were no acute and chronic dietary risks for these agricultural commodities. Acute risk assessments of chlorantraniliprole, chlorfenvinphos, clofentezine, diflubenzuron, etoxazole, fenvalerate, novaluron, pyriproxyfen, spirodiclofen and quinalphos could not perform due to the lack of ARfD values of these compounds in the EU Pesticide and PPDB Databases (Table S1).

Although insecticide residues detected in some products in this study exceeded the MRL levels determined for them; none of these compounds displayed a serious health risk for the consumer. No chronic risk has been determined for any insecticide, either on a product basis or cumulatively. Acute dietary risks were calculated for only 3 crops and 3 insecticides. This has shown that risks may arise from time to time due to wrong agricultural practices in the field. For this reason, it is important for public health to carry out monitoring studies regularly and to reveal the risks, as in this study.

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Supplementary Tables

Table S1. Chemical and toxicological properties of acaricides and insecticides

Pesticide	Mode of action	Acceptable daily intake (mgkg^{-1} bwday^{-1})	Acute reference dose (mgkg^{-1} bwday^{-1})	for mammals oral acute LD_{50} (mg kg^{-1} bwday^{-1})	for mammals dermal LD_{50} (mgkg^{-1} bwday^{-1})	for mammals inhalation LD_{50} (mgkg^{-1} bw)	WHO clasification
Acetamiprid	Insecticide	0.025	0.025	>1.15	2000	146	II
Abamectin	Acaricide/Insecticide	0.0012	0.005	8.7	1914	>0.021	III
Bifenazate	Acaricide	0.01	0.1	>4.4	2000	>5000	U
Bifenthrin	Acaricide	0.015	0.03	54.5	2000	1.01	III
Chlorantraniliprole	Insecticide	1.56	-	>5.1	5000	>5000	U
Chlorfenvinphos	Insecticide	0.0005	-	12	31	0.05	III
Chlorpyrifos	Insecticide	0.001	0.005	66	1250	0.1	III
Chlorpyrifosmethyl	Insecticide	0.01	0.1	>0.67	2000	5000	III
Clofentezine	Acaricide	0.02	-	>5200	2100	>5.2	III
Clothianidin	Insecticide	0.097	0.1	>500	2000	>5.54	III
Cypermethrin	Insecticide	0.05	0.2	3.56	2000	287	II
Cyromazine	Insecticide	0.06	0.1	3387	3100	>3.6	III
Deltamethrin	Insecticide	0.01	0.025	0.6	2000	87	III
Diflubenzuron	Insecticide	0.1	-	>4640	2000	>2.5	III
Emamectin B1a	Insecticide	0.01	0.01	0.582	439	81.5	NL
Ethoprophos	Insecticide	0.0004	0.01	40	7.9	0.123	III
Etoxazole	Acaricide	0.04	-	>1.09	2000	>5000	NL
Fenbutatin oxide	Acaricide	0.05	0.1	>3000	2000	0.046	III
Fenvalerate	Insecticide	0.02	-	451	1000	>0.101	III
Flubendiamide	Insecticide	0.017	0.1	>0.0069	2000	>2000	III
Imidacloprid	Insecticide	0.06	0.08	>0.069	5000	131	II
Indoxacarb	Insecticide	0.005	0.005	>4.2	5000	179	II
Lambdacyhalothrin	Insecticide	0.0025	0.005	0.066	632	56	II
Malathion	Insecticide	0.03	0.3	>5	2000	1778	III
Metaflumizone	Insecticide	0.03	0.13	>5.2	5000	>5000	NL
Methoxyfenozide	Insecticide	0.1	0.1	>5000	5000	>4.3	III
Novaluron	Insecticide	0.01	-	5.15	2000	>5000	U
Phosmet	Insecticide	0.01	0.045	113	1000	>1.52	II
Pirimicarb	Insecticide	0.035	0.1	142	2000	>0.75	III
Pirimiphos methyl	Insecticide	0.004	0.1	>4.7	2000	1414	II
Pyridaben	Acaricide	0.01	0.05	0.62	2000	161	II
Pyriproxyfen	Insecticide	0.1	1.0	>1.3	2000	>5000	U
Spinosad	Insecticide	0.024	0.1	>5.18	5000	>2000	III
Spirodiclofen	Acaricide	0.015	-	>5.03	2000	>2500	NL
Spirotetramat	Insecticide	0.05	1.0	>2000	2000	>4.18	III
Quinalphos	Insecticide	-	-	71	1750	0.45	III
Tau fluvalinate	Insecticide	0.005	0.05	>0.56	2000	546	III
Thiacloprid	Insecticide	0.01	0.03	>1.2	2000	177	II

Class II: Moderately hazardous; Class III: Slightly hazardous; NL: Not listed; U: Unlikely to present an acute hazard.

Table S2. Chronic risk assessments of insecticides for fruits and vegetables in Bursa province

Pesticide	cHQ - long-term dietary risk (chronic)									
	Lettuce	Parsley	Dill	Carrot	Apple	Pear	Orange	Mandarin	Banana	Total cHQ
Acetamiprid	0.012	0.0059	0.0046	-	0.0037	0.0011	0.0042	0.0019	-	0.0334
Abamectin	-	-	0.0470	-	-	0.0035	-	-	-	0.0505
Bifenazate	-	-	-	-	0.0088	-	-	-	-	0.0088
Bifenthrin	-	-	-	-	-	-	-	0.0286	-	0.0286
Chlorantraniliprole	-	-	-	-	0.0001	0.0001	-	-	-	0.0002
Chlorfenvinphos	-	-	-	-	-	-	-	0.1368	-	0.1368
Chlorpyrifos	-	0.0261	-	-	-	-	-	0.0928	-	0.1189
Chlorpyrifos methyl	-	-	0.0014	0.0140	-	-	-	-	-	0.0154
Clofentezine	-	-	-	-	0.0176	-	-	-	-	0.0176
Clothianidin	-	-	0.0001	-	-	-	-	-	-	0.0001
Cypermethrin	-	-	0.0188	-	0.0216	0.0028	-	0.0156	-	0.0588
Cyromazine	0.0002	-	-	-	-	-	-	-	-	0.0002
Deltamethrin	0.0073	0.0033	0.0011	-	0.0048	0.0007	-	-	-	0.0172
Diflubenzuron	-	-	-	-	0.0046	0.0009	-	-	-	0.0055
Emamectin B1a	-	0.1286	-	-	-	-	-	-	-	0.1286
Ethoprophos	-	-	0.1813	-	-	-	-	-	-	0.1813
Etoxazole	-	-	-	-	-	-	-	0.0011	-	0.0011
Fenbutatin oxide	0.0017	0.0003	-	-	-	-	-	-	-	0.0020
Fenvalerate	-	-	-	-	0.0026	-	-	0.0211	-	0.0237
Flubendiamide	-	-	-	-	0.0052	-	-	-	-	0.0052
Imidacloprid	0.0015	0.0003	0.0008	0.0019	-	0.0009	-	-	-	0.0054
Indoxacarb	-	-	-	-	0.0183	-	-	-	-	0.0183
Lambda cyhalothrin	-	-	0.0144	-	-	0.0177	0.0912	-	-	0.1233
Malathion	-	-	0.0015	-	-	-	-	0.0212	-	0.0227
Metaflumizone	-	-	0.0019	-	-	-	-	-	-	0.0019
Methoxyfenozide	-	-	-	-	0.0007	-	-	-	-	0.0007
Novaluron	-	-	-	-	0.0079	0.0012	-	-	-	0.0091
Phosmet	-	-	-	-	-	0.0230	-	-	-	0.0230
Pirimicarb	-	0.0017	0.0049	-	0.0021	-	-	-	-	0.0087
Pirimiphos methyl	-	0.0341	0.0927	-	-	-	-	-	-	0.1268
Pyridaben	-	0.0030	-	-	0.0088	-	-	0.0176	-	0.0294
Pyriproxyfen	-	-	-	-	-	0.0010	-	0.0025	0.0006	0.0035
Spinosad	0.0631	-	-	-	-	-	-	-	-	0.0631
Spirodiclofen	-	-	-	-	0.0037	0.0034	-	0.0033	-	0.0104
Spirotetramat	-	0.0010	0.0017	-	-	-	0.0018	-	-	0.0045
Quinalphos	-	-	-	-	-	-	*	-	*	-
Tau fluvalinate	-	-	-	-	0.0064	-	0.0105	0.0244	-	0.0413
Thiacloprid	-	-	-	-	0.0164	0.0049	-	-	-	0.0213

ARfD and ADI values were taken from EU Pesticide Database (2023); -: Residue not detected in this commodity, *: Not allocated for this insecticide, there was no specified ARfD and/or ADI in EU Pesticide Database (2023).

Table S3. Acute risk assessments of insecticides for fruits and vegetables in Bursa province

Pesticide	aHI -short-term dietary risk (acute)								
	Lettuce	Parsley	Dill	Carrot	Apple	Pear	Orange	Mandarin	Banana
Acetamiprid	45.61	3.40	5.28	-	16.51	8.27	12.66	5.67	-
Abamectin	-	-	1.55	-	-	12.17	-	-	-
Bifenazate	-	-	-	-	2.39	-	-	41.55	-
Bifenthrin	-	-	-	-	-	-	-	-	-
Chlorantraniliprole	-	-	-	-	*	*	-	-	-
Chlorfenvinphos	-	-	-	-	-	-	-	*	-
Chlorpyrifos	-	2.15	-	-	-	-	-	53.83	-
Chlorpyrifos methyl	-	-	0.19	5.01	-	-	-	-	-
Clofentezine	-	-	-	-	*	-	-	-	-
Clothianidin	-	-	0.04	-	-	-	-	-	-
Cypermethrin	-	-	12.23	-	73.86	19.47	-	45.33	-
Cyromazine	0.27	-	-	-	-	-	-	-	-
Deltamethrin	44.01	1.98	1.02	-	-	6.08	-	-	-
Diflubenzuron	-	-	-	-	*	*	-	-	-
Emamectin B1a	-	3.55	-	-	-	-	-	-	-
Ethoprophos	-	-	4.71	-	-	-	-	-	-
Etoxazole	-	-	-	-	-	-	-	*	-
Fenbutatin oxide	1.73	0.06	-	-	-	-	-	-	-
Fenvalerate	-	-	-	-	*	-	-	*	-
Flubendiamide	-	-	-	-	2.39	-	-	-	-
Imidacloprid	2.33	0.09	0.38	8.35	-	7.15	-	-	-
Indoxacarb	-	-	-	-	104.27	-	-	-	-
Lambda cyhalothrin	-	-	4.85	-	-	107.06	137.11	-	-
Malathion	-	-	0.19	-	-	-	-	14.64	-
Metaflumizone	-	-	0.09	-	-	-	-	-	-
Methoxyfenozide	-	-	-	-	3.26	-	-	-	-
Novaluron	-	-	-	-	*	*	-	-	-
Phosmet	-	-	-	-	-	158.2	-	-	-
Pirimicarb	-	0.24	1.92	-	1.95	-	-	-	-
Pirimiphos methyl	-	0.63	1.60	-	-	-	-	-	-
Pyridaben	-	0.25	-	-	4.78	-	-	10.19	-
Pyriproxyfen	-	-	-	-	-	0.79	-	0.16	0.09
Spinosad	31.07	-	-	-	-	-	-	-	-
Spirodiclofen	-	-	-	-	*	*	-	-	-
Spirotetramat	-	0.02	0.52	-	-	-	0.26	-	-
Quinalphos	-	-	-	-	-	-	*	-	*
Tau fluvalinate	-	-	-	-	1.74	-	3.16	7.37	-
Thiacloprid	-	-	-	-	31.49	26.77	-	-	-

ARfD and ADI values were taken from EU Pesticide Database (2023); -: Residue not detected in this commodity, *: Not allocated for this insecticide, there was no specified ARfD and/or ADI in EU Pesticide Database (2023).

Türkiye Entomoloji Dergisi Yayın İlkeleri

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

1. Dergi, entomoloji ve tarımsal zooloji bilim dallarıyla ilişkili konulara açıktır.
2. Dergide Türkçe veya İngilizce yazılmış orijinal araştırmalar yayımlanır.
3. Yayımlanması istenilen eserlerin kısmen veya tamamen herhangi bir yerde yayınlanmamış veya yayımlanmayacak olması zorunludur.
4. Daha önce Kongre/Sempozyum vs. de sözlü/poster bildiri olarak sunulmuş ancak sadece kısa özet olarak basılmış eserler, dipnotta belirtilmesi koşuşuyla kabul edilir.
5. Lisansüstü tezleri veya TÜBİTAK, DPT, BAP gibi çeşitli kurumlarca desteklenen proje bulgularından kısımlar içeren eserler ilgililerinden gerekli izinler alındıktan sonra hazırlanmalı, ilgi durum dipnotta mutlaka belirtilmelidir.
6. Türkiye veya herhangi bir bölge için, başta karantina listesinde bulunan türler olmak üzere, yeni tür kayıtlarını içeren eserler gönderilmeden önce mutlaka ilgili kurumlara bilgi verilmiş olmalıdır.
7. Dergide yayımlanması istenilen eserler, web sayfasında sunulan “eser başvurusu” bölümünde açıklandığı gibi hazırlanarak, üst yazı, imzalı telif hakları formu ve başvuru ücreti dekontu ile dergi e-posta adresine gönderilmelidir.
8. Yayımlanması istenilen eserler web sayfasında sunulan “örnek makale taslağı” kullanılarak, gereksiz tekrar, şekil ve cetvellerden kaçınılarak, özden uzaklaşmayacak şekilde hazırlanmalı ve 16 sayfadan fazla olmamalıdır.
9. Yayın ilkelerine uygun olmayan eserler istenilen şekle göre yeniden düzenlenmek üzere yazara geri gönderilir. Detaylar için web sayfasında sunulan “eser değerlendirme süreci” ne bakınız.
10. Bir eser yayıma kabul edildiğinde, telif hakları formu tüm yazarlar tarafından imzalanıp dergimize gönderilmeden yayımlanmaz. Sorumlu yazara eserin pdf formatında hazırlanmış hali e-posta ile gönderilir, ayrıca telif ücreti ödenmez. Yayımlanan eserlere ait şekil dışı sorumluluklar yazarlarına aittir.

Türkiye Entomoloji Dergisi

Türkiye Entomoloji Dergisi, Türkiye Entomoloji Derneği tarafından yılda dört kez yayınlanır. Dergide, entomoloji ve tarımsal zooloji bilim dallarıyla ilişkili konularda, Türkçe veya İngilizce yazılmış orijinal araştırmalaryayımlanır.

Makale Özetleri, Biological Abstracts, BIOSIS Previews, CABAbstracts, FAOAGRIS, Elsevier Scopus, Global Health, Information Reference Library, Review of Agricultural Entomology, SCI-E, TÜBİTAK/ULAKBİM, VINİTİ, Zoological Record tarafından taranmaktadır.

Yıllık abone bedeli: 150 TL Tek sayı bedeli: 50 TL

Yazışma adresi:

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