

Original article (Orijinal araştırma)

Effect of spinosad, azadirachtin and kaolin on *Stephanitis pyri* (Fabricius, 1775) (Hemiptera: Tingidae) under laboratory conditions

Laboratuvar koşullarında spinosad, azadirachtin ve kaolinin *Stephanitis pyri* (Fabricius, 1775) (Hemiptera: Tingidae) üzerine etkileri

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Abstract

This study was conducted in Diyarbakır (Turkey) in 2019 to determine the effects of natural insecticides, spinosad, azadirachtin and kaolin, on the fourth instar nymphs and adults of *Stephanitis pyri* (Fabricius, 1775) (Hemiptera: Tingidae). The study was conducted under the laboratory conditions; $25 \pm 1^\circ\text{C}$, $65 \pm 5\%$ humidity and 16:8 h L:D photoperiod. The mortality of nymphs 3 and 6 d after treatment with spinosad, azadirachtin and kaolin was 49, 44 and 11%, and 54, 51 and 47%, respectively. The mortality of adults 3 and 6 d after treatment with spinosad, azadirachtin and kaolin was 75, 11 and 14%, and 89, 18 and 12%, respectively. The damage index, feeding damage ratio on the upper surface of the leaf, after spinosad, azadirachtin and kaolin treatment of nymphs was 1.25, 1.50 and 1.50, and adults was 1.00, 2.00 and 2.00, respectively. After the application of spinosad, azadirachtin and kaolin, the number of droppings left by nymphs was 55, 98 and 75, and adults was 15, 98 and 93, respectively. Based these data, spinosad was determined as the most effective product in all parameters except for the fourth instar nymph damage index. However, azadirachtin and kaolin were somewhat inhibitory to nymphs.

Keywords: Control, damage index, Diyarbakır, mortality ratio, natural insecticides

Öz

Bu çalışma doğal insektisitlerden spinosad, azadirachtin ve kaolinin *Stephanitis pyri* (Fabricius, 1775) (Hemiptera: Tingidae)'nin dördüncü dönem nimfleri ve erginler üzerine olan etkilerini tespit etmek amacıyla 2019 yılında Diyarbakır (Türkiye)'de yürütülmüştür. Çalışma $25 \pm 1^\circ\text{C}$ sıcaklık, $\%65 \pm 5$ orantılı nem ve 16:8 (A:K) aydınlatmalı laboratuvar koşullarında yürütülmüştür. Çalışma sonucunda spinosad, azadirachtin ve kaolin uygulaması sonrası toplam ölü nimf oranı üçüncü günde sırasıyla $\%49$, $\%44$ ve $\%11$; altıncı günde sırasıyla $\%54$, $\%51$ ve $\%47$ olarak gerçekleşmiştir. Spinosad, azadirachtin ve kaolin uygulaması sonrası toplam ölü ergin oranı üçüncü günde sırasıyla $\%75$, $\%11$ ve $\%14$; altıncı günde sırasıyla $\%89$, $\%18$ ve $\%12$ olarak gerçekleşmiştir. Yaprak yüzeyinde beslenme sonrası oluşan zarar oranını ifade eden zarar indeksi nimflerde spinosad, azadirachtin ve kaolinde sırasıyla 1.25, 1.50 ve 1.50; erginlerde sırasıyla 1.00, 2.00 ve 2.00 olarak tespit edilmiştir. Beslenme sonucu bıraktıkları dışkı sayısı spinosad, azadirachtin ve kaolinde sırasıyla nimflerde 55, 98 ve 75; erginlerde 15, 98 ve 93 olarak tespit edilmiştir. Bu veriler ışığında nimf zarar indeksi hariç çalışmada incelenen bütün parametrelerde spinosad en etkili ürün olarak tespit edilmiştir. Azadirachtin ve kaolin ise nimfleri baskı altına alma açısından ön plana çıkmıştır.

Anahtar sözcükler: Kontrol, zarar indeksi, Diyarbakır, ölüm oranı, doğal insektisitler

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Received (Alınış): 17.07.2020

Accepted (Kabul ediliş): 17.02.2021

Published Online (Çevrimiçi Yayın Tarihi): 24.02.2021

Introduction

Stephanitis pyri (Fabricius, 1775) (Hemiptera: Tingidae) is one of the most important species causing economic damage in many fruit trees, such as apple, apricot, cherry, hawthorn, pear, peach, walnut, in Turkey including almond [*Prunus amygdalus* (Linnaeus, 1758) (Rosales: Rosaceae)] (Lodos, 1982; Çam, 1993; Gülperçin & Önder, 1999; Schaefer & Panizzi, 2000; Çınar et al., 2004; Aysal & Kivan, 2008; Maral et al., 2013). The nymphs and adults of *S. pyri* are phytophagous. They live on the undersides of leaves and feed by sucking plant sap after piercing the parenchyma tissue with their stylet. Whitish spots appear at the feeding sites. The damaged leaves fall prematurely. Thus, they weaken the plants and cause losses both in quality and yield (Bodenheimer, 1958; Nizamlioğlu, 1961; Göksu, 1964; Drake & Ruhoff, 1965; Lodos, 1982; Péricart, 1983; Lodos & Önder, 1983; Gülperçin & Önder, 1999; Schaefer & Panizzi, 2000; Guilbert, 2001; Demirsoy, 2006). A study was conducted on the density of tingids on almond trees in Diyarbakır, Elazığ and Mardin Provinces and found that *S. pyri* is among the top three tingid species occurring at high densities (Bolu, 2007).

Organophosphate insecticides, such as malathion and dimethoate, are generally used against *S. pyri* in Turkey (Anonymous, 2020). Organophosphates are among the most widely used insecticides today. Pesticides in this group have many undesirable effects on humans and the environment, and can remain in the soil for long periods (Srivastava & Kesavachandran, 2019; Rakhimol et al., 2020). In recent years, IPM has been focusing on minimizing the damage caused by pesticides (Peshin & Pimentel, 2014). In IPM, using bacterial insecticides (e.g., spinosad), botanical insecticides (e.g., azadirachtin) and particle film technology (e.g., kaolin) to control pests has become increasingly important (Rakhimol et al., 2020).

Azadirachtin, a plant-derived insecticide, consisting of compounds from different parts of the neem tree [*Azadirachta indica* A. Juss, 1830 (Sapindales: Meliaceae)]. Azadirachtin is used as a broad-spectrum insecticide as well as bactericide and fungicide. Azadirachtin is effective on more than 400 insect species including species in the family Tingidae. Azadirachtin, which is lethal to insects, negatively affects the metamorphosis, feeding, oviposition and reproduction of insects. Azadirachtin gives useful control of insects that are resistant to synthetic pesticides. Azadirachtin is also an insect growth regulator (Isman, 2006; Saha et al., 2011; Pener & Dhadialla, 2012; Pavela et al., 2013; Sánchez-Ramos et al., 2014; Vacante & Kreiter, 2018; Joseph, 2019; Pamela, 2019).

Spinosad, a bacterial pesticide, is a natural insecticide obtained from the soil-based bacteria *Saccharopolyspora spinosa* Mertz & Yao, 1990 (Actinomycetales: Pseudonocardiaceae) (Vacante & Kreiter, 2018). Spinosad is used against many harmful insect species, including some species in the family Tingidae, and has a range of effects on insects including as a repellent, antifeedant, and growth and mating inhibitor (Isman, 2006; Matthews, 2006; Joseph, 2020).

Particle film technology increasingly used against insect, particularly using kaolin (Akgül & Özgen, 2017). Damage caused by some fruit flies in citrus decreased and less eggs were deposited after kaolin application. Kaolin generally shows repellent effects on insects. After kaolin application, a thin layer is formed on the leaf surface, and this layer prevents the insects from piercing epidermis and depositing eggs. This layer also makes it difficult for insects to find the plant as it changes color perception (Glenn, 2012; Vacante & Kreiter, 2018; Rakhimol et al., 2020).

There have been no studies on the effects of azadirachtin, spinosad and kaolin on *S. pyri*. Access to safe and healthy food is always a primary concern for consumers, so it is important to study the effects of natural insecticides on harmful insects, as these present low risks to human health. This study was conducted to determine the effects of spinosad, azadirachtin and kaolin on the fourth instar nymphs and adults of *S. pyri*. Using the most effective product, identified by this study, when overwintering adults of *S. pyri* become active will contribute lowering the population throughout the growing season. In addition, the findings of this study will also be useful in keeping the population density of nymphs and adults in summer below the economic threshold.

Materials and Methods

Preparing almond sapling for stock culture of *Stephanitis pyri*

The study was conducted in Diyarbakır between August and October 2019. In order to have sufficient *S. pyri* during the study, first suitable conditions were prepared for a stock culture. For this purpose, a two-year-old certified almond sapling obtained from Dicle University Faculty of Agriculture almond plantation (37°53'20.4" N, 40°16'17.0" E) was planted in a pot measuring 50 x 60 cm (height x diameter). In addition to the maintenance of the sapling, nitrogen fertilizer supplements were applied to ensure vegetative growth. The almond sapling was placed in a cage measuring 80 x 80 x 150 cm (length x width x height) covered in insect-proof mesh (Figure 1). The cage was placed in a sun-exposed place at the Diyarbakır GAP International Agricultural Research and Training Center (37°56'41.9" N, 40°15'29.2" E).



Figure 1. Cage used for stock culture (80 x 80 x 150 cm).

Rearing *Stephanitis pyri* under laboratory condition

An 8-year-old almond orchard of the Diyarbakır GAP International Agricultural Research and Training Center, to which pesticide was not applied, was selected for collection of *S. pyri* for the stock culture. At the beginning of August, one branch of the two randomly selected trees was shaken gently, so the insects fell onto white cloth measuring 50 x 50 cm (width x length) and adults were collected with a mouth aspirator. In total, 150 adults were released on the caged almond sapling. Nymphs, hatching from eggs, started to feed under the leaves and were used as the stock culture.

To provide suitable conditions for nymphs and adults, 10 plastic containers, measuring 18 x 10 x 5 cm (length x width x height) were prepared (Figure 2). Two small holes were made on the both ends of the lid with a pin for ventilation. In order to keep the leaves alive as long as possible, the MS growth medium (Murashige & Skoogl, 1962) was used. The growth medium was dispensed into tubes 6.5 x 1.2 cm (height x diameter). A wooden platform measuring 5 x 2 x 3 cm (length x width x height) was prepared so that the

tubes can stand horizontally in the container. The platform was fixed to bottom. Almond leaves were obtained from Dicle University Faculty of Agriculture almond plantation. Healthy leaves, with no apparent diseases and pests were selected. Leafy shoots collected from the plantation were kept in the refrigerator (4°C) in a black plastic bag and replaced every 2 d. These leaves are also used for treatments. The petiole of the leaf was placed in the tube containing the growth medium. The cap of the tube was covered with white modeling clay (Figure 2).



Figure 2. The container used to obtain nymphs and adults of *Stephanitis pyri*: a) top view, and b) side view.

A sufficient number of new generation adults were collected from the cage and brought to the laboratory in a plastic container. These adults were separated into 100 males and 100 females using the methods of Drake & Ruhoff (1965) and Péricart (1983). Five females and five males were placed in each container as indicated in Figure 2. The containers were placed in a growth chamber (Daihan Scientific, Wonju, Gangwon, South Korea) at $25 \pm 1^\circ\text{C}$, $65 \pm 5\%$ RH and 16:8 h L:D photoperiod. The containers were checked daily to see if females had laid eggs. After observing that the females laid enough eggs, the adults were removed from the containers. The containers with growth medium were renewed for the hatching of the eggs. The hatched nymphs were transferred to a container with the help of a soft tip brush. The growth chamber was checked daily and the nymphs were transferred to other containers according to their stages.

Bioassays with fourth instar nymphs and adults

Almond leaves used in the experiment were prepared as described above. In order to keep the leaves alive during the experiment (6 d), moistened cotton was wrapped around the stems (Figure 3). Petri dishes were secured with rubber bands to prevent insects from escaping. The study was conducted in the growth chamber (Daihan Scientific) located in the Laboratory of GAP International Agricultural Research and Training Center at $25 \pm 1^\circ\text{C}$, $65 \pm 5\%$ RH, and 16:8 h L:D photoperiod.

Aysal & Kivan (2008) determined the life parameters of *S. pyri* on apple [*Malus domestica* Borkhausen (Rosales: Rosaceae)] under the laboratory conditions at $26 \pm 1^\circ\text{C}$, 60-70% RH and 16:8 h L:D photoperiod, and they found that the lowest nymph mortality (0%) was observed in the third, fourth and fifth instar stages. Based on these data, the fourth instar was used in this study. Fourth instar nymphs younger than 24 h old were selected for four product replicates and four control replicates for each product. Six fourth instar nymphs were placed in each Petri dish by means of a soft tip brush. In this way, six fourth instar nymphs were placed in each of eight Petri dishes with a total of 48 fourth instar nymphs used for each product. A total of 144 fourth instar nymphs were used for the three products in the study.



Figure 3. Petri dish used in the study.

For adult's selection, nymphs were monitored regularly and adults younger than 24 h old were selected for the study. Six adults, three females and three males, were placed in each Petri dish by using a soft tip brush. Six adults were placed in each of the eight Petri dishes, four product replicates and four control replicates for each product with a total of 48 adults were used for each product. A total of 144 adults, 72 females and 72 males, were used in the study for three products.

Insecticide exposure test

The products used and the doses applied are given in Table 1. A 500-ml capacity hand spray bottle was used to apply the products to the leaves. After the products were prepared, they were sprayed from the same distance until the whole leaf was wet, and the leaves were then allowed to dry. Dried leaves were then transferred to Petri dishes. Pure water was used for the leaves in the control group.

Table 1. Natural insecticides used against *Stephanitis pyri* in the study

Trade name	Active ingredient	Concentration	Recommended maximum field concentration	Supplier	Concentration applied
Nimbecidine	azadirachtin	0,3 g L ⁻¹ (EC)	5 mL L ⁻¹	Agrobrest Grup Tarım İlaçları Toh. İml. İth. İhr. San. ve Tic. A.Ş.- İzmir/Türkiye	1.5 mg L ⁻¹
Laser	spinosad	480 g L ⁻¹ (SC)	0.2 mL L ⁻¹	Dow AgroSciences A.Ş.- Ataşehir/İstanbul	96 mg L ⁻¹
Utelka Kaolin Kili	kaolin	950 gr kg ⁻¹ (WP)	50 mg L ⁻¹	Utelka Maden San. Tic. Ltd. Şti.- Sındırgı/Balıkesir	47.5 mg L ⁻¹

Mortality ratio

Nymphs and adults placed in the growth chamber were observed for 6 d. The Petri dishes were checked on day 3 and 6 and the mortality ratio calculated. For the determination of dead insects, the Petri dishes were taken out of the growth chamber, the lid of the Petri dish was opened and the individuals were observed. Individuals that fell to the bottom of the Petri dish were presumed dead and transferred to another Petri dish. These dead individuals were observed for 30 min and included in the calculations after making sure that they died. After calculating mortality ratios using the method specified in the statistical analysis section of this manuscript, corrected mortalities were found.

Feeding activity

After 6 d, the leaves in the Petri dishes were examined to determine the feeding activity and damage rate of the pest. Tingidae family species leave behind black sticky excrements under the leaf after feeding. In some cases, as a result of intensive feeding, the entire lower surface of the leaf is covered with excrement, which prevents the leaf from photosynthesis and makes it susceptible to disease and pest attacks. The amount of excrement left by *S. pyri* was included in the calculations because it is an important indicator of pest density and damage rate. To measure the feeding activity of *S. pyri*, the lower surfaces of the leaves were examined and the deposits of excrement they left after feeding was counted (Figure 4).



Figure 4. Deposits of excrement on the lower surface of leaf left by fourth instar nymphs and adults of *Stephanitis pyri* after feeding.

Damage rate

Tingidae family species live on the undersides of the leaves and feed on by sucking plant sap by piercing the parenchyma tissue with their stylet. Whitish spots appear at the feeding sites. The density of these whitish spots on the leaf surface is an important indicator of pest density and damage rate. The damage index was based on this parameter using the method developed by Sánchez-Ramos et al. (2014) for the analyzing of feeding damage (Figure 5) on the upper surface of the leaf after feeding fourth instar nymphs and adults: 0, no damage on the leaf surface; 1, a third of the leaf surface is damaged; 2, between one and two thirds of the leaf surface is damaged; and 3, more than two thirds of the leaf surface is damaged.



Figure 5. Feeding damage on the upper surface of the leaf after feeding fourth instar nymphs and adults of *Stephanitis pyri*.

Statistical analysis

Total mortality data of fourth instar nymphs and adults were transformationed [$\sqrt{(x/100)}$]. Corrected mortalities were found by using the method suggested by Abbott (1925).

$$\text{Corrected mortality} = \frac{(\% \text{ observed mortality} - \% \text{ control mortality})}{(100 - \% \text{ control mortality})}$$

All the other parameters were transformed by $\sqrt{(x + 1)}$. The level of significance was $P < 0.05$ in all cases. All parameters were analyzed by means of mixed-model factorial analysis of variance with four replicates. In cases where the examined features were found to be statistically significant ($P < 0.05$), the averages of the applications were grouped by the multiple comparison test, LSD ($P < 0.05$) test. Correlation analyses were made between the examined features. Variance analysis, LSD ($P < 0.05$) and correlation analyses were performed using JMP 7.0 (SAS Institute Inc., Cary, NC, USA) statistical package.

Results

Mortality

The mortality of nymphs with of spinosad, azadirachtin and kaolin on day 3 was 49, 44 and 11% and on day 6 was 54, 51 and 47%, respectively. Maximum mortality of nymphs on the third day was with spinosad and azadirachtin (Table 2).

Table 2. The mortality ratios of fourth instar nymphs of *Stephanitis pyri* on 3 and 6 day after kaolin, spinosad and azadirachtin application (n = 24)

	Mortality (%)		Corrected mortality (%)	
	Day 3	Day 6	Day 3	Day 6
Kaolin	21 ± 7.3 bc	59 ± 11.2	11 b	47
Spinosad	55 ± 5.5 a	64 ± 4.2	49 a	54
Azadirachtin	50 ± 13.0 ab	62 ± 16.1	44 a	51
Control	11 ± 5.9 c	22 ± 8.2		
F (df = 3,9)	48.3	2.46	48.3	2.46
P	0.02	0.12	0.02	0.12
Mean	34	52	35	50
CV (%)	57	49	56	48
LSD (%)	31.3	ns	30.4	ns

The mortality of adults with spinosad, azadirachtin and kaolin on day 3 was 75, 11 and 14%, and on day 6 is 89, 18 and 12%, respectively. Maximum mortality of adults on day 3 and 6 was with spinosad (Table 3).

Table 3. The adult mortality ratios of *Stephanitis pyri* on 3 and 6 day after kaolin, spinosad and azadirachtin application (n = 24)

	Mortality (%)		Corrected mortality (%)	
	Day 3	Day 6	Day 3	Day 6
Kaolin	14 ± 4.7 b	18 ± 3.2 b	14 b	12 b
Spinosad	75 ± 5.6 a	90 ± 2.6 a	75 a	89 a
Azadirachtin	12 ± 7.4 b	23 ± 7.0 b	11 b	18 b
Control	1 ± 4.3 b	6 ± 5.0 b		
F (df = 3,9)	268	45.0	268	47.0
P	<0.0001	<0.001	<0.001	<0.001
Mean	25	34	33	34
CV (%)	51	32	51	32
LSD (%)	20.8	17.6	20.3	17.6

Damage index

The damage index of spinosad, azadirachtin and kaolin for nymphs is 1.25, 1.50 and 1.50, respectively and the only significant difference was between the treatments and the control (Table 4).

The damage index of spinosad, azadirachtin and kaolin for adults was 1.00, 2.00 and 2.00, respectively, and, there was a significant difference between spinosad and the control (1.00 versus 2.53).

Table 4. Damage index* for *Stephanitis pyri* with kaolin, spinosad and azadirachtin application (n = 24)

	Fourth instar nymphs	Adults
Kaolin	1.50 ± 0.38 a	2.00 ± 0.37 a
Spinosad	1.25 ± 0.16 a	1.00 ± 0.10 b
Azadirachtin	1.50 ± 0.47 a	2.00 ± 0.36 a
Control	1.88 ± 0.20 a	2.53 ± 0.10 a
F	0.476	4.21
Df	0.70	0.04
P	1.53	1.88
Mean	50	33
CV (%)	ns	0.99
LSD (%)	ns	0,99

* Damage index: 0, no damage on the leaf surface; 1, a third of the leaf surface is damaged; 2, between one and two thirds of the leaf surface is damaged; and 3, more than two thirds of the leaf surface is damaged.

Feeding activity

After application of spinosad, azadirachtin and kaolin, the deposits of excrement left by nymphs were 55, 98 and 75, respectively, with kaolin (75 versus 167) and spinosad (55 versus 167) being significantly different from the control (Table 5). After the application of spinosad, azadirachtin and kaolin, the deposits of excrements left by adults were 15, 98 and 93, respectively, with spinosad significantly different (15 versus 121) from the control (Table 5).

Table 5. The deposits of excrement per leaf left by fourth instar nymphs and adults of *Stephanitis pyri* after kaolin, spinosad and azadirachtin application (n = 24)

	Fourth instar nymphs	Adults
Kaolin	75 ± 19.6 b	93 ± 9.2 a
Spinosad	55 ± 8.4 b	15 ± 5.5 b
Azadirachtin	98 ± 28.6 ab	98 ± 19.4 a
Control	167 ± 14.1 a	121 ± 6.2 a
F (df = 3, 9)	4.82	12.1
P	0.029	0.002
Mean	99	82
CV (%)	45	32
LSD (%)	70.8	42.4
LSD (%)	70,78	42,410

Correlation between variables

Negative correlations were found between the deposits of excrement left after feeding and the total mortality and between the damage index and the total mortality. Positive correlations were found between the damage index and the deposits of excrement left after feeding (Table 6).

Table 6. Correlation analysis of the three response variables measured in this study for fourth instar nymphs and adults of *Stephanitis pyri* after kaolin, spinosad and azadirachtin application

Comparison	Fourth instar nymphs		Adults	
	R	P	R	P
Excrement vs total mortality (%)	-0.930	<0.001	-0.871	<0.001
Damage index vs total mortality (%)	-0.810	<0.001	-0.685	0.0034
Damage index vs excrement	0.810	<0.001	0.822	<0.001

Discussion

In the present study, spinosad was determined as the most effective product in all parameters except the nymph damage index. Spinosad suppressed the feeding activity of *S. pyri* and as a result, the lowest damage index values were obtained. No studies on the effects of spinosad on *S. pyri* have previously been published. However, in a study conducted under the laboratory conditions on the repellency of some insecticides, including spinosad, on *Stephanitis pyrioides* (Scott, 1874) (Hemiptera: Tingidae), spinosad was found to be a repel *S. pyrioides* (Joseph, 2020). In the present study, the repellency of spinosad was not studied. It is considered that, however, the significant suppressive effect of *S. pyri* on feeding activity is a potential indicator of repellency. In another study, spinosad with added 1% petroleum caused a low mortality rate of 10% in adults of avocado lace bug, *Pseudacysta perseae* (Heidmann, 1908) (Hemiptera: Tingidae), which was probably due to the feeding habits of this pest (Humeres et al., 2009). The results of that study differ from the present study probably as a consequence of different doses and different target pest.

In the present study, it was determined that azadirachtin had useful effects on mortality rates of fourth instar nymphs. In terms of total mortality of fourth instar nymphs on day 3, azadirachtin showed a significant difference from spinosad as compared to the control. No studies on the effects of azadirachtin on *S. pyri* have previously been published. Sánchez-Ramos et al. (2014) found that azadirachtin caused a high mortality (97%) in the fourth instar nymphs, and 32% in adults of *Monosteira unicastata* (Mulsant & Rey, 1852) (Hemiptera: Tingidae), one of the most important pests of almonds in Spain, at similar application rates to the present study. The researchers reported that azadirachtin being a growth regulatory was contributing to nymphal mortality. According to Joseph (2019), azadirachtin caused transovarial activity and as a result, there was a decrease in the number of nymphs hatching from eggs of adults with azadirachtin applied as a topical spray compared to the control. In another study injecting azadirachtin in the trunks of *Platanus* sp. under natural conditions, it was found that the density of nymphs and adults of *Corythucha ciliata* (Say, 1832) (Hemiptera, Tingidae) decreased significantly over 2 years compared to the control (Pavela et al., 2013). Similarly, azadirachtin was found to cause a high mortality of fourth instar nymphs in the present study.

In the present study, it was determined that kaolin had less impact on both fourth instar nymph and adult mortality compared to spinosad and azadirachtin. Although, kaolin significantly reduced on the feeding of fourth instar nymphs compared to the control, It did not significantly reduce the damage index for fourth instar nymphs and adults, and the feeding of adults. No studies on the effect of kaolin on *S. pyri* have previously been published. In a study of the effects of kaolin on nymphs and adults of *M. unicastata*, the mortality of fourth instar nymphs and adults on day 6 was 43 and 44%, respectively (Sánchez-Ramos et al., 2014) and kaolin significantly suppresses the reproduction and feeding of *M. unicastata*. In the present study, it was similarly determined that after application of kaolin, feeding of nymphs decreased, and kaolin increased the mortality fourth instar nymph and adult. In a field study with kaolin in 2009 and 2010, Marcotegui et al. (2015), determined that the density of *M. unicastata* halved in 2009 and damage decreased by 26%, and in 2010, the density decreased by a third and the damage decreased by 11%. Since we did not perform any application under the field conditions in the present study, it was not possible

to compare our data with that study. However, it is considered that the pest density may have decreased due to the fact that kaolin suppresses nymphs similarly in the present study.

The present study, spinosad was determined to be the most effective product in all parameters except for the fourth instar nymphs damage index. Spinosad gave higher mortality of adults than nymphs. Therefore, it is considered that spinosad can be used to control overwintering adults as the reemerge in spring and also to decrease the population density in the summer when the adult density is high and the generation time is short. Kaolin, which had significant effects on the feeding of the fourth instar nymphs, and azadirachtin, which caused high mortality in the fourth instar nymphs, are considered to be able to decrease the population density of *S. pyri* if applied on first generation nymphs. To confirm this suggestion, it is recommended that the effects of spinosad, kaolin, and azadirachtin on *S. pyri* and its natural enemies be studied under field conditions. In addition, the effects of these products on nymphs and adults of *S. pyri* should be studied in detail by contact application under both laboratory and field conditions, because only the leaf exposure method was used in the present study.

Acknowledgment

We thank Diyarbakır GAP International Agricultural Research and Training Center for provision of the laboratory used to conduct this study.

References

- Abbott, W. S., 1925. A method of computing the effectiveness of an insecticide. *Journal of Economic Entomology*, 18 (2): 265-267.
- Akgül, B. & İ. Özgen, 2017. The application areas of clay minerals in agriculture. *International Journal of Innovative Engineering Applications*, 1 (2): 18-26.
- Anonymous, 2020. Republic of Turkey Minister of Agriculture and Forestry, plant protection product database. (Web page: <https://bku.tarim.gov.tr/Zararli/Details/379>) (Date accessed: July 2020).
- Aysal, T. & M. Kivan, 2008. Development and population growth of *Stephanitis pyri* (F.) (Heteroptera: Tingidae) at five temperatures. *Journal of Pest Science*, 81 (3): 135-141.
- Bodenheimer, F. S., 1958. Türkiye'de Ziraate ve Ağaçlara Zararlı Olan Böcekler ve Bunlarla Savaş Hakkında Bir Etüt. Bayur Matbaası, Ankara, 346 s (in Turkish).
- Bolu, H., 2007. Population dynamics of Lacebugs (Heteroptera: Tingidae) and its natural enemies in almond orchards of Turkey. *Journal of the Entomological Research Society*, 9 (1): 33-37.
- Çam, H., 1993. Some studies on the heteropterous species collected on mahaleb, sweet and sour cherries trees in Tokat and surrounding area. *Journal of Agricultural Faculty of Gaziosmanpaşa University*, 10 (1): 32-42.
- Çınar, M., İ. Çimen & H. Bolu, 2004. The cherry pests, their natural enemies and observations on some important species in Elazığ and Mardin provinces of Turkey. *Turkish Journal of Entomology*, 28 (3): 213-220.
- Demirsoy, A., 2006. Yaşamın Temel Kuralları-Entomoloji (Omurgasızlar-Böcekler). Meteksan Yayınları Cilt- II /Kısım-II, Ankara, 945 s (in Turkish).
- Drake, C. J. & F. A. Ruhoff, 1965. *Lagebugs of the World, A Catalog (Hemiptera: Tingidae)*. Smithsonian Institution, Washington, 710 pp.
- Glenn, D. M., 2012. The mechanisms of plant stress mitigation by kaolin-based particle films and applications in horticultural and agricultural crops. *Horticultural Science*, 47 (6): 710-711.
- Göksu, M. E., 1964. Sakarya ve Kocaeli Bölgeleri Meyve Ağaçlarında Zarar Yapan Armut Kaplanının (*Stephanitis pyri* Fabr.) Biyolojisi ve Mücadelesi Üzerinde Araştırmalar. T.C. Tarım Bakanlığı Göztepe Zirai Mücadele Enstitüsü Yayınları, İstanbul, 58 s (in Turkish).
- Guilbert, E., 2001. Phylogeny and evolution of exaggerated traits among the Tingidae (Cimicomorpha, Heteroptera). *Zoologica Scripta*, 30 (4): 313-324.

- Gülperçin, N. & F. Önder, 1999. Some investigations on the biology and natural enemies of *Stephanitis pyri* (F.) (Heteroptera: Tingidae) Bornova (Turkey). Turkish Journal of Entomology, 23 (1): 51-56.
- Humeres, E. C., J. G. Morse, R. Stouthamer, W. Roltsch & M. S. Hoddle, 2009. Evaluation of natural enemies and insecticides for control of *Pseudacysta perseae* (Hemiptera: Tingidae) on avocados in Southern California. Florida Entomologist, 92 (1): 35-42.
- Isman, M. B., 2006. Botanical insecticides, deterrents and repellents in modern agriculture and an increasingly regulated world. Annual Review of Entomology, 51 (1): 45-66.
- Joseph, S. V., 2019. Transovarial effects of insect growth regulators on *Stephanitis pyrioides* (Hemiptera: Tingidae). Pest Management Science, 75 (8): 2182-2187.
- Joseph, S. V., 2020. Repellent effects of insecticides on *Stephanitis pyrioides* Scott (Hemiptera: Tingidae) under laboratory conditions. Crop Protection, 127: 104985.
- Lodos, N., 1982. Türkiye Entomolojisi II (Genel Uygulamalı ve Faunistik). Ege Üniversitesi Ziraat Fakültesi Yayınları No: 429, Bornova İzmir, 580 s (in Turkish).
- Lodos, N. & F. Önder, 1983. Preliminary List of Tingidae with Notes on Distribution and Importance of Species in Turkey. Ege Üniversitesi Ziraat Fakültesi Yayınları, İzmir, 51 s.
- Maral, H., M. R. Ulusoy & H. Bolu, 2013. Faunistic studies on Tingidae (Hemiptera) species of Diyarbakir, Mardin and Elazığ provinces. Turkish Bulletin of Entomology, 3 (4): 139-155.
- Marcotegui, A., I. Sánchez-Ramos, S. Pascual, C. E. Fernández, G. Cobos, I. Armendáriz, A. Cobo & M. González-Núñez, 2015. Kaolin and potassium soap with thyme essential oil to control *Monosteira unicastata* and other phytophagous arthropods of almond trees in organic orchards. Pest Management Science, 88 (4): 753-765.
- Matthews, G. A., 2006. Pesticides: Health, Safety and the Environment. Blackwell Publishing, Oxford, UK, 248 pp.
- Murashige, T. & F. A. Skoog, 1962. A revised medium for rapid growth and bioassays with tobacco tissue cultures. Plant Physiology, 15 (3): 473-497.
- Nizamlioğlu, K., 1961. Türkiye Ziraatında Zararlı Olan Böcekler ve Mücadelesi. Ziraat Mücadele Enstitüsü, İstanbul, 510 s (in Turkish).
- Pamela, G. M., 2019. Pesticidal natural products - status and future potential. Pest Management Science, 75 (9): 2325-2340.
- Pavela, R., M. Žabka, V. Kalinkin, E. Gotenev, A. Gerus, A. Shchenikova & T. Chermenskaya, 2013. Systemic applications of azadirachtin in the control of *Corythucha ciliata* (Say, 1832) (Hemiptera, Tingidae), a pest of *Platanus* sp. Plant Protection Science, 49 (1): 27-33.
- Pener, M. P. & T. S. Dhadialla, 2012. Insect Growth Disruptors (An Overview of Insect Growth Disruptors; Applied Aspects). Academic Press, Elsevier, Oxford, UK, 533 pp.
- Péricart, J., 1983. Hémiptères Tingidae Euro-Méditerranéens. Fédération Française des Sociétés de Sciences Naturelles, Faune de France, Vol. 69, 626 pp.
- Peshin, R. & D. Pimentel, 2014. Integrated Pest Management, Experiences with Implementation, Global Overview. Springer, Netherlands, Vol. 4, 574 pp.
- Rakhimol, K. R., T. Sabu, V. Tatiana & K. Jayachandran, 2020. Controlled Release of Pesticides for Sustainable Agriculture. Springer Nature, Switzerland, 268 pp.
- Saha, S., S. Walia & B. S. Parmar, 2011. Exploring the diversity of neem bio actives as economic benign pesticides: a reappraisal. Toxicological Environmental Chemistry, 93 (8): 1508-1546.
- Sánchez-Ramos, I., S. Pascual, A. Marcotegui, C. E. Fernández & M. González-Núñez, 2014. Laboratory evaluation of alternative control methods against the false tiger, *Monosteira unicastata* (Hemiptera: Tingidae). Pest Management Science, 70 (3): 454-461.
- Schaefer, W. C. & A. R. Panizzi, 2000: Heteroptera of Economic Importance. CRC Press, Washington D.C., USA, 824 pp.
- Srivastava, A. K. & P. C. Kesavachandran, 2019. Health Effects of Pesticides. CRC Press, Taylor & Francis Group, Boca Raton, USA, 182 pp.
- Vacante, V. & S. Kreiter, 2018. Handbook of Pest Management in Organic Farming. CAB International, Boston, USA, 577 pp.