https://doi.org/10.30910/turkjans.1372048

TÜRK TARIM ve DOĞA BİLİMLERİ DERGİSİ



TURKISH JOURNAL of AGRICULTURAL and NATURAL SCIENCES

www.dergipark.gov.tr/turkjans

Araştırma Makalesi

Toxic, Repellent, and Oviposition-Inhibiting Effects of Almina Diatomaceous Earth on *Tetranychus urticae* Koch, 1836 (Acari: Tetranychidae)



¹Bursa Uludag University, Faculty of Agriculture, Plant Protection Department, 16059, Nilufer/Gorukle/BURSA

Corresponding Author: e-posta : hilalsusurluk@uludag.edu.tr

Geliş Tarihi: 06.10.2023 Düzeltme Geliş Tarihi: 06.11.2023 Kabul Tarihi: 06.11.2023

ABSTRACT

Tetranychus urticae Koch, 1836 (Acari: Tetranychidae) is one of the most important pests of agricultural fields worldwide. In this study, the leaf disc method was used to determine whether local diatomaceous earth (Almina) has a toxic, repellent, and oviposition-inhibiting effect on *T. urticae*. Adult females of *T. urticae* were placed on leaf discs immersed in 10%, 5%, 2.5%, and 1.25% concentrations (w/v) of diatomaceous earth. Considering the mortality rates after 24, 48, 72, and 96 hours, respectively, the highest mortality rate was 33.56% at 10% concentration. At the same concentrations, the highest repellent effects were found to be 64.54%, 42.10%, 20.35%, and 19.46% after 1 hour, respectively. Although the number of eggs laid by females increased over time at all concentrations, it was statistically less than the control at 10% and 5%. In light of the data obtained from this study, further studies are planned to increase the effectiveness of Almina diatomaceous earth by mixing it with botanical extracts or entomopathogens in the control of *T. urticae*.

Key words: : Diatomaceous earth, oviposition-inhibiting, repellent, *Tetranychus urticae*, toxicity

Tetranychus urticae Koch, 1836 (Acari: Tetranychidae) üzerinde Almina diatom toprağının toksik, uzaklaştırıcı ve yumurtlama engelleyici etkileri

ÖZ

Tetranychus urticae Koch, 1836 (Acari: Tetranychidae) dünya çapında tarım alanlarının en önemli zararlılarından biridir. Bu çalışmada, *T. urticae* üzerinde lokal bir diatom toprağının (Almina) toksik, uzaklaştırıcı, ve yumurta engelleyici etkisinin olup olmadığını anlamak için yaprak disk metodu kullanılmıştır. Diatom toprağının %10, %5, %2,5 ve %1,25 konsantrasyonlarına (w/v) daldırılan yaprak diskler üzerine *T. urticae* ergin dişileri yerleştirilmiştir. Sırasıyla 24, 48, 72 ve 96 saat sonraki ölüm oranlarına bakıldığında en yüksek ölüm oranı %10 konsantrasyonda %33,56 olarak belirlenmiştir. Aynı konsantrasyonlarda en yüksek uzaklaştırıcı etkiler sırasıyla 1 saat sonra % 64.54, % 42.10, % 20.35 ve % 19.46 olarak bulunmuştur. Tüm konsantrasyonlarda dişilerin bıraktıkları yumurta sayıları zamanla artış gösterse de, % 10 ve %5'te kontrole göre istatistiksel olarak daha az bulunmuştur. Bu çalışmadan elde edilen veriler ışığında, *T. urticae* ile mücadelede Almina diatom toprağının bitkisel ekstraktlar veya entomopatojenlerle karıştırılarak etkinliğinin arttırılmasına yönelik daha ileri çalışmaların yapılması planlanmıştır.

Anahtar kelimeler: Diatom toprağı, yumurtlamayı engelleme, uzaklaştırıcı, Tetranychus urticae, toksisite

INTRODUCTION

Tetranychus urticae Koch, 1836 (Acari: Tetranychidae) is among the most important pests that damage fields and greenhouse crops worldwide. Adult and pre-adult periods damage plants by sucking plant sap. Its damage causes mostly yellowing and curling in mostly young leaves. Furthermore, nymphs and adults can form webs, and plants can be completely covered by these mite-inhabited webs. These symptoms cause either decreased product quality or loss of crop (Erdoğan et al., 2010). The short life cycle, being polyphagous, and many progenies in a short time complicate pest control. The use of acaricide is the most preferred pest control

method by growers. However, its biology and high reproductive capacity cause many problems, such as developing pesticide resistance (Van Leeuwen et al., 2010; Susurluk and Gürkan, 2022). Considering the harmful effects of pesticides on humans and the environment, as well as the pests' resistance to pesticides, it is inevitable to search for alternative control methods. Diatomaceous earth (DE) has the potential to be one of the most important materials used for this purpose. DE is the only mineral of biological origin. Since the 1930s, diatomaceous earth has been used as an effective insecticide substitute. Today, most DE is found in Europe, and Türkiye has great potential for DE resources (Çetin and Taş, 2012). DE is obtained from the fossilized sedimentary layers of diatoms with siliceous shells from phytoplankton living in lakes and seas. DE particles are thought to adhere to the insect cuticle and induce death by desiccation or dehydration in insects (Zeni et al., 2021). In addition, abrasions caused by DE particles on the insect cuticle with the insect's movement also indirectly cause the insect's death structure. The epicuticular layer in the insect cuticle limits insect water loss and prevents drying. DE adheres to the lipids in the epicuticle and prevents it from preventing water loss (Başkaya, 2020). Considering the effect of DE on arthropods, this practice is considered within the scope of physical control measures. The most important advantage of DE is that it is obtained from organic material; therefore, it is harmless to the environment and has low toxicity for other living organisms (Zeni et al., 2021; Özcan and Tunaz, 2022). Most of the studies evaluating the efficacy of DEs on economically important arthropods have been on stored product pests. DE has many commercial preparations such as Drycide, Insecto[®], Perma Guard, Untreated, Silicosec, and Diafil 610, which are generally used around the world (Athanassiou et al., 2003; Ziaee and Khashaveh, 2007; Wakil et al., 2010; Alagöz and Sağlam, 2022). In studies with the Acarina order, different formulations of diatomaceous earth are generally focused on the families Dermanyssidae (Kilpinen and Steenberg, 2009; Steenberg and Kilpinen, 2014; Kilpinen and Steenberg, 2016; Alves et al., 2019; Alves et al., 2020; Ulrichs et al., 2020), and Macronyssidae (Mullens et al., 2012; Martin and Mullens, 2012; Murillo and Mullens, 2016). There are few studies on the effectiveness of DE on T. urticae (Shah and Appleby, 2019; Başkaya, 2020). This study aims to investigate the diatomaceous earth's potential in controlling T. urticae. For this, a local DE's acaricidal and repellent effects on T. urticae, its effect on reproductive potential, and its use in control were investigated.

MATERIALS AND METHODS

Tetranychus urticae

Tetranychus urticae culture was obtained from Ankara University, Faculty of Agriculture, Department of Plant Protection in 2022. The climate rooms ($25\pm2^{\circ}$ C, 50-60% humidity, and 16 hours of light: 8 hours of darkness) of Bursa Uludag University, Faculty of Agriculture, Department of Plant Protection were used to grow bean (*Phaseolus vulgaris* L.) (Fabales: Leguminosae) plants. This study was carried out at the same university in 2023. The plants were grown in small plastic pots (13×11) containing perlite and peat (ratio of 1:1) under the same conditions. Bean seeds (Magnum F₁) were obtained from May-Agro Ltd. Sti. (Antalya, Türkiye).

Diatomaceous earth product

Almina brand diatomaceous earth (Minitalya Madencilik A.Ş. Antalya, Türkiye) was used in the experiments. The basic material of Almina is obtained from a region with rich geothermal water resources. It is known that the diatom shells, which constitute the main component, are rod-like, generally round, and half-moon shaped, and the main raw material of diatoms is hydrated amorphous silica (SiO₂.NH₂O) with little clay (Figure 1). Mineral ratios according to total quantitative chemical analysis are 85.97% SiO₂, 0.66% CaO, 2.36% Al₂O₃, 2.61% Fe₂O₃, 0.91% MgO, 0.75% K₂O, <0.010 % Na₂O, and 0.28 % TiO₂ (Argetest, Yenimahalle, Ankara). The mean particle diameter is between 12-20 μ m. In the experiments, diatomaceous earth was prepared by mixing it with distilled water.



Figure 1. Scanning electron microscope image of Almina DE

Toxic effects of the diatomaceous earth on Tetranychus urticae females

The leaf disc method was used to determine the toxic effect of the DE on *T. urticae*. For this, 1.5 cm diameter discs were cut from the bean leaves by using a cork borer. The discs were immersed in different concentrations of the DE for 4-5 seconds and dried for about half an hour. The dried leaf discs were then placed in a 6 mm Petri dish containing moistened cotton discs. Then, at least 10 adult *T. urticae* females were placed on each leaf disc. The females used in the experiments are one- to three-day-old. Petri dishes were covered with parafilm after the lids were closed. Petri dishes were incubated in the above-mentioned climate chamber. The numbers of alive and dead were recorded after 24, 48, 72, and 96 hours, respectively. In the experiments, 10% (w/v), 5% (w/v), 2.5% (w/v), 1.25% (w/v), and 0% (w/v) (control) concentrations of DE were studied. The concentrations were determined by preliminary trials. Since Almina diatomaceous earth formed a thick layer at higher DE concentrations applied on the leaf discs, it took a long time for the leaf discs to dry, which affected the efficiency of the experiments. That's why 10% was chosen as the highest concentration. The experiments were carried out in 4 replications with at least ten adult females per replicate for each concentration. The experiment was repeated at least four times.

Repellent effects of the diatomaceous earth on Tetranychus urticae females

The method of Akyazı et al. (2015) was used with minor modifications to evaluate the repellency of the diatomaceous earth on *T. urticae*. For this experiment, discs of 1.5 cm in diameter were cut from the bean leaves with the help of a cork borer. Half of the discs were immersed in diatomaceous earth concentration and the other half in water for control purposes for 4-5 seconds and dried for half an hour. The discs were then placed in 6-mm Petri dishes with moistened cotton discs. Then, 30 adult females of *T. urticae* were placed on the midrib of each leaf disc, and the Petri dishes were covered with parafilm. In the experiments, the concentrations of 10% (w/v), 5% (w/v), 2.5% (w/v), and 1.25% (w/v) of the suspension were studied. The experiments were made with eight replications. The orientations of adult females were examined after 1, 2, and 24 hours, and counts were made. The repellent effect was calculated with the formula of Mozaffari et al. (2013). % Repellency= (C-T/N) ×100 where T= number of mites (treatment), C= number of mites (control), and N= number of mites (total). According to Juliana and Su (1983), percent repellency values were classified from 0 to V (Table 1). Positive values indicate repellency.

Table 1. The mean repellency values (%) are assigned to repellency classes (Juliana and Su, 1983).

Repellency Class	Repellency (%)		
0	>-0.1 to < 0.1		
1	0.1 to 20		
II	20.1 to 40		
111	40.1 to 60		
IV	60.1 to 80		
V	80.1 to 100		

Fecundity of Tetranychus urticae females on the DE-treated plant surface

The leaf disc method was used to determine the effects of the diatomaceous earth on the fecundity of adult females of *T. urticae*. For this purpose, females at the deutonymph chrysalis stage were collected from culture. As mentioned above, 1.5 cm-diameter discs were cut from bean leaves, and the discs were dipped in the above-mentioned concentrations of the DE, and dried. A deutonymph was placed on each of the discs and allowed to adult. Daily hatching eggs were then counted. For each concentration, the cumulative number of eggs laid by a female over 5 days was calculated. The percentage of emerged larvae was also recorded. The experiments were repeated 67, 89, 40, 41, and 16 times for the 10% (w/v), 5% (w/v), 2.5% (w/v), 1.25% (w/v), and 0% (w/v) (control) concentrations, respectively.

Statistical analysis

The cumulative mortality data means after 24, 48, 72, and 96 hours were analyzed by one-way ANOVA (P < 0.05), and differences between the means were separated using a Tukey multiple comparison test. Statistical analysis was performed using JMP 16.0.0 software. The mean numbers of the mites in both halves of diatomaceous earth-applied and untreated (control) bean leaf discs were separated using the t-test (p < 0.05). The average of the cumulative number of eggs laid by adult females during the first 5 consecutive days was subjected to analysis of variance (ANOVA) (p < 0.05), and the differences between characters were compared with student t-tests. Cumulative mortality and fecundity experiments were compared individually each time. All graphs were created using GraphPad Prism[®] Version 8.0.1.

RESULTS

Toxic effects of the diatomaceous earth on Tetranychus urticae females

The average mortality rates caused by different concentrations of diatomaceous earth in adult females of *T. urticae* are shown in Figure 2. Mortality rates observed after 24 hours at 10% and 5% concentrations were 15.01% and 8.01%, respectively, and were statistically significant compared to the control (Figure 2) (F =4.78; df =7, 100; p < 0.0001). The difference between 2.5% and 1.25% concentrations as well as with control was statistically similar. After 48 hours, mortality rates increased to 22.96%, 12.62%, 12.44%, and 1.73% at 10%, 5%, 2.5%, and 1.25% concentrations, respectively, and were significant except for the 1.25% concentration compared to the control group (F =8. 23; df =7, 91; p < 0.0001). High to low mortality rates at the same concentrations after 72 hours were 26%, 27.89%, 22.5%, and 7.14%, respectively. At this time, the difference among the highest three concentrations was found statistically the same, but except for %1.25 concentration, higher mortality rates were observed than the control groups (F =59.58; df =7, 51; p < 0.0001). Accordingly, the highest mortality rates were found to be %33.56, %32.52, %26.66, and %26.66 after 96 hours of exposure at 10%, %5, %2,5, and %1,25 concentrations. The difference between the concentrations and their control groups was statistically important after the 96-hour exposure period (F =92.29; df =7, 51; p < 0.0001) but, except for %10, the other concentrations did not differ from each other statistically (Figure 2).



Figure 2. Average mortality rates (%) of different concentrations of diatomaceous earth on adult females of *Tetranychus urticae*. The difference between means showing the same letter for concentrations in each period and their control groups is not statistically significant (p > 0.05; student's t-test)

Repellent effect of the diatomaceous earth on Tetranychus urticae females

The repellent effects of Almina diatomaceous earth on *T. urticae* adult females after 1, 2, and 24 hours are shown in Table 2. The diatomaceous earth at 10% concentration showed 64.54%, 53.63%, and 51.81% repellent effects on *T. urticae* adult females after 1, 2, and 24 hours, respectively. At this concentration, the number of mites oriented to the control side of the leaf discs after 1, 2, and 24 hours was statistically higher than the number of mites oriented to the diatomaceous-treated side (Table 2). At 5% concentration, the repellent effect was found to be 42.10%, 26.67%, and 15.23%, respectively, at the same time. The repellent effect was statistically significant in the first 2 hours and decreased to class I at the end of 24 hours. After 1, 2, and 24 hours, the repellent effects were found to be 20.35%, 10.22%, and -6.66%, respectively, for 2.5%, 19.46%, -5.40%, and -21.62%, respectively, at 1.25% concentrations. At 2.5% and 1.25% concentrations, the repellent effect decreased over time, and even the opposite attracted adult females at the end of 24 hours (Table 2).

Table 2. Repellent effect (%) (1, 2, and 24 hours after treatment) of the diatomaceous earth on adult females of *Tetranychus urticae**.

Hours	Percentage of repellency and average number of mite orientations on leaves							
	Concentrations (%) (w/v)	Untreated	Treated	t value	p-value	Repellency % (Repellency class)		
1 h	10	22.62±1.56	4.87±1.07	9.32	<0001*	64.54 (IV)		
	5	20.25±1.89	8.25±1.27	5.24	<0001*	42.10 (III)		
	2.5	17.00±0.53	11.25±0.79	5.99	<0001*	20.35 (II)		
	1.25	16.87±1.58	11.37±1.28	2.69	=0.01*	19.46 (I)		
2 h	Concentrations (%) (w/v)	Untreated	Treated	t value	p-value	Repellency % (Repellency class)		
	10	21.12±1.82	6.37±1.45	6.32	<0001*	53.63 (III)		
	5	16.62±2.41	9.62±1.75	2.34	=0.03*	26.67 (II)		
	2.5	15.50±1.63	12.62±1.48	1.30	=0.21	10.22 (I)		
	1.25	13.12±0.98	14.62±1.41	-0.86	=0.40	-5.40 (0)		
24 h	Concentrations (%) (w/v)	Untreated	Treated	t value	p-value	Repellency % (Repellency class)		
	10	20.87±2.01	6.62±1.19	6.08	<0001*	51.81 (III)		
	5	15.12±1.95	11.12±1.38	1.66	=0.11	15.23 (I)		
	2.5	13.12±1.20	15.00±1.50	-0.97	=0.34	-6.66 (0)		
	1.25	10.87±1.65	16.87±2.31	-2.11	=0.05	-21.62 (0)		

Means with () are significantly different between treated and untreated by t-test (mean \pm S.E., p <0.05); S.E. means the standard error.

Effects of the diatomaceous earth on the fecundity of Tetranychus urticae females

After 24 hours, the mean number of eggs laid at 10%, 5%, and 2.5% concentrations were 3.34, 4.07, and 4.25, respectively, and the difference between them was statistically insignificant, but the mean number of eggs laid at all four concentrations was significantly lower than the control (F= 8.50; df= 4, 295; p < 0.0001) (Figure 3). After 48 hours, there was no statistical difference between the number of eggs laid at 10% and 5% concentrations, and a statistically significant decrease was observed compared to the control (F = 8.60; df = 4, 275; p < 0.0001). The differences between the four concentrations applied after 72, 96, and 120 hours were not statistically significant compared to each other but were found to be significant compared to the control (Figure 3). The number of eggs laid by surviving female individuals at all applied concentrations increased

proportionally with the elapsed time (Figure 3). Looking at the larval emergence that emerged after 96 hours, it was observed that there were no significant differences in % larval emergence between the control and treatment groups (Table 3).

Time	After 24 hours							
Concentrations	10%	5%	2.5%	1.25%	Control			
Emerged Larva %	_	-	-	-	-			
Time	After 48 hours							
Concentrations	10%	5%	2.5%	1.25%	Control			
Emerged Larva %	_	_	_	-	-			
Time	After 72 hours							
Concentrations	10%	5%	2.5%	1.25%	Control			
Emerged Larva %	_	-	-	-	-			
Time	After 96 hours							
Concentrations	10%	5%	2.5%	1.25%				
Emerged Larva %	0.29	0.20	_	-				
Emerged Larva % (control)	0.70	0.69	0.94	0.64				
Time	After 120 hours							
Concentrations	10%	5%	2.5%	1.25%				
Emerged Larva %	5.73	6.52	4.45	4.64				
Emerged Larva % (control)	5.67	4.75	5.19	5.61				

Table 3. Larval emergence (%) in the control and treatment groups after 24, 48, 72, 96, and 120 hours



Figure 3. Effects of the diatomaceous earth on the fecundity of *Tetranychus urticae* females. The difference between means showing the same letter for concentrations in each period and their control groups is not statistically significant (p > 0.05; student's t-test)

DISCUSSION

In this study, the effectiveness of local diatomaceous earth on adult females of two-spotted spider mites was evaluated. With a general evaluation; a low toxic effect was found in the experiments. However, statistically more significant repellency activity was found at all four concentrations than in the control, especially for the first hour. The repellency effect increased with the increase in concentration but decreased as time progressed. When the effect of diatomaceous earth on the egg-laying of females was examined, at 10% and 5% concentrations, females laid fewer eggs than controls.

The insecticidal effect of DEs is generally related to their SiO₂ content (latrou et al., 2010). In this study, the SiO₂ content of Almina diatomaceous earth was found to be 85.97%. However, the toxicity percentages obtained are not compatible with the SiO₂ content. Again, low toxicity (24.6%) occurred in adult females of two-spotted spider mites in diatomaceous earth with a SiO₂ content of 67% (Shah and Appleby, 2019). Contrary to these results, the residual effects of 0.2%, 0.4%, and 0.8% concentrations of 10 μ m diameter diatomaceous earth named Turco 0010 on *T. urticae* adult females were found to be 4%, 6%, and 15% after 24 h (Başkaya, 2020). The DE concentrations used in our study were considerably higher than the concentrations in the study of Başkaya (2020). However, lower mortality rates were obtained in the present study. Korunic (1997) explained that the effectiveness of DE depends primarily on the physical properties of its powder, not its chemical composition. This situation can be explained by the differences in the chemical and physical properties of the Almina diatomaceous earth used.

The cuticle characteristics of the insect species to which DE is applied affect its susceptibility to DE (Athanassiou et al., 2005). Studies have shown that diatomaceous earth will damage the cuticles of soft-bodied organisms such as mites more quickly, so mites will have a higher mortality rate in a shorter period than insects (Kılıç, 2022). In this study, DE was used mixed with water. However, Kılıç (2022) tested the dust formulation of DE. The results obtained from this study show that both the species and the formulation types of DE are significant in effectiveness. In this study, the highest mortality rate was observed at 10% concentration (33.56%) at the 96th hour in four different concentrations applied on *T. urticae* adult females, and the mites who did not die continued their lives in the following days. The diatomaceous earth particles used in this study may not have adhered to the epicuticle layer and therefore did not cause water loss in the mites. Flanders (1941) stated that creatures that can recover the water lost, such as mites, are more resistant than insects that metabolize water from their food. Due to the size of a particle, the ejection of the dust from the cuticle with the movement of the insect can also reduce the mortality rate (Subramanyam and Roesli, 2000). In studies conducted with stored product mites, it has been reported that different DE formulations do not eliminate all mite populations (Collins and Cook, 2006a; Nesvorna and Hubert, 2014). In studies, an 8-24 mg/kg dose of Detia diatomaceous earth suppressed 50% of the population (Nesvorna and Hubert, 2014). The residual mortality rate in DE (Fisiocontrol) water-based suspension was 97.39% in all periods for Dermanyssus gallinae (De Geer, 1778) (Acari: Dermanyssidae), a parasitic mite species. The same type of diatomaceous earth combined with mechanical cleaning caused a 90% reduction in the mite population in 21 days (Alves et al., 2020). Again, when the powder De (Diamol) and the entomopathogenic fungus Beauveria bassiana (Balsamo) were applied together, it showed a synergistic effect and showed a mortality rate of 89.1% (Steenberg and Kilpinen, 2014). A dust formulation (Detech® Dust) of a local Turkish diatomaceous earth in Tyrophagus putrescentiae (Schrank, 1781) (Acari: Acaridae), a cosmopolitan mite species, caused 100% mortality in an average of 24 hours (Kılıç, 2022). A water-soluble formulation of DE in all periods of Onythonyssus sylvarium (Canestrini and Fanzago 1877) (Acari: Macronyssidae), a parasitic mite species, significantly decreased the mite population when applied only for 2 consecutive weeks (Mullens et al., 2012). Diatomaceous earth DE cide showed 24.6% contact toxicity in adult females of T. urticae (Shah and Appleby, 2019). Similarly, granite dust showed an acaricidal effect of 8–16% in two-option experiments after contact with T. urticae (Faraone et al., 2020). Again, in studies with Lepidoglyphus destructor (Schrank, 1781) (Acarina: Glycyphagidae) and Acarus siro (L., 1758) (Acarina: Acaridae), it was stated that large variations were observed between repetitions in terms of diatomaceous earth activity (Collins and Cook, 2006a).

The effect of DEs on mites varies according to formulations, mite species, and biological periods (Nesvorna and Hubert, 2014). In this study, only adult females of the mite were used in the experiments. The sensitivity of the pre-adult period to the applied diatomaceous earth may be different. Similarly, in trials with *T. putrescentiae* (Acari: Acaridae), it was found that the adult period is more tolerant to diatomaceous earth applied than the pre-adult period (latrou et al., 2010). The more the insect moves, the more dust particles will stick to its body. As a result, the mortality rate of insects will be higher due to water loss. According to Rigaux et al. (2001), there was a direct relationship between insect activity and mortality when Protect-It diatomaceous earth was applied to different *Tribolium castaneum* (Herbst, 1797) (Coleoptera: Tenebrionidae) strains. In this study, it was observed that in *T. urticae*, adult females moved less on diatom-treated leaf discs than the control. Fewer dust particles may have adhered to their bodies due to less movement, resulting in lower mortality rates. In addition, the insecticidal effects of diatomaceous earth may vary according to abiotic factors (temperature, applied concentration, and formulation form) (Şen et al., 2019). In general, studies on cereals have reported that water-based formulations of DE preparations have less effect than dust formulations (Fields and Korunic, 2000; Collins and Cook, 2006b; Wakil et al., 2006). The surface tension differences between DE particles in different formulations may affect the adhesion of particles to the insect cuticle. The fact that the DE

we used in our study is a water-wettable powder formulation may explain the low toxicity. However, more studies are needed to prove this.

According to our research, this is the first study on the repellent effect of diatomaceous earth in T. urticae, and there are very few studies on the repellent or attractant effect of DE on stored product pests. In this study, T. urticae adult females preferred to orient to the control side in the first hour when diatomaceous earth was applied to half of the bean leaf discs at 10%, 5%, 2.5%, and 1.25% concentrations. However, at 5%, 2.5%, and 1.25% concentrations, the repellent effect decreased over time, and the adult females moved to the diatomaceous-applied side of the leaf. The high repellent effect, which can be seen even at the end of the first 24 hours, especially at 10% concentration, is very important in terms of dispersal of the T. urticae population, moving to different hosts, and preventing damage. Korunic (2013) stated that DE dusts are insect repellent, and the quality of repellent depends on the dose. Similarly, it has been reported that a DE product called Mitex has a repellent effect on T. castaneum larvae (Hossain et al., 2010). It was also determined that Mitex controls both T. castaneum and Sitophilus oryzae L., 1763 (Col: Curculionidae) populations (Kabir et al., 2011). In our study, since 10% and 5% concentrations may be physically more repulsive, mites may have oriented to the control side of the leaf. Insects often tend to avoid dusty surfaces. Mites, too, may have moved away from the environment because the surface is dusty. The presented results are also compatible with Eastin and Burden (1960) and Ebeling (1971). The researchers considered that DE deposits are repellent for cockroaches. However, in an application made with granite rock powder material in T. urticae, foliar and soil applications of DE did not show a repellent effect, on the contrary (Faraone et al., 2020).

There are very few studies examining the effect of diatomaceous earth on the oviposition of *T. urticae* (Shah and Appleby, 2019). In the study of these researchers, diatomaceous earth applied to *T. urticae* females as contact and spray did not have a significant effect on female oviposition. In a 24-72 hours period, the number of eggs laid by females increased over time and the difference with the control group was found to be statistically the same (Shah and Appleby, 2019). In our study, egg-laying was evaluated for 120 hours and larval hatching was observed. Contrary to the study by Shah and Appleby (2019), the number of eggs laid by female individuals at 10% and 5% concentrations were found to be less in the first 120 hours of oviposition compared to the control. In all concentrations applied during this period, larvae hatching percentages did not differ greatly between applied concentrations and control.

As a result, further experiments will be required to determine why this DE on *T. urticae* has a low toxic effect. Additionally, in future studies, the same concentrations of this diatomaceous earth will be tested by mixing it with botanical extracts or entomopathogens. In conclusion, the moderate repellent effect, toxicity and reduced fecundity of Almina diatomaceous earth at 10% and 5% concentrations seem promising in the future as a physical control method in pest control programs for *T. urticae*.

Acknowledgments: The authors thank Alperen Kaan Bütüner (Bursa Uludag University, Faculty of Agriculture, and Plant Protection Department) for his advice and the statistical analysis.

Conflict of Interest Statement: The authors declare that they have no conflict of interest.

Contribution Rate Statement Summary of Researchers: H.S. investigation, writing, review, and editing; M.İ. investigation, writing, review, and editing.

Author Orchid Numbers

Hilal SUSURLUK[:] ORCID^(D): 0000-0002-8329-8855

Merve ILKTAN[:] ORCID^(D): 0009-0007-9999-5034

REFERENCES

Akyazı, R., Soysam, M. and Hassan, E. 2015. Toxic and repellent effects of *Prunus laurocerasus* L. (Rosaceae) extracts against *Tetranychus urticae* Koch (Acari: Tetranychidae). *Turkish Journal of Entomology*, 39 (4): 367-380.

- Alagöz, V. and Sağlam, Ö. 2022. Insecticidal efficacy of some Turkish diatomaceous earth deposits against rice weevil, Sitophilus oryzae I. (Coleoptera: Curculionidae) on paddy. Journal of Tekirdag Agricultural Faculty, 19 (2): 446-455.
- Alves, L. F. A., de Oliveira, D. G. P., Pares, R. B., Sparagano, O. A. E. and Godinho, R. P. 2020. Association of mechanical cleaning and a liquid preparation of diatomaceous earth in the management of poultry red mite, *Dermanyssus gallinae* (Mesostigmata: Dermanyssidae). *Experimental and Applied Acarology*, 81 (2): 215-222.
- Alves, L. F. A., Oliveira, D. G. P., Kasburg, C. R. and Nardelli, M. S. 2019. Acaricidal activity of inert powders against the poultry red mite *Dermanyssus gallinae* (De Geer, 1778) (Mesostigmata: Dermanyssidae). *Archives of Veterinary Science*, 24 (2): 81-92.
- Athanassiou, C. G., Vayias, B. J., Dimizas, C. B., Kavallieratos, N. G., Papagregoriou, A.S. and Buchelos, C.Th. 2005. Insecticidal efficacy of diatomaceous earth against *Sitophilus oryzae* (L.) (Coleoptera: Curculionidae) and *Tribolium confusum* du Val (Coleoptera: Tenebrionidae) on stored wheat: influence of dose rate, temperature and exposure interval. *Journal of Stored Products Research*, 41 (1): 47-55.
- Athanassiou, C. G., Kavallieratos, N. G., Palyvos, N. E. and Buchelos, C. T. 2003. Three-dimensional distribution and sampling indices of insects and mites in horizontally stored wheat. Applied Entomology and Zoology, 38 (3): 413-426.
- Başkaya, M. 2020. Insecticidal, Acaricidal, and Synergistic Activity of Diatomaceous Earth. The Graduate School of Natural and Applied Science of Selçuk University the Degree of Master of Science in Department of Plant Protection, (Unpublished) MSc Thesis, Selçuklu, Konya, pp. 74.

Çetin, M. and Taş, B. 2012. A natural mineral with Biological Origin: Diatomite. *Tübav Bilim Dergisi*, 5 (2): 28-46.

- Collins, D. A. and Cook, D. A. 2006a. Laboratory studies evaluating the efficacy of diatomaceous earths, on treated surfaces, against stored-product insect and mite pests. *Journal of Stored Products Research*, 42 (1): 51-60.
- Collins, D. A. and Cook, D. A. 2006b. Laboratory evaluation of diatomaceous earths, when applied as dry dust and slurries to wooden surfaces, against stored-product insect and mite pests. *Journal of Stored Products Research*, 42 (1): 197-206.
- Eastin, J. L. and Burden, G. S. 1960. Tests with five silica dusts against German cockroaches. *Florida Entomologist*, 43 (3): 99-102.
- Ebeling, W. 1971. Sorptive dusts for pest control. Annual Review of Entomology, 16 (1): 123-158.
- Erdoğan, P., Saltan, G. and Sever, B. 2010. Acaricidal effect of *Capsicum annum* L. extracts on two-spotted spider mite *Tetranychus urticae* Koch (Arachnida: Tetranychidae). *Plant Protection Bulletin*, 50 (1): 35-43.
- Faraone, N., Evans, R., LeBlanc, J. and Hillier, N. K. 2020. Soil and foliar application of rock dust as natural control agent for two-spotted spider mites on tomato plants. *Scientific Reports*, 10 (1): 12108.
- Fields, P. and Korunic, Z. 2000. The effect of grain moisture content and temperature on the efficacy of diatomaceous earths from different geographical locations against stored-product beetles. *Journal of Stored Products Research*, 36 (1): 1-13.
- Flanders, S.F. (1941). Dust as an inhibiting factor in the reproduction of insects. *Journal of Economic Entomology*, 34 (3): 470-472.
- Hossain, M. M., Reza, A. M. S. and Parween, S. 2010. Age-related response of *Tribolium castaneum* (Herbst) larvae to diatomaceous earth at different exposure periods. *Journal of Bio-Science*, 18: 40-43.
- Iatrou, S. A., Kavallieratos, N. G., Palyvos, N. E., Buchelos, C. T. and Tomanović, S. 2010. Acaricidal effect of different diatomaceous earth formulations against *Tyrophagus putrescentiae* (Astigmata: Acaridae) on stored wheat. *Journal of Economic Entomology*, 103 (1): 190-196.
- Juliana, G. and Su, H. C. F. 1983. Laboratory studies on several plant materials as insect repellents for protection of cereal grains. *Journal of Economic Entomology*, 76 (1): 154-157.
- Kabir, S. M. H., Das, D. R., Faruki, S. I., Reza, A. S. R. and Parween, S. 2011. Control of population build-up of *Tribolium castaneum* (Herbst) and *Sitophilus oryzae* L. by diatomaceous earth. *Journal of the Asiatic Society of Bangladesh* (Sci.), 37 (1): 15-23.
- Kilpinen, O. and Steenberg, T. 2009. Inert dusts and their effects on the poultry red mite (*Dermanyssus gallinae*). *Experimental and Applied Acarology*, 48 (1-2): 51-62.
- Kilpinen, O. and Steenberg, T. 2016. Repellent activity of desiccant dusts and conidia of the entomopathogenic fungus *Beauveria bassiana* when tested against poultry red mites (*Dermanyssus gallinae*) in laboratory experiments. *Experimental and Applied Acarology*, 70 (3): 329-341.
- Korunic, Z. (2013). Diatomaceous earths Natural Insecticides. *Pesticides and Phytomedicine* (Belgrade), 28 (2): 77-95.

- Kılıç, N. 2022. Efficacy of dust and wettable powder formulation of diatomaceous earth (Detech[®]) in the control of *Tyrophagus putrescentiae* (Schrank) (Acari: Acaridae). *Insects*, 13 (10): 857.
- Martin, C. D. and Mullens, B. A. 2012. Housing and dustbathing effects on northern fowl mites (*Ornithonyssus sylviarum*) and chicken body lice (*Menacanthus stramineus*) on hens. *Medical and Veterinary Entomology*, 26 (3): 323-333.
- Mozaffari, F., Abbasipour, H., Garjan, A. S., Saboori, A. and Mahmoudvand, M. 2013. Toxicity and oviposition deterrence and repellency of *Mentha pulegium* (Lamiacaeae) essential oils against *Tetranychus urticae* Koch (Tetranychidae). *Journal of Essential Oil Bearing Plants*, 16 (5): 575-581.
- Mullens, B. A., Soto, D., Martin, C. D., Callaham, B. L. and Gerry, A. C. 2012. Northern fowl mite (*Ornithonyssus sylviarum*) control evaluations using liquid formulations of diatomaceous earth, kaolin, sulfur, azadirachtin, and *Beauveria bassiana* on caged laying hens. *Journal of Applied Poultry Research*, 21 (1): 111-116.
- Murillo, A. C. and Mullens, B. A. 2016. Timing diatomaceous earth-filled dustbox use for management of northern fowl mites (Acari: Macronyssidae) in cage-free poultry systems. *Journal of Economic Entomology*, 109 (6): 2572-2579.
- Nesvorna, M. and Hubert, J. 2014. Effect of diatomaceous earth-treated wheat on population growth of stored product mites under laboratory test. *International Journal of Acarology*, 40 (4): 269-273.
- Özcan, K. and Tunaz, H. 2022. BGN-1 kodlu Türk Diatom Toprağının Alman Hamamböceği (Blatella germanica L.)'nin Erginlerine Karşı Ölüm Etkisi. Kahramanmaraş Sütçü İmam Üniversitesi Tarım ve Doğa Dergisi, 25 (5): 1061-1067.
- Rigaux, M., Haubruge, E. and Fields, P. G. 2001. Mechanisms for tolerance to diatomaceous earth between strains of *Tribolium castaneum*. *Entomologia Experimentalis et Applicata*, 101 (1): 33-39.
- Şen, R., Işıkber, A. A., Bozkurt, H. and Sağlam, Ö. 2019. Effect of temperature on insecticidal efficiency of local diatomaceous earth against stored-grain insects. *Turkish Journal of Entomology*, 43 (4): 441-450.
- Shah, R. and Appleby, M. 2019. Testing the contact and residual toxicity of selected low-risk pesticides to *Tetranychus urticae* Koch and its Predators. *Sarhad Journal of Agriculture*, 35 (4): 1113-1121.
- Steenberg, T. and Kilpinen, O. 2014. Synergistic interaction between the fungus *Beauveria bassiana* and desiccant dusts applied against poultry red mites (*Dermanyssus gallinae*). *Experimental and Applied Acarology*, 62 (4): 511-524.
- Subramanyam, B. and Roesli, R. 2000. "Inert Dusts". *In: Alternatives to Pesticides in Stored-Product IPM* (Eds. B. Subramanyam & D. W. Hagstrum)., Kluwer Academic Publishers, Boston, UK, 321-380.
- Susurluk, H. and Gürkan, M.O. 2022. The effects of lambda-cyhalothrin and bifenthrin resistance on the fitness of *Tetranychus urticae* Koch (Acari: Tetranychidae). *Systematic and Applied Acarology*, 27 (3): 525-537.
- Ulrichs, C., Han, Y. J., Abdelhamid, M. T. and Mewis, I. 2020. Management of the poultry red mite, *Dermanyssus gallinae*, using silica-based acaricides. *Experimental and Applied Acarology*, 82 (2): 243-254.
- Van Leeuwen, T., Vontas, J., Tsagkarakou, A., Dermauw, W. and Tirry, L. 2010. Acaricide resistance mechanisms in the two-spotted spider mite *Tetranychus urticae* and other important Acari: a review. *Insect Biochemistry and Molecular Biology*, 40 (8): 563-572.
- Wakil, W., Ashfaq, M., Shabbir, A., Javed, A. and Sagheer, M. 2006. Efficacy of diatomaceous earth (protect-it) as a protectant of stored wheat against *Rhyzopertha dominica* (F.) (Coleoptera: Bostrichidae). *Pakistan Entomologist*, 28 (2): 19-24.
- Wakil, W., Ashfaq, M., Ghazanfar, M. U. and Riasat, T. 2010. Susceptibility of stored-product insects to enhanced diatomaceous earth. *Journal of Stored Products Research*, 46 (4): 248-249.
- Zeni, V., Baliota, G.V., Benelli, G., Canale, A. and Athanassiou, C. G. 2021. Diatomaceous earth for arthropod pest control: Back to the future. *Molecules*, 26 (24): 7487.
- Ziaee, M. and Khashaveh, A. 2007. Effect of five diatomaceous earth formulations against *Tribolium castaneum* (Coleoptera: Tenebrionidae), *Oryzaephilus surinamensis* (Coleoptera: Silvanidae) and *Rhyzopertha dominica* (Coleoptera: Bostrychidae). *Insect Science*, 14 (5): 359-365.