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Research Article/Araştırma Makalesi

Effects of a native diatomaceous earth on *Oryzaephilus surinamensis* (L., 1758) (Coleoptera: Silvanidae), and *Acanthoscelides obtectus* (Say, 1831) (Coleoptera: Chrysomelidae)

Oryzaephilus surinamensis (L., 1758) (Coleoptera: Silvanidae), ve Acanthoscelides obtectus (Say, 1831) (Coleoptera: Chrysomelidae) üzerinde yerel diatom toprağının etkileri

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ABSTRACT

Pesticides are widely used to prevent damage caused by pests during the storage of agricultural products under warehouse conditions. However, in recent years, alternative methods for controlling pests have gained significance due to the emergence of toxic effects of pesticides on non-target organisms. Recently, Diatomaceous Earth (DE) is widely utilized for control storage pests. Therefore, it is considered that a local Diatomaceous Earth Almina could be used for the potential control of *Oryzaephilus surinamensis* (L., 1758) (Coleoptera: Silvanidae) and *Acanthoscelides obtectus* (Say, 1831) (Coleoptera: Chrysomelidae). Almina® DE was applied at varying doses (125, 250, 500, 750, and 1000 ppm) (mg DE/ kg grains), and mortality rates were assessed at 24, 48, 72, and 96 hours. The highest mortality rates achieved were 100% for *O. surinamensis* after 96 hours with the 1000 ppm dose and for *A. obtectus* after 48 hours with doses of 500, 750, and 1000 ppm DE. In addition, the lowest LT_{50} and LT_{90} values were determined as 20.5, and 45.6 hours at 1000 ppm for *O. surinamensis*. For *A. obtectus*, LT_{50} and LT_{90} values at 250 and 125 ppm were 14.2 and 56.5 hours, and 51.4 and 95.7 hours, respectively. Consequently, Almina® DE can be considered a good alternative to synthetic chemicals to control two important stored product pests, *O. surinamensis* and *A. obtectus*.

Key Words: Diatomaceous earth, Effect, *Acanthoscelides obtectus*, *Oryzaephilus surinamensis*

ÖZ

Tarımsal ürünlerin depo koşullarında saklanması sırasında zararlı böceklerin ürün kaybına neden olmaması için yaygınlıkla pestisitler kullanılmaktadır. Ancak pestisitlerin hedef dışı organizmalar üzerinde oluşturduğu toksik etkilerin ortaya çıkması ile zararlılarla mücadelede alternatif yöntemler son yıllarda önem kazanmaktadır. Son yıllarda, depo zararlılarının kontrolünde Diatom Toprağı (DE) yaygınlıkla kullanılmaktadır. Bu nedenle *Oryzaephilus surinamensis* (L., 1758) (Coleoptera: Silvanidae) ve *Acanthoscelides obtectus* (Say, 1831) (Coleoptera: Chrysomelidae)'un potansiyel kontrolü amacıyla yerel Almina® Diatom toprağı kullanılabileceği düşünülmektedir. Bu çalışmada 125, 250, 500, 750, 1000 ppm (mg DE/kg tahıl) dozunda DE belirtilen türlere uygulanmıştır. 24, 48, 72 ve 96 saat sonunda ölüm oranları belirlenmiştir. *O. surinamensis*'de elde edilen en yüksek ölüm oranı 96 saat sonunda 1000 ppm doz uygulanan bireylerde 100%, *A. obtectus* içinse en yüksek ölüm oranı 48 saat sonra 500, 750 ve 1000 ppm DE uygulanan bireylerde 100% olarak belirlenmiştir. Ayrıca, *O. surinamensis* için en düşük LT_{50} ve LT_{90} değerleri 1000 ppm de sırasıyla 20.5 ve 45.6 saat olarak belirlenmiştir. *A. obtectus* için 250 ve 125 ppm'deki LT_{50} ve LT_{90} değerleri ise sırasıyla 14.2 ve 56.5 saat ve 51.4 ve 95.7 saat olarak bulunmuştur. Sonuç olarak Almina® DE, iki önemli depolanmış ürün zararlısı *O. surinamensis* ve *A. obtectus* ile mücadelede sentetik kimyasallara iyi bir alternatif olarak düşünülebilir.

Anahtar Kelimeler: Diatom toprağı, Etki, *Acanthoscelides obtectus*, *Oryzaephilus surinamensis*

Introduction

The increasing world population has heightened the importance of agricultural production and further emphasized the need for food (Gilland, 2002; Crist et al., 2017; Zsögön et al., 2022). Therefore, research on factors leading to product loss in agricultural fields and post-harvest storage conditions has been on the rise. Among the foremost factors causing reduced yield in agricultural fields and storage conditions are agricultural pests (Eickhout et al., 2006; García-Oliveira et al., 2022; Floret et al., 2023). Pesticides are one of the most commonly used products to protect agricultural products from post-harvest pests. However, in recent years, the determination of the toxic effects of pesticides used in chemical control on non-target organisms (such as bees, fish, and humans) has led to restrictions on their use in agricultural areas and storage conditions. Moreover, residues of pesticides used for pest control, especially under storage conditions, can directly reach humans. For this reason, alternative methods of chemical control have gained importance in recent years.

Oryzaephilus surinamensis (L., 1758) (Coleoptera: Silvanidae), also known as the Sawtoothed Grain Beetle, is a widely distributed insect species worldwide. It causes yield losses in various products such as grain crops, nuts, chocolate, and tobacco (Weston & Rattlingourd, 2000; Trematerra & Sciarretta, 2004; Nika et al., 2020; Abdel-Banat et al., 2023). Adults of this species can rapidly reproduce, especially in warm and humid conditions, leading to productivity losses in storage facilities. Female beetles can live for approximately 5-10 months under suitable conditions and can lay nearly 300 eggs during their lifespan (Arthur, 2000; Trematerra & Sciarretta, 2004; Nika et al., 2020; Nika et al., 2021). These eggs hatch within 2 to 3 days at 28°C. The larvae of this pest, like the adults, typically feed on grain kernels, contributing to productivity losses in storage conditions (Finkelman et al., 2006; Klys & Przystupinska, 2015).

Similarly, another species that causes yield losses in food products both in the field and in storage conditions is *Acanthoscelides obtectus* (Say, 1831) (Coleoptera: Chrysomelidae). This pest is commonly known to feed on bean seeds, but it also feeds on the seeds of peas, lentils, and many other leguminous plants. *A. obtectus* larvae feed by entering plant leaves and seeds (Papachristos & Stamopoulos, 2002; Parsons & Credland, 2003; Şen et al., 2020). These pests become adults in storage facilities, producing new generations and perpetuating their damage. Considering climatic conditions, *A. obtectus* can produce an average of 5 generations throughout the year. This pest diminishes product quality, yield, and the germination capacity of seeds by creating holes in the grains (Franco et al., 2005; Jovanović et al., 2007; Freitas et al., 2016; Hervet et al., 2023).

To control both pests, pesticides are widely used in storage conditions (Haddi et al., 2018). However, the use of pesticides in food products, especially after harvest, is restricted due to their toxic effects on non-target organisms. In addition, regulations implemented by the European Union in recent years have supported alternative methods to the use of pesticides (Bütüner & Susurluk, 2023).

Diatomaceous earth has become increasingly popular, particularly for controlling harmful insects in storage conditions. Diatomaceous earth (DE) is a type of natural soil obtained from the remains of fossilized algae (diatoms) found in marine or freshwater sources. DE has been used for pest control purposes in storage conditions since the early 1930s (Quarles, 1992; Wakil et al., 2010; Bello et al., 2014; Zeni et al., 2021). It is known that DE particles adhere to the exoskeleton of insects, causing their death due to drying or dehydration. Additionally, studies have shown that DE causes abrasions of insect cuticles, leading to deformations and ultimately death in insects (Vayias et al., 2009; Shah & Khan, 2014; Korunić et al., 2020; Wakil et al., 2023).

Studies on the effect of diatomaceous earth on *O. surinamensis* and *A. obtectus* pests are quite limited. This study aims to determine the effectiveness of a local diatomaceous earth in the control of both pests. Therefore, this study is important for the potential control of *O. surinamensis* and *A. obtectus* pests.

Materials and Methods

Cultures of *Oryzaephilus surinamensis* and *Acanthoscelides obtectus*

The cultures of *O. surinamensis* and *A. obtectus* were cultivated in 2.5 L plastic containers in climate chambers maintained at 26±2°C, 65±5 % humidity. Cracked wheat grains served as the cultivation medium for *O. surinamensis*, while white, medium-sized Dermason variety bean grains were employed for the cultivation of *A. obtectus*, as documented in studies by Soares et al. (2015) and Hassan et al. (2020). Emerging adult mixed-sex individuals (< one week old) were used in the trials (Astuti et al., 2018). To achieve this objective, containers were prepared with a population of 100 mixed-sex individuals aged 1-2 days. These containers were furnished with beans and cracked wheat for *A. obtectus* and *O. surinamensis*, respectively. After, approximately 7 days (for mating and egg-laying), all adult insects were extracted from the containers, allowing the laid eggs to develop into F_1 offspring. The progression of the culture was regularly monitored at two-day intervals, and experiments were initiated with newly emerged adults. Each trial was carried out under the above controlled atmosphere conditions and its continuity was ensured.

Diatomaceous earth

In this study, Almina® Diatomaceous Earth was utilized in a water-dispersible powder formulation, which is produced by a local company (Minitalya Mining Company, Antalya, Türkiye). The raw material for this compound is known to be obtained from regions rich in geothermal water sources. The general composition of the DE is as follows: 0.28% TiO_2, 85.97% SiO_2, 2.36% Al_2O_3, 0.66% CaO, 0.91% MgO, 2.61% Fe_2O_3, 0.75% K_2O, and less than 0.010% Na_2O (Argetest, Ankara, Türkiye). The average particle size of DE varies between 12-20 µm. Also, the particle shapes of the diatomaceous earth used are mixed (angular, oval, etc.) (Figure 1). In this study, different doses of the DE were mixed into bean and wheat grains.

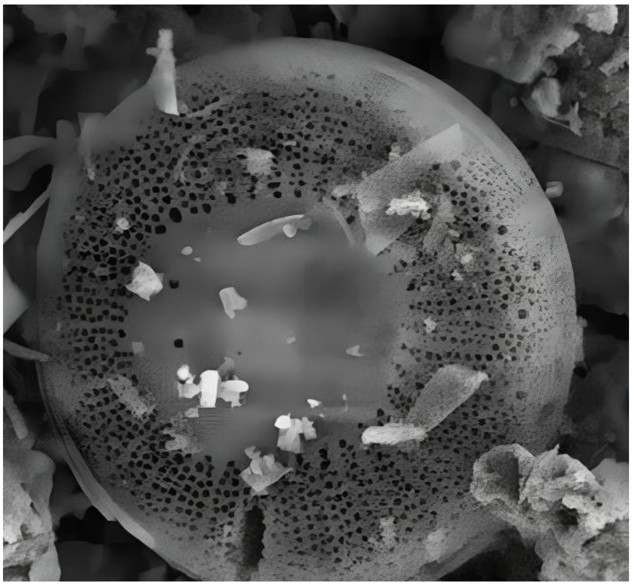

Figure 1. Electron microscope image of the general structure of the diatomaceous earth used in the study.

Experimental design

First of all, cracked wheat grains (30 g / replicate / dose rate) for *O. surinamensis*, and bean grains (30 g/ replicate / dose rate) for *A. obtectus* were placed in small glass jars (6.5 cm in diameter, 7 cm in height) (Freitas et al., 2016). Then, 125, 250, 500, 750, and 1000 ppm (mg DE kg grains^{-1}) DE doses were added to these jars separately. Each jar was shaken by hand for approximately 2-3 minutes to ensure even distribution. In each jar, 20 adults of *O. surinamensis*, and *A. obtectus* used in the experiment were released. Then, lids with holes to facilitate air circulation were placed over the jars. Underneath these lids, filter papers were placed to prevent the insects from escaping. After 24, 48, 72, and 96 hours, the numbers of live and dead individuals in the jars were determined. As a control group, jars containing only wheat and bean grains were used, and adult individuals were released into them. Similarly, the numbers of live and dead individuals in the control group jars were observed after 24, 48, 72, and 96 hours. If an insect could not move when touched with a small brush, it was considered dead (Jovanović et al., 2007). The experimental setup adhered to a randomized parcel design, implemented with a minimum of three replications for each dose.

Statistical analyses

Cumulative mortality rates at 24, 48, 72 and 96 hours were analyzed using two-factor analysis of variance (ANOVA) and means were compared using LSD (Least Significant Differences) test ($P < 0.05$). ANOVA analysis was conducted using JMP®16.0 software, while the LT_{50} and LT_{90} values were determined using Probit analysis and Log-probit method (POLO-PLUS ver.2.0) (LeOra Software). All graphs were produced using GraphPad Prism® Version 8.0.1.

Results and Discussion

According to the obtained results, when examining the mortality of *O. surinamensis* adults under different doses and at different time intervals, the highest mortality for 24 hours was observed in individuals subjected to a dose of 1000 ppm of the DE. This rate was determined to be 60%. The mortality for 750, 500, 250, and 125 ppm for 24 hours were determined as 45, 45, 16.67 and 8.33%, respectively. In the control group (0 ppm), no mortality was observed. After 48 hours, the highest mortality was determined to be 91.67% at 1000 ppm. The mortality for other doses were 80.00, 61.67, 25.00, and 21.67% for 750, 500, 250, and 125 ppm, respectively. This value was obtained as 3.33% in the control group. When the mortality of individuals were examined at 72 hours, the highest mortality was observed in individuals subjected to a dose of 1000 ppm. This rate was determined as 96.67%. At 750 ppm, this rate was 93.33%. For 500, 250, and 125 ppm, these values were determined as 88.33, 50.00, and 46.67%, respectively. For the control group, this value was found to be 10.00%. After 96 hours, all individuals subjected to a dose of 1000 ppm of DE were found to have died (100%). The mortality for 750, 500, 250, and 125 ppm were determined as 96.67%, 86.67%, 71.67%, and 61.67%, respectively. For control group, this value was determined as 10.00% for 96 hours (Figure 2). The data exhibits statistically significant differences ($F = 39.06$; $df = 23, 48$; $p < 0.0001$).

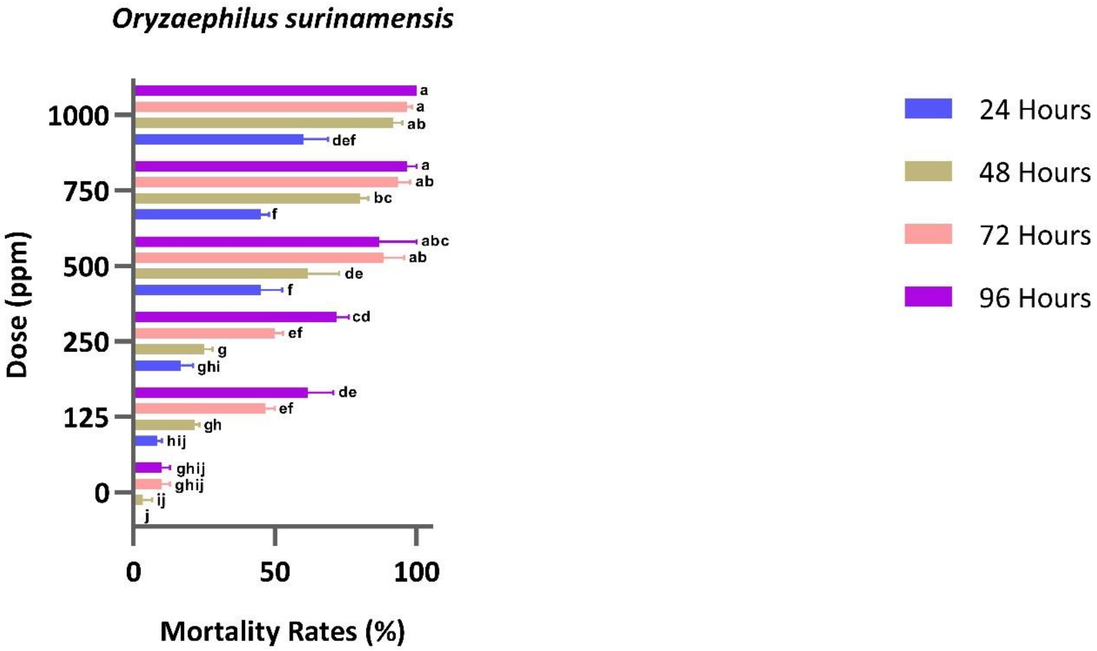

Figure 2. The mortality rates of *Oryzaephilus surinamensis* induced by Almina DE were examined. Statistical analysis was conducted separately for each species (Mean±SE). Doses of the DE with the same letters following the means are not significantly different ($p < 0.05$).

When examining the mortality of *A. obtectus*, the highest mortality for 24 hours was determined to be 93.33% in individuals subjected to a dose of 1000 ppm of DE. For individuals exposed to a dose of 750 ppm of DE, this rate was determined to be 91.67%. The mortality for 500, 250, and 125 ppm were determined as 81.67, 75.00 and 10.00%, respectively. No dead individuals were encountered in the control group. After 48 hours, when the mortality for 1000, 750, and 500 ppm were examined, the mortality for these doses was determined to be 100%. For 250 and 125 ppm, these values were obtained as 90.00, and 31.67%, respectively. In the control group, no dead individuals were encountered. When the mortality rates were examined at 72 hours, similar to the rates at 48 hours, the mortality rates for 1000, 750, and 500 ppm were determined to be 100%. For 250 and 125 ppm, these values were obtained as 95.00, and 78.33%, respectively. In the control group, the mortality rate was determined to be 1.67%. After 96 hours, the highest mortality rates were obtained for 1000, 750, 500, and 250 ppm, which were all 100%. For 125 ppm, this value was 93.33%, while in the control group, it was 3.33% (Figure 3). All of these data show statistically significant differences ($F = 215.82$; $df = 23, 48$; $p < 0.0001$).

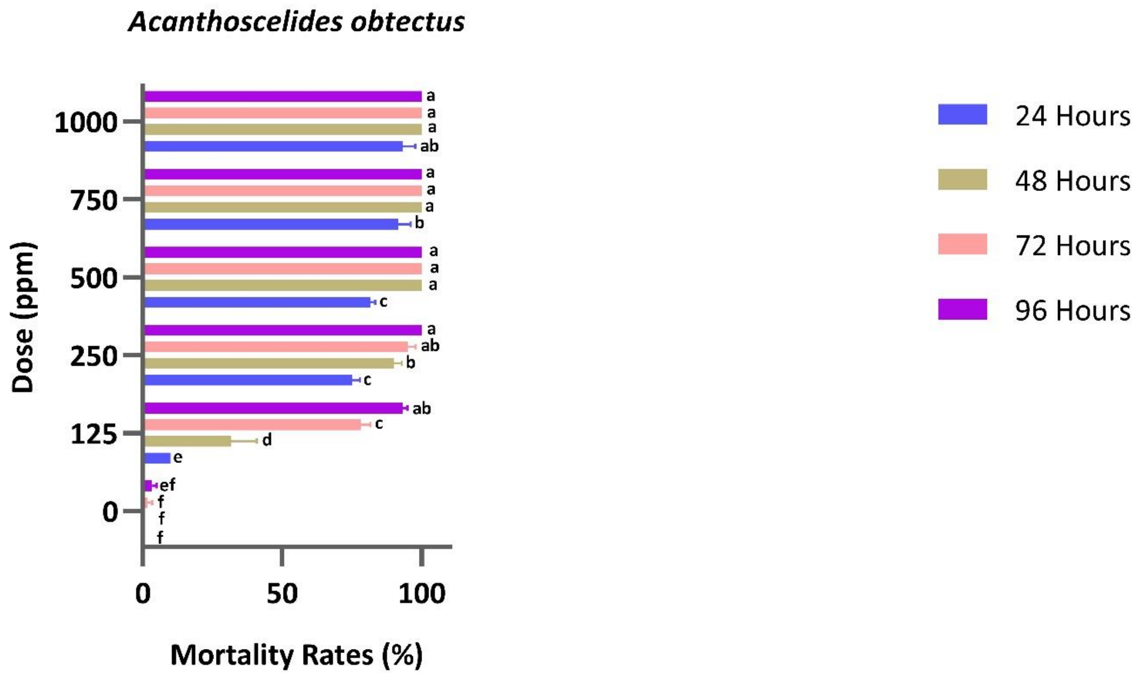

Figure 3. The mortality rates of *Acanthoscelides obtectus* induced by Almina DE were examined. Statistical analysis was conducted separately for each species (Mean±SE). Doses of the DE with the same letters following the means are not significantly different ($p < 0.05$).

For the species that were subjected to testing, all primary factors (dosage and time) and their respective interactions (time × dose) were found to be statistically significant with a p-value of less than 0.001 (Table 1).

Table 1. ANOVA parameters for the primary factors and their interactions related to the mortality rates of *Oryzaephilus surinamensis* and *Acanthoscelides obtectus*.

Species	*Oryzaephilus surinamensis*			*Acanthoscelides obtectus*		
Source	Df	*F*	*p*	df	*F*	*p*
Whole Model	23	39.06	<0.001	23	215.82	<0.001
Dose	5	128.66	<0.001	5	845.99	<0.001
Times	3	70.05	<0.001	3	96.91	<0.001
Dose x Times	15	2.99	0.0020	15	29.55	<0.001

Additionally, in this study, the LT_{50} and LT_{90} values of Almina DE used in different doses and on different species were determined. In *O. surinamensis*, the lowest LT_{50} value was observed at 1000 ppm at 20.551 hours. However, according to the fiducial limits, there was no difference in LT_{50} values between 1000, 750, and 500 ppm. The highest LT_{50} value for *O. surinamensis* was determined at a dose of 125 ppm (78.626 hours). However, the fiducial limits of LT_{50} values did not show a difference between 250, and 125 ppm. When LT_{90} values were examined, it was seen that there was no difference between 500, 250, and 125 ppm doses, and the lowest LT_{90} value was obtained at the highest 1000 ppm dose.

The LT_{50} and LT_{90} values could not be calculated for *A. obtectus* due to the very high mortality rate at doses of 1000, 750, and 500 ppm. (Table 2). LT_{50} value was 14.262 hours at 250 ppm. In other words, for *A. obtectus*, 50% of the adults tested at a dose of 250 ppm died in less than 24 hours. Similarly, 90% of *A. obtectus* adults died at approximately 57 hours (LT_{90} value) at the same dose. On the other hand, the highest LT_{50} (51.496 hours) and LT_{90} (95.749 hours) values were found at 125 ppm.

Table 2. The LT_{50} and LT_{90} values were determined for *Oryzaephilus surinamensis* and *Acanthoscelides obtectus*.

O. surinamensis

Dose	Slope (±SE)	LT_{50} (95% Fiducial Limits)	LT_{90} (95% Fiducial Limits)	χ^2 (df)
1000 ppm	3.699 (±0.609)	20.551 (15.023-24.792)	45.638 (39.176-56.429)	7.748 (10)
750 ppm	3.313 (±0.469)	26.282 (20.630-30.966)	64.045 (54.651-80.548)	8.957 (10)
500 ppm	2.573 (±0.410)	29.306 (12.605-40.419)	92.283 (64.304-279.752)	28.217 (10)
250 ppm	2.583 (±0.414)	68.197 (58.597-82.490)	213.791 (151.819-402.111)	9.077 (10)
125 ppm	2.929 (±0.460)	78.626 (68.183-95.636	215.330 (156.191-386.773	6.271 (10)

A. obtectus

Dose	Slope (±SE)	LT_{50} (95% Fiducial Limits)	LT_{90} (95% Fiducial Limits)	χ^2 (df)
1000 ppm*				
750 ppm*				
500 ppm*				
250 ppm	2.141 (±0.488)	14.262 (2.407-23.110)	56.591(41.500-108.978)	14.465 (10)
125 ppm	4.758 (±0.512)	51.496 (45.342-57.861)	95.749 (81.961-121.415)	12.095 (10)

* Not calculated; S.E. means Standard Error; LT means Lethal Time.

In this study, the effects of a local diatomaceous earth on two important stored product pests were evaluated under laboratory conditions. Based on the study, it was determined that the death rate of pests increased with an increase in DE dose in ppm and the duration of exposure of insects to DE (hours) in the control of *O. surinamensis* and *A. obtectus* adults. Therefore, this study holds promise for the potential control of these pests.

The sensitivity of different target species to diatomaceous earth applications also varies (Zeni et al., 2021). In the present study, 90% of mortality occurred in the bean weevil at a dose of 250 ppm within the first 48 hours. Time is of the essence as very small damages to stored products cause large economic losses. The high mortality seen in a short period has yielded very promising results in the control of this insect. Chireceanu et al., (2022) in their study, reached high mortality rates in beans treated with diatomaceous earth (900 ppm) only after 7 days. In their study, adult mortality rates were found to be 93, 96, and 98.5% in the Buzau, Bucharest, and Bacau populations, respectively. Similarly, Gad et al., (2020) obtained a 93.88% mortality rate after 7 days when they applied the *Trichoderma harzianum* Rifai fungus together with diatomaceous earth (800 ppm of DE, and 2.1 x 10 (7) spores/kg of *T. harzianum*) to the bean weevil. Viteri Jumbo et al., (2019), similar to our study, stated that over 90% of mortality occurred in *A. obtectus* at temperatures above 30 ºC (at 0.50 - 1 g/kg DE doses) within 48 hours. In the study conducted by Prasantha et al., (2019), similar to other studies, it was determined that an increase in DE dose resulted in an increased mortality rate among *A. obtectus* individuals. Inhibition of mating and rapid death ultimately means reduced damage to the crop. Therefore, the results obtained for bean weevil are very important.

In this study, mortality rates over 90% were observed in *O. surinamensis* at a dose of 750 ppm in 72 hours. A similar study conducted by Arthur (2000) exposed *O. surinamensis* adults to DE particles added to their food sources at different temperatures (22, 27, and 32 ºC) for durations ranging from 4 to 72 hours. The results obtained indicated that as the exposure duration to DE increased, mortality rates also increased. Similarly, in the study conducted by Baliota et al., (2022), as the dose rate and duration increased in *O. surinamensis*, the mortality rate also increased. While 13.3% of deaths occurred at 25 ºC at the highest dose of 1000 ppm on day 1, this rate reached 100% on day 7. In a study by Ziaee et al., (2016), adult *O. surinamensis* was exposed to wheat, barley, and rice treated with DE doses of 300, 600, 1000, 1500, and 2000 ppm. On the 2nd, 5th, 10th, and 14th days, both dead and live individuals were recorded. The results showed that as the dose and duration of exposure to DE increased, there was an increase in mortality rates. At higher concentrations, wax adsorption and corrosivity caused by DE occur faster, resulting in faster deaths (Shams et al., 2011). In this regard, the results obtained from these studies are in line with the findings of the present study.

In general, since DE does not act quickly depending on the difference in environmental conditions and insect species, control of the pest may take a few days (Korunić et al., 2020). In our study, temperature and humidity values were found to be quite suitable for both stored product pests, and deaths were observed in a short time. *A. obtectus* adults were found to be more sensitive to the diatomaceous earth than *O. surinamensis*. Bello et al., (2006), in their study where they applied different combinations of DE and *Beauveria bassiana* (Balsamo) Vuillemin, stated that *A. obtectus* was more sensitive to *Sitophilus oryzae* L. (Coleoptera: Curculionidae) in terms of both mortality rate and median lethal time values. In our study, the reason for this sensitivity is thought to be the mobility of the insect, apart from species differences. Insect mobility is a very important factor affecting the effectiveness of DE (Zeni et al., 2021). In the experiments, it was observed that *A. obtectus* adults were more mobile than *O. surinamensis* adults. The high mortality observed in *A. obtectus* may be due to the insect coming into contact with more DE due to its movement and therefore dying from water loss. Similarly, Rigaux et al., (2001) stated that there was a direct relationship between insect activity and mortality rate when they applied diatomaceous earth to *Tribolium castaneum* (Herbst., 1797) (Coleoptera: Tenebrionidae) races.

In a study conducted by Ziaee et al., (2007), five different DE dust formulations with varying particle sizes (5 µm, 8.2 µm, 11.7 µm, 8-12 µm, 13-15 µm) were mixed into the food sources of *O. surinamensis*, *T. castaneum*, and *Rhyzopertha dominica* (Fabricius, 1792) (Coleoptera: Bostrichidae) adults and the mortality rates of individuals were examined. The results revealed that the mortality of *O. surinamensis* individuals increased with the duration of exposure to DE doses. These findings align with the outcomes of the present study.

The effectiveness of DE depends on many parameters apart from the applied target species and insect mobility as mentioned above. The most important component of diatomaceous earth, and associated with its insecticidal effect is its silicon dioxide content (Korunić, 1997; Iatrou et al., 2010). The SiO_2 content used in this study (85.97%) is quite high, and the insecticidal effect seen on both stored product pests may be related to the SiO_2 content. In addition, the insect's eating speed, cuticle waxes, adhesion of DE to the cuticles and the food it eats, and water absorption from the hindgut (Korunić & Fields, 2006; Shams et al., 2011) are other factors that play a significant role in the effectiveness of DE. These factors should also be studied in future studies.

In recent years, regulations have been imposed to limit the use of pesticides in agricultural fields and storage facilities for chemical pest control due to the toxic effects of pesticides on non-target organisms. One of the main reasons for these restrictions is the residues of pesticides on stored products, which can directly impact human health. In this regard, the use of Almina DE in storage conditions holds promise as an alternative for pest control. This study has achieved a high level of success in controlling *O. surinamensis*, and *A. obtectus* adults under laboratory conditions using Diatomaceous earth. With effective research and studies in the coming years, diatomaceous earth is expected to be used extensively in storage conditions.

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

Authors' Contribution: HS designed the study and set up the experiments, HS and AKB conducted the study, HS and AKB analyzed the data, and HS and AKB wrote the article.

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