Türk. entomol. derg., 2024, 48 (3): 353-365
DOI: http://dx.doi.org/10.16970/entoted.1518849

ISSN 1010-6960
E-ISSN 2536-491X

Original article (Orijinal araştırma)

Insecticidal efficacy of local diatomaceous earth compositions with different particle sizes against stored grain pests

Farklı partikül boyutlarına sahip yerel diatom toprağı kompozisyonların depolanmış tahıl zararlılarına karşı insektisidal etkinliği

Songül HENTEŞ[1*] **Ali Arda IŞIKBER[2]**

Abstract

In this study, the efficacy of a mixture of local diatomaceous earth (DE) with different diatomite compositions and particle sizes against stored-grain pests, *Sitophilus oryzae* L., 1763 (Coleoptera: Curculionidae), *Tribolium confusum* du Val, 1863 (Coleoptera: Tenebrionidae), and *Rhyzopertha dominica* (F., 1792) (Coleoptera: Bostrichidae) adults was investigated in 2023 in the Entomology Laboratory of the Department of Plant Protection, Faculty of Agriculture, KSU. In biological tests, 500 and 1000 ppm concentrations (mg DE/kg wheat) of different particle sizes (≤ 20, 20-75, 75-150, and ≥ 150 µm) of DE mixture and original DE (≤ 250 µm) were applied to wheat. The highest mortality rate for all insect species tested at 500 ppm was obtained from DE treatments of ≤20 µm. At 1000 ppm, 100% mortality was observed for *T. confusum* and *S. oryzae* in almost all particle size DE-treatments, whereas no local DE treatments resulted in 100% mortality for *R. dom*inica adults. Moreover, the greatest reduction in the number of F1 generation adults for *S. oryzae* and *R. dominica* was obtained in the DE treatment of ≤20 µm at 500 and 1000 ppm. In conclusion, local DE mixture treatments with ≤ 20 µm particle size were found to be highly effective in the control of stored-grain pests.

Keywords: Diatomite composition, local diatomaceous earth, particle size, stored-grain insect pests

Öz

Bu çalışmada, farklı diyatomit bileşimlerine ve partikül boyutlarına sahip yerel diyatom toprağı (DE) karışımının depolanmış tahıl zararlıları *Sitophilus oryzae* L, 1763 (Coleoptera: Curculionidae), *Tribolium confusum* du Val, 1863 (Coleoptera: Tenebrionidae) ve *Rhyzopertha dominica* (F., 1792) (Coleoptera: Bostrichidae) erginlerine karşı etkinliği 2023 yılında KSÜ Ziraat Fakültesi Bitki Koruma Bölümü Entomoloji Laboratuvarında araştırılmıştır. Biyolojik testlerde, DE karışımı ve orijinal DE'nin (≤ 250 µm) farklı partikül boyutlarının (≤ 20, 20-75, 75-150 ve ≥ 150 µm) 500 ve 1000 ppm konsantrasyonları (mg DE/kg buğday) buğdaya uygulanmıştır. Test edilen tüm böcek türleri için 500 ppm'de en yüksek ölüm yüzdesi ≤20 µm DE uygulamalarından elde edilmiştir. 1000 ppm'de, *T. confusum* ve *S. oryzae* için neredeyse tüm partikül boyutu DE uygulamalarında %100 ölüm gözlenirken, hiçbir yerel DE uygulaması *R. dominica* erginleri için %100 ölümle sonuçlanmamıştır. Ayrıca, *S. oryzae* ve *R. dominica* için F1 nesli ergin sayısındaki en büyük azalma 500 ve 1000 ppm'de ≤20 µm DE uygulamasında elde edilmiştir. Sonuç olarak, ≤ 20 µm partikül boyutuna sahip yerel DE karışımı uygulamalarının depolanmış tahıl zararlılarının kontrolünde oldukça etkili olduğu bulunmuştur.

Anahtar sözcükler: Diatomit kompozisyonu, yerel diatomit toprağı, partikül büyüklüğü, depolanmış tahıl zararlıları

[1] Kahramanmaraş Sütçü İmam University, Agriculture Faculty, Plant Protection Department Avşar Campus, 46100 Kahramanmaraş, Türkiye

* Corresponding author (Sorumlu yazar) e-mail: songulhentes@ksu.edu.tr

Received (Alınış): 19.07.2024 Accepted (Kabul ediliş): 15.10.2024 Published Online (Çevrimiçi Yayın Tarihi): 16.10.2024

Introduction

Cereals are among the most important food sources of plant products. In 2023, global cereal production increased by about 3.8 million metric tons, reaching a total of 2,819 million metric tons. Turkey's cereal production was approximately 42.2 million metric tons (FAOSTAT, 2024). These cereals are stored in warehouses for periods that can range from a few weeks to several years, making them susceptible to infestation by various insect pests. Post-harvest insect infestations can lead to substantial losses in grain production (Reichmuth et al., 2007; Mason & McDonough, 2012). Each year, 10-30% of global grain production is lost due to storage insects, which compromise both the quantity and quality of the grain during the post-harvest period (Wijayaratne & Rajapakse, 2018). In many parts of the world, chemical control is the most commonly used method against stored grain pests. Currently, residual contact insecticides (chlorpyriphos-methyl, pirimiphos-methyl, and deltamethrin + piperonyl butoxide (PBO)) and fumigants (phosphine and methyl bromide) are widely used in the chemical control of stored-grain insects. These synthetic insecticides have a rapid lethal effect on insects; however, the use of synthetic insecticides and fumigants in the control of stored grain insect pests causes yearly resistance problems (Zettler & Cuperus 1990; Benhalima et al., 2004; Pimentel et al., 2010). There is a growing need to develop environmentally friendly insect pest control methods, which are considered safer or less harmful (Arthur et al., 2007). This need is driven by concerns over health risks from toxic chemicals (Arthur, 1996), potential side effects on non-target organisms (Fields, 1992), and detrimental impacts on the environment (Zettler & Keever, 1994; Benhalima et al., 2004; Phillips & Throne, 2010; Pimentel et al., 2010; Alkan & Gökçe, 2012).

Diatomaceous earth (DE) is sediment formed from fossilized siliceous shells of unicellular microscopic algae of the algae class. The cell walls of the diatomite are composed of amorphous silica (SiO_2 +H_2O). DE is a natural source of dry matter that can be used as an insecticide (Korunic, 1998). The physical mode of action of DE in insects involves acting on the insect's cuticle, causing rapid drying, ultimately resulting in death due to dehydration (Ebeling, 1971; Korunic, 1998). DE is safe for mammals, demonstrated by an oral LD_{50} value greater than 5000 mg/kg body weight in rats, and it does not leave toxic residues in products (Anonymous, 1991; Quarles, 1992). As a result, it is classified as a food additive and falls within the GRAS (Generally Recognized as Safe) category according to the US EPA (FDA, 1995). DEs can be used as an alternative to conventional insecticides, particularly in protecting stored grains from insect pests where extensive research on a wide range of stored-product insect species has been done, for extended periods due to their ability to remain on treated crops for an extended period (Quarles, 1992; Korunic, 1998; Subramanyam & Roesli, 2000; Kavallieratos et al., 2007). Numerous studies have been also conducted to evaluate the efficacy of local DEs in Turkey against various stored-grain pests and as a result, some micronized local DE deposits showed high efficacy against various stored-grain insect pests at a concentration of 1000 ppm (1 g DE /1 kg wheat) (Doğanay, 2013; Alagöz, 2016; Bayram, 2019; Bağrıaçık, 2020; Sağlam et al., 2022).

The effectiveness of DE in controlling insect pests is influenced by several biotic and abiotic factors, including the diatomite shell morphology, DE's physicochemical properties, the type of grain treated, the specific insect species, as well as environmental conditions like temperature and relative humidity (Korunic, 1998). One crucial physicochemical property of DEs that affect its efficacy against insect pests is DE particle size. Ideally, DE should be composed of high-purity amorphous silica with uniformly small particles, minimal clay content, and less than 1% crystalline silica (Quarles, 1992; McLaughlin, 1994; Vayias et al., 2009). DE rocks should be finely ground, ensuring that diatoms are well separated and ideally remain physically intact (Quarles, 1992). The effectiveness of DE in absorbing lipids and killing insects is also affected by the size, shape, and surface characteristics of the diatom species, along with the uniformity of the particles and the formulation's purity (Korunic, 1997, 2013). DE rocks should be finely milled to ensure that diatoms are well separated and, if feasible, maintain their physical integrity (Quarles, 1992). Additionally, the effectiveness of DE in lipid absorption and insect control depends on the diatom size, shape, and surface features, as well as the uniformity of the particles and the purity of the formulation (Korunic, 1997, 2013). However, Baliota & Athanassiou (2020) reported that the insecticidal properties of DEs depend on particle shape rather than particle size, and that DE particle size does not necessarily mean higher efficacy. Research has shown that modifying diatomite composition and particle size can improve the insecticidal effectiveness of DE against stored-grain pests (Vayias et al., 2009; Ziaee et al., 2013; Baliota & Athanassiou, 2020). It is important to study whether adjusting the physicochemical properties, such as diatom composition and particle size, of local DEs can enhance their effectiveness against stored grain pests at lower concentrations. This study aimed to evaluate how different compositions and particle sizes of local DE affect its insecticidal efficacy against stored-grain pests *Sitophilus oryzae* L., 1763 (Coleoptera: Curculionidae) (rice weevil), *Tribolium confusum* Jacquelin du Val., 1863 (Coleoptera: Tenebrionidae) (confused flour beetle), and *Rhyzopertha dominica* (F., 1792) (Coleoptera: Bostrichidae) (lesser grain borer).

Materials and Methods

Test Insect species and their culture

Insect species used in biological tests were obtained from stock cultures in the Entomology Laboratory of the Department of Plant Protection of Kahramanmaraş Sütçü Imam University. Elbistan soft bread wheat with 11%-12% moisture content was used as food for *S. oryzae* and *R. dominica* cultures and wheat flour was used as food for *T. confusum*. Whole wheat and wheat flour used for the culture of test insects were kept in a -20ºC deep freezer for one week in case of insect contamination. For *S. oryzae*, jars were filled one-third full with wheat, and adult insects were added. The jars were kept in a dark climate chamber at 26±1ºC and 65±5% relative humidity for one week to allow the adults to lay eggs. After this period, the adults were removed using 70 mesh (210 µm) metal sieves (Retsch™ Test Sieves, Germany). The mouths of the jars were covered with fine muslin cloth and placed in the climate chamber for the emergence of the new generation. Two-week-old, mixed-sex (1:1 sex ratio) adults from this new generation were used in the biological tests. For *R. dominica*, 300-400 adults were initially placed in 200 g of wheat flour and allowed to lay eggs for two days. Afterward, the adults and eggs were separated using 35 mesh (500 µm) and 70 mesh (212 µm) sieves (Retsch™, Germany). Adults and eggs were collected using the 500 µm and 212 µm sieves, respectively, and the flour was transferred into a collection container. The eggs collected from the 212 µm sieve were placed in 1-liter sterile glass jars containing 250 g of wheat, and the jar mouths were covered with fine muslin cloth. The cultures were maintained at 30±1ºC and 65±5% relative humidity under dark conditions. Two-week-old new generation mixed-sex (1:1) *R. dominica* adults were used in the biological tests. For *T. confusum* culture, 250 g of wheat flour and 5% dry yeast (inactive) were added to 1-liter sterile glass jars, and 250-300 mixed-sex (1:1) *T. confusum* adults were introduced into the jars. The jars were covered with fine muslin cloth and stored in a completely dark climate chamber at 26±1ºC and 65±5% relative humidity for one week to allow the adults to lay eggs. After one week, the adults were removed using metal sieves, and two-week-old new-generation adults were used in the biological tests. All insect culture jars were covered with fine muslin cloth to ensure proper ventilation.

Preparation of binary mixture of local diatomaceous earths

The local DEs used in the biological tests were obtained from diatomite rocks with BHN-1 and CBN-1 codes collected from two DE reserves in Turkey. To ensure a representative sample, 10 random splits were made at different points within each DE reserve, and groove DE samples were collected. Raw DE samples, weighing at least 5 kg, were collected from each DE reserve and transported to the laboratory for processing. After coarse grinding (2 mm) of the natural raw DE rocks in a laboratory-scale jaw crusher (Alfa Test Equipment, Ankara), the coarse ground DE samples were dried in an oven (MEMMERT UN 75 Memmert Gmb, Germany) at 100±10ºC for three days until 3-5% moisture content of DE samples were reduced. The dried DEs were micronized using a laboratory-scale ball mill (RAM1107; Rantek Test Solutions, Ankara, Turkey). Powder formulations of the micronized BHN-1 and CBN-1 local DEs were obtained after sieving them using an automatic oscillating metal sieve with a mesh size of 250 µm (Retsch, Germany). The resulting powder formulations are shown in Figure 1. Scanning electron microscopy (SEM) images and physicochemical properties of the powder formulations of BHN-1 and CBN-1 local DEs are presented in Figure 2 and Table 1, respectively. To prepare a binary mixture of BHN-1 and CBN-1 local DEs at a 1:1 ratio, each local DE was weighed and mixed using a high-shear powder mixer (PRMG-5, Prism Pharma Machinery, India) at 1500 rpm for 15 min to ensure uniform mixing. The resulting local DE mixture was placed in 1 L capacity glass bottles with tightly closed metal caps and stored at room temperature until use in biological tests. This mixture was used as the original DE in biological tests. Metal sieves of three different sizes (20, 75, and 150 µm) were used to separate the original DE mixtures into four different particle sizes. By sieving the original DE mixture (≤ 250 µm) through a metal sieve with three different mesh sizes, a local DE mixture with four different particle sizes (≤20, 20-75, 75-150 and ≥150 µm) was obtained.

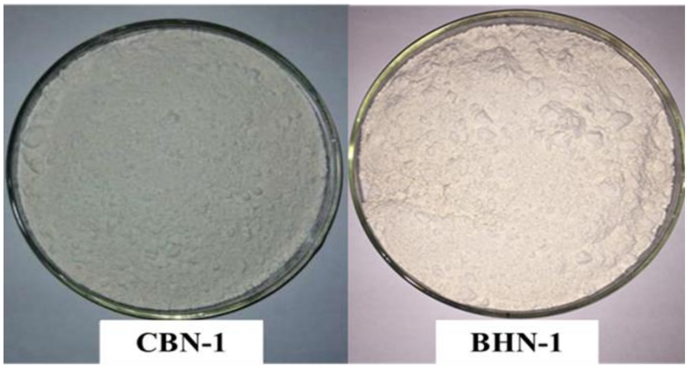

Figure 1. Micronized powder formulations of CBN-1 and BHN-1 diatomaceous earth to form local diatomaceous earth binary mixture used in biological tests.

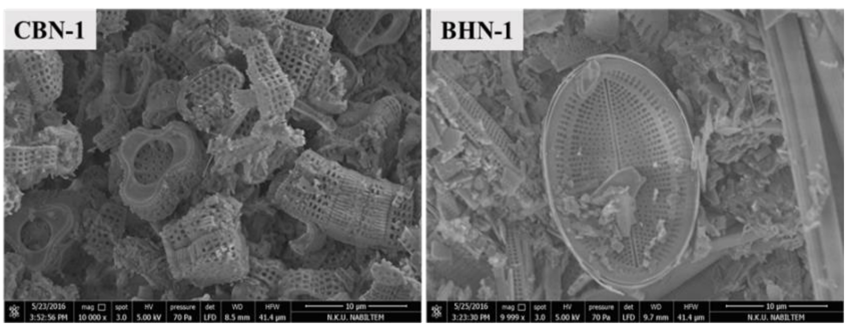

Figure 2. Scanning electron microscopy (SEM) images of CBN-1 and BHN-1 diatomaceous earth at 10000 x magnification [Scanning Electron Microscope (SEM) (FEI, QUANTA FEG 250) analysis of local DE samples was performed at Tekirdağ Namık Kemal University, Scientific and Technological Research Application and Research Centre Centre (NABILTEM].

Table 1. Silicon dioxide (SiO_2) content, median particle sizes, pH values, and adhesion rates on wheat of CBN-1 and BHN-1 diatomaceous earths to constitute the local diatomaceous earth binary mixture used in biological tests

Diatomaceous earth	SiO_2 ratio (%)[1]	Median particle size (µm)[2]	Mean pH ±S.E	Adhesion rate (%) ± S.E[3]
CBN-1	80.75	13.09	8.1 ± 0.01	75.0 ± 1.3
BHN-1	91.40	20.05	7.8 ± 0.06	89.3 ± 0.4

[1] Quantitative chemical analysis was conducted by the Accredited Mineralogy and Petrography Laboratory of General Directorate of Mineral, Research and Exploration of Turkey (MTA), and atomic absorption spectroscopy (AAS) was also used to analyze the elements following melting and acid removal processes.
[2] The median particle diameter value corresponding to 50% of the total particle volume in the volumetric cumulative particle size distribution. Particle size analysis was conducted using the laser light diffraction technique by the Accredited Mineralogy and Petrography Laboratory of General Directorate of Mineral, Research and Exploration, Turkey (MTA).
[3] Adherence rate of DE on wheat kernels were determined using the method described by Korunic (1997) at the Stored Product Insects Laboratory of Kahramanmaraş Sütçü Imam University.

Biological tests

In the biological tests, 500 and 1000 ppm (mg DE/kg wheat) concentrations of the binary mixture of local DE with particle sizes ≤ 20 µm, 20 -75 µm, 75-150 µm, ≥ 150 µm, and original DE (≤ 250 µm) were applied in powder form to wheat. For each concentration and particle size used in the biological tests, 1 kg of wheat was weighed using a balance (EGE-M-30 BARTES, Turkey) and placed in 3 L jars. Then, for the desired concentrations, 500 and 1000 ppm local DE mixtures were weighed using a precision microbalance (AUW220D, Shimadzu Corporation, Japan) and added to 1 kg of wheat in 3 L jars. This was done separately for each of the tested particle sizes of the DE mixture. To ensure a homogenous distribution of the weighed local DE mixture on wheat, the jars were tightly closed and shaken by hand for 3 min. Wheat that was not treated with DE was used as the control. The DE treatment was repeated three times for each insect species. Sub-samples of 50 g DE-treated wheat were randomly collected from each lot (1 kg of DE-treated wheat) and transferred into 100 mL (8.3 cm x 4.5 cm) glass vials. Subsequently, 30 mixed-sex and 14-days-old adults of each insect species were transferred to glass vials containing the DE-treated wheat. The openings of the vial were tightly covered with muslin cloth, which allowed for an air inlet and outlet. Each DE treatment with three replicates was placed in 80 L (26 × 36.5 × 15 cm) lockable plastic storage containers with plastic grids underneath and 65±3% r.h. which was prepared with saturated Sodium nitrate (Na NO_2) solution (Greenspan, 1977). Plastic containers were placed in an incubator at 25±1ºC. After 14 d of treatment, live and dead adults were counted. Subsequently, all live and dead individuals were removed from the vials, and the glass vials were kept in the dark in a climate chamber of 26 ± 1ºC and 65 ± 5 % r.h. for additional 45 days to determine the number of F1 generation adults of *S. oryzae* and *R. dominica*. After 45 days, the grains in the treatment vials were sieved and the number of F1 progeny was counted.

Data evaluation and statistical analysis

When mortality was observed in the control group, Abbott's formula (Abbott, 1925) was applied to adjust the mortality rates. All mortality data for each DE treatment were normalized using the arcsine square root transformation and then subjected to three-way analysis of variance (ANOVA) with main factors: insect species, DE concentration, and DE particle size by using SAS 9 (SAS Inst., 2009) statistical software. Mean percentage mortalities for each species DE particle size, and DE concentrations were separated by using the Tukey's HSD test (Tukey's Honestly Significant Difference Test) at a 5% significance level. The differences in number of adult emergences of the new generation from the control were tested using Dunnett's test ($p = 0.005$). Adult progeny in each insect species-DE particle size combination was compared with adult progeny in the control vials using Dunnett's test at a 5% significant level. The formula was used to determine the percentage reduction in the number of adults in new generation: ((Nc-Nt) / Nc) x 100, where, Nc and Nt were the number of adult progenies in the control and DE treatments, respectively. The data on percentage reduction in the progeny adult emergence data for each insect species was evaluated using one-way analysis of variance (ANOVA) with main factor of DE particle size. The means of percentage reduction in the number of adults of new generation for each insect species and DE particle size were separated by using the Tukey's HSD test at a 5% significance level.

Results

Insect mortality

Particle size, insect species and DE concentration of the local DE mixture had a significant effect on mortality rate as shown in the statistical analysis ($p<0.0001$) Statistically significant interactions were observed between insect species and DE particle size, insect species and DE concentration, DE particle size and concentration ($p<0.0001$). However, the interaction effect between insect species, DE particle size and concentration was not significant ($p=0.468$) (Table 2).

Table 2. Results of three-factor analysis of variance applied to the percentage mortalities of *Sitophilus oryzae, Tribolium confusum*, and *Rhyzopertha dominica* adults exposed to 500 and 1000 ppm concentrations of local diatomaceous earth mixture with different particle sizes for 14 days in biological tests conducted on wheat

Sources	Degrees of freedom	Sum of squares	Mean squares	F value	p value
Insect species (B)	2	10826.6	5413.28	270.50	<0.0001
Particle size (P)	4	3382.7	845.68	42.26	<0.0001
DE dose (D)	1	7946.2	7946.24	397.07	<0.0001
B * P	8	1777.3	222.16	11.10	<0.0001
B * D	2	851.8	425.89	21.28	<0.0001
P * D	4	999.1	249.77	12.48	<0.0001
B * P * D	8	155.4	19.42	0.97	0.468
Error	60	1200.7	20.01		
Total	89	27139.8			

None of the local DE treatments with different particle sizes at 500 ppm for 14 d resulted in 100% mortality of *S. oryzae*, *T. confusum*, and *R. dominica* adults (Figure 3). For *T. confusum*, the highest percentage of death (93.1%) was obtained at a particle size ≤ 20 µm of local DE, whereas the lowest percentage of mortality (67.2%) was achieved in the local-origin DE treatment. Percentage of death did not differ significantly between the ≤ 20 µm and 20-75 µm particle sizes of local DE treatments. However, the mortality rate for the ≤ 20 µm particle size was significantly higher than those for the 75-150 µm, ≥ 150 µm, and original DE (≤ 250 µm) treatments. For *S. oryzae*, the highest percentage of death (86.5%) was obtained at the local DE particle size of ≤ 20 µm, whereas the lowest mortality rate (65.1%) was achieved at the local DE particle size of ≥ 150 µm (Figure 3). Although there was no statistical difference between the percentage of deaths obtained from ≤ 20 µm, 20-75 µm and 75-150 µm particle sizes of the local DE and original DE (≤ 250 µm) treatments, the percentage of mortality obtained from the local DE ≤ 20 µm particle size was significantly higher than that obtained from the local DE particle of ≥ 150 µm. For *R. dominica*, the highest mortality rate (80.9%) was obtained at a local DE particle size of ≤ 20 µm, whereas the lowest percentage (43.9%) was achieved at the local DE of ≥ 150 µm. The mortality rate in the ≤ 20 µm particle size of the local DE treatment was significantly higher than those obtained in the 20-75 µm and 75-150 µm particle sizes of the local DE and original DE (≤ 250 µm) treatments (Figure 3). However, there was no statistical difference between the percentage of mortalities in the 20-75 µm and 75-150 µm particle sizes of the local DE and original DE (≤ 250 µm) treatment.

Local DE with all particle sizes at 1000 ppm resulted in 100% mortality for *S. oryzae* and *T. confusum*, whereas 100% adult mortality for *R. dominica* was not achieved in any of the DE treatments. At 1000 ppm, the highest percentage mortality for *R. dominica* (85.3%) was obtained in the local DE with a particle size ≤ 20 µm, whereas the lowest percentage mortality (56.1%) was achieved in the local DE with a particle size ≥ 150 µm. The mortality rate for *R. dominica* obtained from local DE with ≤ 20 µm particle size was significantly higher than that obtained from local DE with 75-150 µm and ≥ 150 µm particles, whereas there was no significant difference between those obtained from local DE with ≤ 20 µm, 20-75 µm particle size, and original DE (Figure 3).

Significant differences in mortality rate between the insect species tested were detected at both 500 and 1000 ppm (Figure 4). At 500 ppm, no significant difference was observed in the mortality rates for *T. confusum* and *S. oryzae* adults exposed to all DE treatments, whereas the mortality rates for *T. confusum* and *S. oryzae* adults were significantly higher than those of *R. dominica.* At 1000 ppm, no significant difference was observed in the mortality rates for *T. confusum* and *S. oryzae* adults exposed to all DE treatments, whereas the percentage of death for *T. confusum* and *S. oryzae* adults were significantly higher than that for *R. dominica*. (Figure 4).

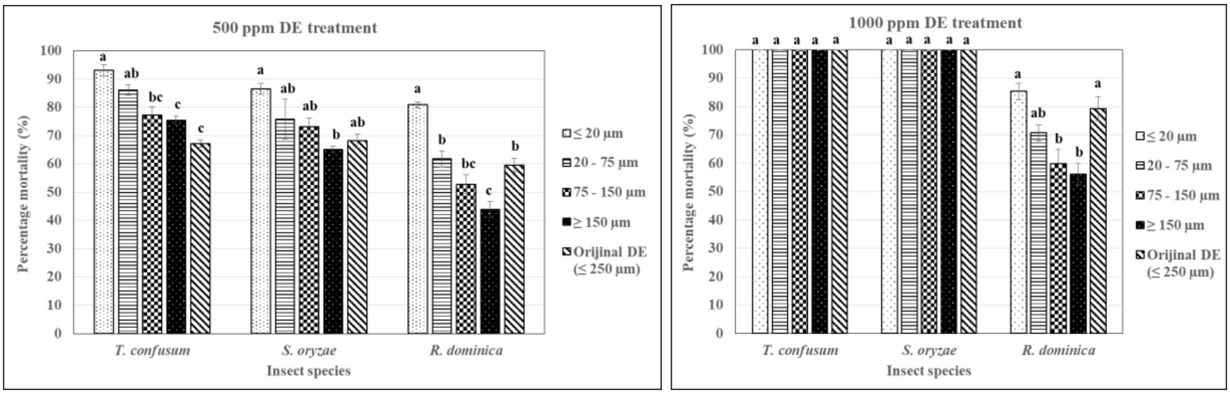

Figure 3. The percentage mortality (mean±S.E) of *Sitophilus oryzae*, *Tribolium confusum*, and *Rhyzopertha dominica* adults exposed to 500 and 1000 ppm concentrations of local diatomaceous earth mixture with different particle sizes on wheat for 14 days (Differences between means are presented according to Tukey's HSD test at 5% significance level. For a given species, means with same lowercase letters are not significantly different).

The mortality rates (mean±S.E) for *S. oryzae*, *T. confusum*, and *R. dominica* adults exposed to 500 and 1000 ppm concentrations of local diatomaceous earth mixture with different particle sizes on wheat for 14 days (Differences between means are presented according to Tukey's HSD test at a 5% significance level. For a given species, means with same lowercase letters are not significantly different) (Figure 4).

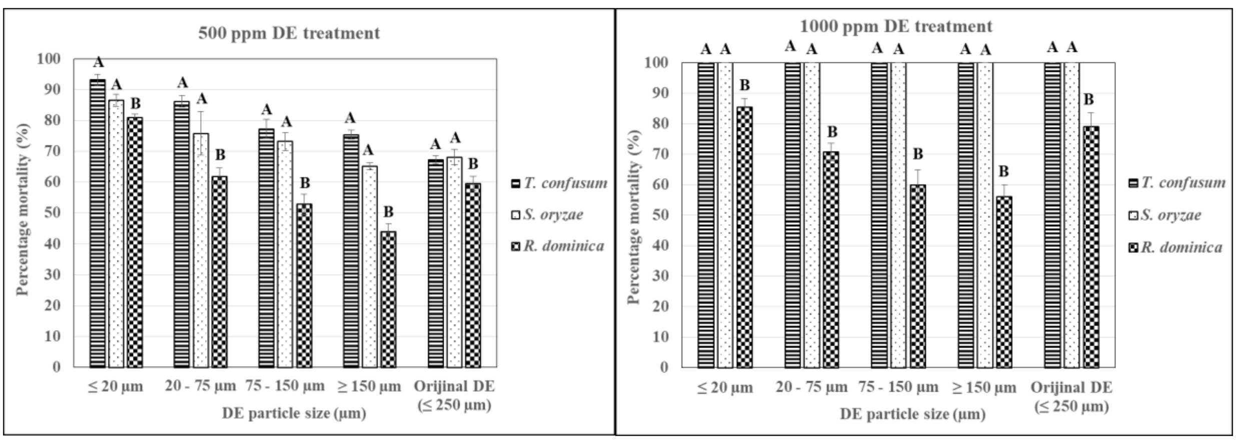

Figure 4. Effect of local diatomaceous earth mixture with different particle sizes at 500 and 1000 ppm concentrations on the percentage mortality (mean±S.E) of *Sitophilus oryzae*, *Tribolium confusum*, and *Rhyzopertha dominica* adults in wheat (differences between means are presented according to Tukey's HSD test at the 5% significance and any statistically significant differences between the species were indicated by different capital letters above the bars). For a given DE particle size, means with same uppercase letters are not significantly different)

The DE concentration had a significant effect on percentage mortality of *T. confusum* and *S. oryzae*. For both species, the mortality rates at 1000 ppm were higher than those at 500 ppm for all DE treatments with varying particle sizes, except for the local DE treatment with ≤ 20 µm particle size for *T. confusum* (Figure 5). However, for *R. dominica*, no significant differences were found among the DE concentrations in all DE treatments, except for the original DE treatment. In the original local DE treatment, the mortality rates at 1000 ppm were higher than that at 500 ppm (Figure 5).

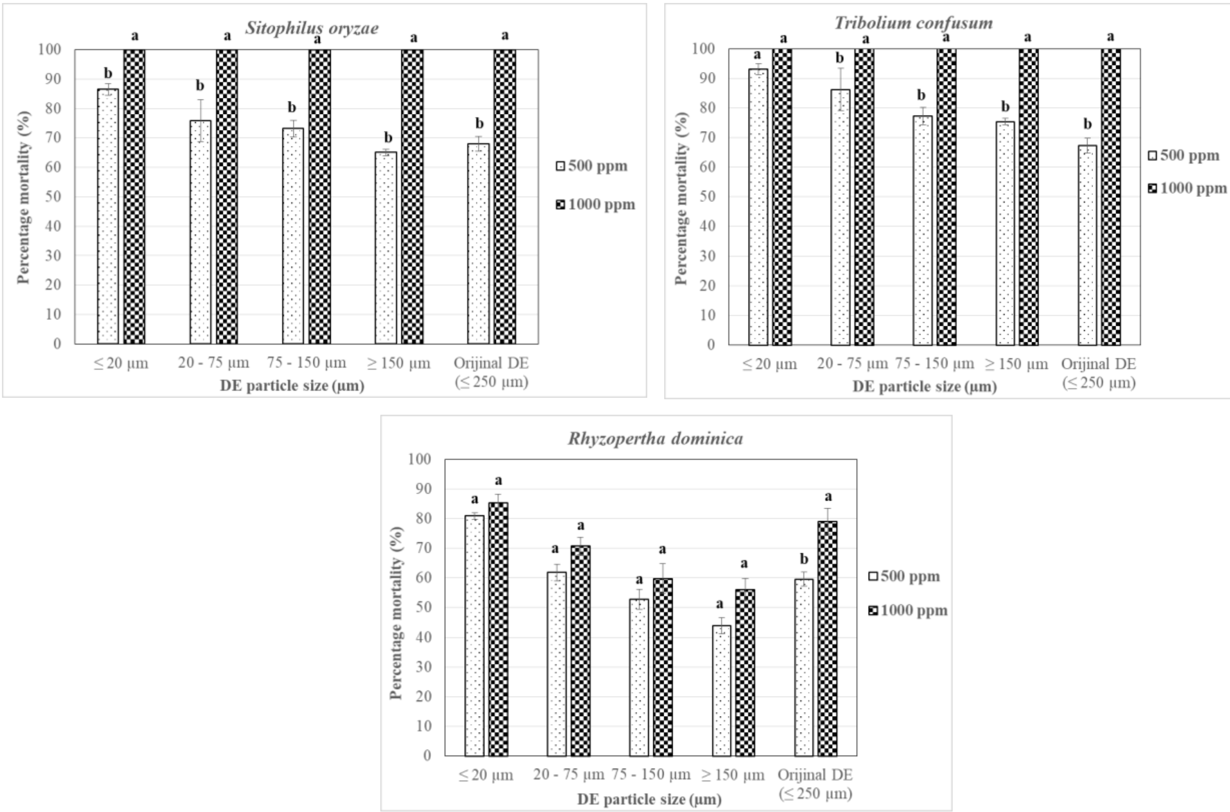

Figure 5. Effect of application concentration of local diatomaceous earth with different particle sizes on the percentage mortality (mean±S.E) of *Sitophilus oryzae, Tribolium confusum*, and *Rhyzopertha dominica* adults in wheat (differences between means are presented according to Tukey's HSD test at a 5% significance level. For a given DE particle size, means with same lowercase letters are not significantly different)

F1 progeny

According to Dunnett's test for *R. dominica*, there were significant differences between the number of new-generation adults obtained from each DE treatment at 500 and 1000 ppm, and the control treatment (Table 3). At both 500 and 1000 ppm, the lowest number of new-generation adults was obtained in the local DE with particle size ≤ 20 µm, whereas the highest number of new-generation adults was observed in the local DE with particle size ≥ 150 µm. At 500 ppm concentration, the highest reduction rate in the number of new-generation adults (75%) was obtained with the local DE ≤ 20 µm particle-size treatment, whereas the lowest reduction rate (42.7%) was observed with the local DE ≥ 150 µm particle-size treatment. The reduction rates in the number of new-generation adults in the local DE with ≤ 20 µm particle size treatment were significantly higher than those in all the other DE treatments. Similarly, the reduction rates in the number of new-generation adults in the local DE with 20-75 µm particle size treatment were significantly higher than those in the local DE with 75-150 µm, ≥ 150 µm, and local-origin DE treatments. The lowest rates of reduction in the number of new-generation adults were obtained in the local DE with ≥ 150 µm and original DE treatments. For the 1000 ppm concentration, the highest reduction rate in the number of new-generation adults (94%) was achieved in the local DE with ≤ 20 µm particle size treatment, whereas the lowest reduction rate (88.6%) was observed in the DE with ≥ 150 µm particle size treatment. The reduction rates in the number of new-generation adults in the local DE with ≤ 20 µm particle size treatment were significantly higher than those in all the other DE treatments. Similarly, the reduction rates in the number of new-generation adults in the local DE with 20-75 µm particle size treatment were significantly higher than those in the local DE with 75-150 µm, ≥ 150 µm particle size, and original DE treatments. The lowest rates of reduction in the number of new-generation adults were achieved in the local DE with ≥ 150 µm and original DE treatments. In addition, there was no significant difference between the rates of decrease in the number of new-generation adults in the local DE with 75-150 µm, ≥ 150 µm particle size, and the original DE.

Table 3. Mean numbers of new generation of *Rhyzopertha dominica* adults and reduction rates (%) in the number of adults as a result of biological tests at 500 and 1000 ppm concentration of local diatomaceous earth mixture with varying particle sizes on wheat

DE particle size (µm))	500 ppm DE treatment			1000 ppm DE treatment		
	Number of new generation adults ± S.E	p value**	Reduction rate in the number of adults (%) ± S.E***	Number of new generation adults ± S.E	p value**	Reduction rate in the number of adults (%) ± S.E***
≤ 20 µm	53.3±4.03 (216± 1.4)*	<0.0001	75.4±1.8 A	11.6±2.18 (216 ±1.4)*	<0.0001	94.6±0.5 A
20- 75 µm	74.3±3.05 (216 ± 1.4)	<0.0001	66.1±1.4 B	21.2±2.51 (216 ± 1.4)	<0.0001	90.1±1.1 AB
75-150µm	95.6±4.8 (216 ± 1.4)	<0.0001	55.7±2.2 C	23.6±1.45 (216 ± 1.4)	<0.0001	89.07±0.6 B
≥ 150 µm	123.6±1.8 (216 ± 1.4)	<0.0001	42.7±0.8 D	24.6±1.85 (216 ± 1.4)	<0.0001	88.6±0.8 B
Original DE	108.3±4.4 (216 ± 1.4)	<0.0001	49.8.±0.3 DC	22.1±0.57 (216 ± 1.4)	<0.0001	89.7.±0.8 B
	F and p value		$F_{4,10}$ = 59.82; p<0.0001	F and p value		$F_{4,10}$ = 6.45; p=0.008

* Values in parentheses are the average number of new generation adults obtained from the control treatment.

** Dunnett's test at a 5% significance level was applied to compare the number of new generation adults in DE treatments with that in the control.

*** One-way analysis of variance (ANOVA) was applied to the data, and the differences between the means were determined according to Tukey's HSD test at a 5% significance level. Means with same uppercase letters are not significantly different.

According to Dunnett's test for *S. oryzae*, there were significant differences between the number of new-generation adults in each DE treatment and the control treatments at 500 and 1000 ppm (Table 4). At 500 ppm, the lowest number of new-generation adults was obtained in local DE with ≤ 20 µm particle size, whereas the highest number of new-generation adults was achieved in local DE with 75-150 µm, ≥ 150 µm particle size, and original DE treatments. At 500 ppm, the highest reduction rate in the number of new-generation adults (91%) was obtained in local DE with ≤ 20 µm particle size, whereas the lowest reduction rate (85%) was observed in local DE with ≥ 150 µm particle size and original DE treatments. At 500 ppm, the reduction rates in the number of new-generation adults in the local DE with ≤ 20 µm particle size treatment were significantly higher than those in all the other DE treatments. Similarly, the reduction rates in the number of new-generation adults in the local DE with 20-75 µm particle size treatment were significantly higher than those in the local DE with 75-150 µm, ≥ 150 µm, and original DE treatments. At 500 ppm, the lowest rate of reduction in the number of new-generation adults were obtained in the original DE treatment (85.1%). At 1000 ppm, the local DE with ≤ 20 µm and 20-75 µm particle size treatments resulted in the complete suppression of F1 progeny production. No significant difference in reduction rates for the number of new generations in the local DE ≤ 20 µm, 20-75 µm, 75-150 µm, and ≥ 150 µm particle size treatment were detected, whereas, the reduction rates in the number of new-generation adults for the original DE treatment were significantly lower than those of the other DE treatments.

Table 4. Mean numbers of new generation of *Sitophilus oryzae* adults and reduction rates (%) in the number of adults as a result of biological tests conducted at 500 and 1000 ppm concentration of local diatomaceous earth mixture with varying particle sizes on wheat

DE particle size (µm)	500 ppm DE treatment			1000 ppm DE treatment		
	Number of new generation adults ± S.E	p value**	Reduction rate in the number of adults (%) ±S.E.***	Number of new generation adults ± S.E	p value**	Reduction rate in the number of adults (%) ±S.E.***
≤ 20 µm	23.1±1.52 (280 ±1.3)*	<0.0001	91.7.3±0.7 A	0±0 (280 ± 1.3)*	<0.0001	100±0 A
20- 75 µm	29.6±2.60 (280 ± 1.3)	<0.0001	89.4.2±1.2 B	0±0 (280 ± 1.3)	<0.0001	100±0 A
75-150 µm	38.2±3.21 (280 ± 1.3)	<0.0001	86.3±1.4 C	3.1±0.57 (280 ± 1.3)	<0.0001	98.8±0.2 A
≥ 150 µm	42±0.57 (280 ± 1.3)	<0.0001	85.5±0.2 C	5±0.58 (280 ± 1.3)	<0.0001	98.2±0.4 A
Original DE	41.6±1.20 (280 ± 1.3)	<00001	85.1±0.7 C	8.3±0.8 (280 ± 1.3)	<0.0001	97.03±1.7 B
	F and p value		$F_{4,10}$=39.70; p<0.0001	F and p value		$F_{4,10}$ = 44.73; p<0.0001

* Values in parentheses are the average number of new generation adults obtained from the control treatment.

** Dunnett's test at a 5% significance level was applied to compare the number of new generation adults in DE treatments with that in the control.

*** One-way analysis of variance (ANOVA) was applied to the data, and the differences between the means were determined according to Tukey's HSD test at a 5% significance level. Means with same uppercase letters are not significantly different.

Discussion

In the present study, the DE particle size had a significant effect on insect mortality. Therefore, the efficiency of the local DE formulation increases with decreasing DE particle size. Local DE formulations with small particle sizes (generally ≤ 20 µm) were more effective than those with large particle sizes. Similarly, several previous studies concluded that DE treatments with low particle sizes are more effective against various stored grain pest species. For instance, DE formulations with particle sizes ≤ 10 µm exhibited high insecticidal activity against *Sitophilus granarius* (L., 1758) (Coleoptera, Curculionidae) (Aldryhim, 1990; McLaughlin, 1994; Korunic & Fields, 2006; Saez & Fuentes Mora, 2007). Another study tested DEs with particle sizes ≤ 45 µm, 45-150 µm, and ≤ 150 µm against *R. dominica*, *Chriptolestes ferrugineus* (Stephens, 1831) (Coleoptera, Cucujoidea: Laemophloeidae), and *S. oryzae* adults, and found that the smallest particle size was the most effective (Vayias et al., 2009). Ziaee et al. (2013) also found that DE treatment with particle sizes ≤ 37 µm was more efficacious against *S. granarius* than treatment with larger particle sizes. These results are consistent with the findings of the present study.

Particle size distribution is a factor that greatly affects the insecticidal efficacy of inert dusts, particularly DE (Korunic, 1997). DE with smaller particles has a higher toxicity against the tested insect species compared to those with higher particles, mainly because of three reasons: (a) larger surface area and higher particle number per mass unit, (b) better and more uniform dispersity or coverage on the grains, and (c) increased contact with insect cuticles. Previous studies have highlighted the significance of particle size in DEs, indicating that smaller particles can adhere more effectively to insect bodies, leading to faster degradation of the waxy cuticular layer and increased water loss (Robinson, 2005; Ziaee et al., 2013). Consistent with these findings, Vayias et al. (2009) reported that DE efficacy is generally inversely proportional to the particle size, with smaller particles providing a larger contact area with insects. Ziaee et al. (2013) also found that particles in the 0-37 µm range successfully adhered to insect bodies. These studies collectively suggest that larger surface areas and higher surface-to-volume ratios enhance the reactivity of DE particles, significantly impacting their toxicity to insect species.

Local DE particles ≤ 45 µm exhibited stronger insecticidal properties against the tested insect species than larger particles. In our study, the presence of larger particles (≥ 150 µm), such as rocks, sand, and large diatoms, negatively impacted DE performance. Consequently, it is important to eliminate these larger particles during DE processing and purification. However, Korunic & Ormesher (1998) found no correlation between mean particle size < 15 µm and diatom shape with insecticidal activity. In some cases, DE formulations from Elassona, Greece, with particle sizes of 45-150 µm proved to be more effective than DE samples with smaller particles (Vayias et al., 2009). Baliota & Athanassiou (2020) observed that particle shape affects insecticidal value of DE, where smaller particles do not necessarily increase efficacy. This suggests that insecticidal value of DE depends on factors beyond particle size, such as active surface area, oil adsorption, inner pore diameter, moisture content, SiO_2 content, and tapped density (Korunic, 1997).

The effectiveness of local DE treatments with varying particle sizes differed among the insect species tested. At concentrations of 500 and 1000 ppm, there were no significant differences in the mortality rates of *T. confusum* and *S. oryzae* adults across all local DE treatments. However, the mortality rates for T. confusum and *S. oryzae* were significantly higher compared to *R. dominica*. At 1000 ppm, all local DE treatments achieved complete mortality for *T. confusum* and *S. oryzae*, while *R. dominica* did not reach 100% mortality with any of the local DE treatments. At 500 ppm, none of the local DE treatments with all particle sizes achieved complete mortality for any species, whereas the highest mortality rates of *T. confusum (*93%)*, S. oryzae (*86%), and *R. dominica* (80%) were observed in local DE treatments with the smallest particle size (≤ 20 µm). Our findings suggest that the local DE mixture is highly effective against *T. confusum*, which is one of the most tolerant stored-grain insects to DE (Korunic, 1998; Arthur, 2000). Kavallieratos et al. (2007) noted that three commercially available modified DE formulations at 500 ppm with 7 days exposure resulted in low mortality rates of *T. confusum* adults, ranging from 5% to 39%. According to these results, our local DE mixture appeared to be more effective against *T. confusum* adults than PyriSec, Insecto, or Protect-It. Athanassiou et al. (2007) reported that PyriSec, Insecto and Protect-It on wheat at 500 ppm resulted in mortality rates of *S. oryzae* and *R. dominica* adults ranging from 92% to 99% after 7 days exposure. Based on these mortality results, it appears that our local DE mixture is slightly less effective against *S. oryzae* and *R. dominica* than PyriSec, Insecto and Protect-It. These findings clearly indicate that the blends of local DEs can be a potential source for the development of commercial products, despite the slightly lower mortality rates in *S. oryzae* and *R. dominica* compared to the three commercial DE formulations.

Subramanyam and Roesli (2000) suggest that, under practical cereal storage conditions, it is often more critical to prevent the formation of progeny than to focus exclusively on achieving direct lethal effects of DE on adult insects. In our study, aligned with the mortality rates observed at 500 and 1000 ppm, there was a statistically significant difference in the number of new-generation adults between the control treatments and those with different particle sizes for all insect species. Specifically, *S. oryzae* showed complete progeny inhibition with local DE at ≤ 20 µm and 20-75 µm particle sizes only at 1000 ppm, whereas for *R. dominica*, progeny production was not inhibited in any of the local DE treatments at either concentration. These results are consistent with those of previous studies. Similar findings in controlling *S. oryzae* progeny have been observed with several commercially available DE formulations, such as SilicoSec, Insecto, and Protect-It (Subramanyam & Roesli, 2000; Athanassiou et al., 2003, 2005, 2008). In our study, *R. dominica* offspring were present at all intervals, regardless of DE particle size and dose. Similarly, Athanassiou et al. (2011) observed that the commercial DEs tested could not completely eliminate *R. dominica* progeny production in wheat, maize, rice, and barley.

Diatom geometric properties vary between species, with an increase in pore number, size, and surface distribution enhancing insecticidal capability and cuticular wax absorption. Diatom particles can erode insect cuticles, resulting in increased water loss and death (Ebeling, 1971; Subramanyam & Roesli, 2000). Small particles, when they come into contact with pests, limit their movement and lead to death (Robinson, 2005). Our findings confirm that smaller particles are more effective, particularly against stored grain pests. Diatom particles are prepared for commercial use by quarrying, drying, and milling, processes that mainly decrease moisture content and the average particle size. As a result, milling could play a key role in defining the insecticidal effectiveness of DE formulations against stored-grain pests. In conclusion, this study emphasizes the critical role of particle size in affecting the insecticidal effectiveness of DE formulations and stresses the need to account for these factors during the DE production process.

References

Abbott, W. S., 1925. A method of computing the effectiveness of an insecticide. Journal of Economic Entomology, 18 (28): 65-267.

Alagöz, V., 2016. Insecticidal Activity of Various Turkish Diatomaceous Earths against Rice Weevil (*Sitophilus oryzae* L.) and Confused Flour Beetle (*Tribolium confusum* du Val.) on Paddy and Rice. Tekirdağ Namık Kemal University, Institute of Science and Technology, Department of Plant Protection, (Unpublished) Master Thesis, Tekirdağ. 59 pp. (in Turkish with abstract in English).

Aldryhim, Y. N., 1990. Efficacy of the amorphous silica dust, Dryacide, against *Tribolium confusum* Duv. and *Sitophilus granarius* (L.) (Coleoptera: Tenebrionidae and Curculionidae). Journal of Stored Products Research, 26 (4): 207-210.

Alkan, M. & A. Gökçe, 2012. Contact and behavioural effects of stem and flower extracts of *Tanacetum abrotanifolium* (L.) DRUCE (Asteraceae) on *Sitophilus granarius* and *Sitophilus oryzae* (Col., Curculionidae). Turkish Journal of Entomology, 36 (3): 377-389.

Anonymous, 1991. EPA R.E.D. FACTS: Silicon Dioxide and Silica Gel: 21T-1021, 1-4, September 1991.

Arthur, F. H., 1996. Grain protectants: current status and prospects for the future. Journal of Stored Products Research, 32 (4): 293-302.

Arthur, F. H., 2000. Toxicity of diatomaceous earth to red flour beetles and confused flour beetles (Coleoptera: Tenebrionidae): effects of temperature and relative humidity. Journal of Economic Entomology, 93 (2): 526-532.

Arthur, F. H., R. C. Bautista & T. J. Siebenmorgen, 2007. Influence of growing location and cultivar on *Rhyzopertha dominica* (Coleoptera: Bostrichidae) and *Sitophilus oryzae* (Coleoptera: Curculionidae) infestation of rough rice. Insect Science, 14 (3): 231-239.

Athanassiou, C. G., B. J. Vayias, C. B. Dimizas, N. G. Kavallieratos, A. S. Papagregoriou & C. Th. Buchelos,. 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., N. G. Kavallieratos & C. M. Meletsis, 2007. Insecticidal effect of three diatomaceous earth formulations, applied alone or in combination, against three stored-product beetle species on wheat and maize. Journal of Stored Products Research, 43 (4): 330-334.

Athanassiou, C. G., N. G. Kavallieratos, B. J. Vayias & E. C. Panoussakis, 2008. Influence of grain type on the susceptibility of different *Sitophilus oryzae* (L.) populations, obtained from different rearing media to three diatomaceous earth formulations. Journal of Stored Products Research, 44 (3): 279-284.

Athanassiou, C. G., N. G. Kavallieratos, B. J. Vayias, Z. Tomanovi, C. A. Petrovic, V. Rozman, C. Adler, Z. Korunic & D. Milovanovic, 2011. Laboratory evaluation of diatomaceous earth deposits mined from several locations in central and southeastern Europe as potential protectants against Coleopteran grain pests. Crop Protection, 30 (3): 329-339.

Athanassiou, C. G., N. G. Kavallieratos, F. C. Tsaganou, B. J. Vayias, C. B. Dimizas & C. T. Buchelos, 2003. Effect of grain type on the insecticidal efficacy of SilicoSec against *Sitophilus oryzae* (L.) (Coleoptera: Curculionidae). Crop Protection, 22 (10): 1141-1147.

Bağrıaçık, O., 2020. Efficacy of Crop Surface Application of Local Diatomaceous Earth on Wheat and Maize against Some Stored Grain Pests. Kahramanmaraş Sütçü İmam University, Institute of Science and Technology, Department of Plant Protection, (Unpublished) Master Thesis, Kahramanmaraş, 85 pp (in Turkish with abstract in English).

Baliota, G. V. & C. G. Athanassiou, 2020. Evaluation of a Greek diatomaceous earth for stored product insect control and techniques that maximize its insecticidal efficacy. Applied Sciences, 10 (18): 6441.

Bayram, A., 2019. Determination of the Repellent Effect of Local Diatom Earths against Some Stored Grain Pests. Kahramanmaraş Sütçü İmam University, Institute of Science and Technology, Department of Plant Protection (Unpublished) Master Thesis, Kahramanmaraş, 69 pp (in Turkish with abstract in English).

Benhalima, H., M. Q. Chaudhry, K. A Mills & N. R. Price, 2004. Phosphine resistance in stored-product insects collected from various grain storage facilities in Morocco. Journal of Stored Products Research, 40 (3): 241-249

Doğanay, S. I., 2013. Determination of the Effectiveness of Various Diatomaceous Earths Against Stored Grain Pests, *Sitophilus granarius* (L.) and *Rhyzopertha dominica* (F.). Kahramanmaraş Sütçü İmam University, Institute of Science and Technology, Department of Plant Protection, (Unpublished) Master Thesis, Kahramanmaraş, 55 pp (in Turkish with abstract in English).

Ebeling, W., 1971. Sorptive dust for pest control. Annual Review of Entomology, 16 (1): 123-158

FAOSTAT, 2024. Food and Agriculture Organization of the United Nations. (Web page: http://faostat.fao.org/site) (Date accessed: 18.03.2024).

FDA (Food and Drug Administration, USA), 1995. Specifications for diatomaceous earths as a maximum 2% animal feed additive. 21 CFR Section 573.340.

Fields, P. G., 1992. The control of stored-product insects and mites with extreme temperatures. Journal of Stored Products Research, 28 (2): 89-118.

Greenspan, L., 1977. Humidity fixed points of binary saturated aqueous solutions. Journal of Research of the National Bureau of Standards-A. Physics and Chemistry, 81A (1): 89-96.

Kavallieratos, N. G., C. G. Athanassiou, B. J. Vayias & S. N. Maistrou, 2007. Influence of temperature on susceptibility of *Tribolium confusum* (Coleoptera: Tenebrionidae) populations to three modified diatomaceous earth formulations. Florida Entomologist, 90 (4): 616-625.

Korunic, Z. & P. G. Fields 2006. "Susceptibility of three species of *Sitophilus* to diatomaceous earth, 681-686". In: Proceedings of the Ninth International Working Conference on Stored-Product Protection (Eds. İ. Lorini, B. Bacaltchuk, H. Beckel, D. Deckers E. Sundfeld, J. P dos Santos, J. D. Biagi, J. C. D'A., Celaro, L. R. Faroni, L. Bortolini, de O. F. M. R. Sartori, M. C Elias, R. N. C Guedes, da R. G. Fonseca & V. M. Scussel) (15-18 October, 2006, Campinas, Sao Paulo, Brazil), Brazilian Post-Harvest Association, Campinas, Brazil, 1359 pp.

Korunić, Z. & P. Ormesher, 1998. "Evaluation and standardized testing of diatomaceous earth, 738-744". In: Proceedings of the 7th International Working Conference on Stored-product Protection (14-19 October 1998, Beijing, China), 2003 pp.

Korunic, Z., 1997. Rapid assessment of the insecticidal value of diatomaceous earths without conducting bioassays. Journal of Stored Products Research, 33 (3): 219-229.

Korunic, Z., 1998. Diatomaceous earths, a group of natural insecticides. Journal of Stored Products Research, 34 (3-4): 87-97.

Korunic, Z., 2013. Diatomaceous earths-Natural insecticides. Pesticides Phytomedicine, 28 (2): 77-95.

Mason, L. J. & M. McDonough, 2012. Biology, behavior, and ecology of stored grain and legume insects. Stored Product Protection, 1 (7): 7-20.

McLaughlin, A., 1994. "Laboratory trials on desiccant dust insecticides, 638-645". In: Proceedings 6th International Working Conference on Stored-Product Protection, Canberra, Australia, 1312 pp.

Phillips, T. W. & J. E. Throne, 2010. Biorational approaches to managing stored-product insects. Annual Review of Entomology, 55 (2010): 375-397.

Pimentel, M. A., F. H. Faroni, da Silva, M. D. Batista & R. N. Guedes, 2010. Spread of phosphine resistance among Brazilian populations of three species of stored product insects. Neotropical Entomology, 39 (1): 101-107

Quarles, W., 1992. Diatomaceous earth for pest control. IPM Practitioner, 14 (5/6): 1-11.

Reichmuth, Ch., M. Schöller & Ch. Ulrichs, 2007. Stored Product Pests in Grain. Morphology- Biology-Damage-Control. AgroConcept Verlagsgesellschaft, Bonn, 170 pp.

Robinson, W. H., 2005. Handbook of Urban Insects and Arachnids: A Handbook of Urban Entomology Cambridge University Press, New York, USA, 472 pp.

Saez, A. & V. H. Fuentes Mora, 2007. Comparison of the desiccation effects of marine and freshwater diatomaceous earths on insects. Journal of Stored Products Research, 43 (4): 404-409.

Sağlam, Ö., A. Bayram, A. A. Işıkber, R. Şen, H. Bozkurt & S. Henteş, 2022. Insecticidal and repellency effects of a Turkish diatomaceous earth formulation (Detech®) on adults of three important pests of stored grain. Turkish Journal of Entomology, 46 (1): 75-88.

SAS, 2009. SAS Institute Inc./ STAT R User's Guide, Version 6, 4th Ed. SAS Institute Inc., Cary, NC.

Subramanyam Bh. & R. Roesli. 2000. "Inert Dusts, 321-373". In: Alternatives to Pesticides in Stored- Product IPM. (Eds. Bh. Subramanyam & D. W. Hagstrum). Kluwer Academic Publishers, Dordrecht, 429 pp.

Vayias, B. J., C. G. Athanassiou, Z. Korunic & V. Rozman, 2009. Evaluation of natural diatomaceous earth deposits from south-eastern Europe for stored-grain protection: The effect of particle size. Pest Management Science: Formerly Pesticide Science, 65 (10): 1118-1123.

Zettler, L. J. & D. W Keever, 1994. Phosphine resistance in cigarette beetle (Coleoptera: Anobiidae) associated with tobacco storage in the Southeastern United States. Journal of Economic Entomology, 87 (3): 546-550.

Zettler, L. J. & G. W. Cuperus, 1990 Pesticide resistance in *Tribolium castaneum* (Coleoptera: Tenebrionidae) and *Rhyzopertha dominica* (Coleoptera: Bostrichidae) in wheat. Journal of Economic Entomology, 83 (5): 1677-1681.

Ziaee, M., S. Moharramipour & K. Dadkhahipour, 2013. Effect of particle size of two Iranian diatomaceous earth deposits and a commercial product on *Sitophilus granarius* (Col.: Dryophthoridae). Journal of Entomological Society of Iran, 33 (2): 9-17.