

REPELLENCY OF LOCAL DIATOMACEOUS EARTH ON WHEAT AGAINST SOME COLEOPTERAN STORED-PRODUCT PESTS

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ABSTRACT

In this study, the repellent effect of a local diatomaceous earth (DE) formulation (Demite®) was evaluated on monolayer and bulk wheat against three coleopteran stored-grain pests: the lesser grain borer (*Rhyzopertha dominica* (Fabricius), Coleoptera: Bostrichidae), the confused flour beetle (*Tribolium confusum* Jacquelin du Val, Coleoptera: Tenebrionidae), and the rice weevil (*Sitophilus oryzae* (L.), Coleoptera: Curculionidae). Two-choice tests were conducted to assess insect behavior on treated and untreated surfaces at a DE concentration of 1000 ppm. The experiments measured insect preferences on monolayer wheat surfaces and within bulk wheat at specific observation intervals. The repellency tests were performed under controlled conditions: 25 ± 1 °C temperature, $65 \pm 5\%$ relative humidity, and complete darkness in an insect growth room. The repellency of Demite® significantly varied between application methods. On monolayer wheat, the strongest response was observed for *T. confusum* (97%), followed by *S. oryzae* (72%) and *R. dominica* (56%). In contrast, bulk wheat tests showed higher repellency for *S. oryzae* (90%) and *T. confusum* (89.3%), while *R. dominica* exhibited lower responsiveness (53.3%). Significant differences in repellency among the insect species were detected. *Sitophilus oryzae* and *T. confusum* exhibited strong repellency across all observation periods, whereas *R. dominica* was less affected by DE-treated wheat. In conclusion, the local DE formulation demonstrated notable repellent properties, making it a valuable tool in stored grain pest control strategies.

Keywords: Demite®, Integrated pest management, Monolayer wheat, Repellent effect, Two-choice tests.

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Published first online October 08, 2025

Published final November 30, 2025

INTRODUCTION

Diatomaceous earth (DE) is an inert, lightweight dust derived from amorphous sediment, consisting of fossilized remains of unicellular algae. Its low-density composition varies in color from white to dark gray. DE typically contains 80–93% silicon dioxide, with the remaining percentage comprised of organic matter, clay minerals, quartz, and calcium and magnesium carbonates (Korunic, 1998; Stathers *et al.*, 2004). Diatomaceous earth has demonstrated significant potential as a non-toxic insecticide for controlling stored grain pests. Its insecticidal action is primarily physical and works through various mechanisms. Proposed mechanisms include cuticle abrasion (Vayias *et al.*, 2009), absorption of cuticular waxes from the epicuticle surface (Subramanyam and Roesli, 2000; Mewis and Ulrichs, 2001; Prasantha, 2003), damage to the digestive tract (Smith, 1969), blockage of spiracles and tracheae (Webb, 1945), and surface enlargement combined with dehydration (Zacher and Kunike, 1931). Recent studies

highlight the primary mode of action of absorption of epicuticular lipids and fatty acids, which leads to desiccation in arthropods (Golob, 1997; Fields, 1998; Mewis and Ulrichs, 2001; Prasantha, 2003). Moreover, DE remains effective in treated grains over extended periods, presenting a viable alternative to traditional insecticides for long-term pest control (Bukhari and Khan, 2025).

This environmentally friendly, non-chemical method prevents pest resistance, making DE an appealing and sustainable solution. Numerous studies have confirmed its efficacy against a wide range of stored product pests (Subramanyam *et al.*, 1994; Korunic, 1998; Subramanyam and Roesli, 2000; Wakil *et al.*, 2006; Athanassiou *et al.*, 2007; Kostyukovsky *et al.*, 2010; Saglam *et al.*, 2022). There are more than 30 DE formulations, derived from natural deposits, that are now commercially available worldwide (Subramanyam and Roesli, 2000; Fields and Korunic, 2000; Kavallieratos *et al.*, 2005; Losic and Korunic, 2018). Studies consistently show these formulations to be highly effective against a

variety of stored-grain insect species (Subramanyam *et al.*, 1994; Korunic, 1998; Subramanyam and Roesli, 2000; Wakil *et al.*, 2006; Kostyukovsky *et al.*, 2010; Sağlam *et al.*, 2020; Athanassiou *et al.*, 2025). Preliminary evidence suggests that Turkey is rich in natural diatomaceous earth reserves, with significant deposits located in the Central and Western Anatolian regions (Özbey and Atamer, 1987; Mete, 1988; Sıvacı and Dere, 2006; Çetin and Taş, 2012). Turkey's DE reserves are estimated at approximately 125 million tons. Studies evaluating local DE samples from various Turkish regions have shown promising results in managing stored product pests (Doğanay, 2013; Işıkber *et al.*, 2016; Akçalı *et al.*, 2018; Şen *et al.*, 2019).

When using DE for grain protection, several critical factors must be considered. These include the application amount, the type of grain, the specific insect species being targeted, grain moisture levels, relative humidity, temperature, and the duration of storage (Quarles, 1992). The type of grain is particularly important because DE does not adhere as effectively to corn as it does to other grains. Additionally, qualitative factors such as insect preferences may influence effectiveness. Key metrics for determining DE's effectiveness include its level of repellency and the duration of that effect, its toxicity to insects and the longevity of that toxicity, as well as the amount of grain damaged (Quarles, 1992; Mohan and Fields, 2002). Potential behavioral responses of target insect species should also be addressed during the development of any new pest control agent. Repellence to DE appears to be a widespread phenomenon in stored-grain pest control. DE has been shown to repel various insects, including stored-product beetles such as *Sitophilus oryzae*, *Tribolium castaneum*, *T. confusum*, *Rhyzopertha dominica*, and *Cryptolestes ferrugineus* (Quarles, 1992; Rigaux *et al.*, 2001; Mohan and Fields, 2002; Sağlam *et al.*, 2022). It is also effective against cockroach species (*Blattella germanica*, *Periplaneta americana*, and *Blatta orientalis*) (Faulde *et al.*, 2006a, b), silverfish (*Lepisma saccharina*) (Faulde *et al.*, 2006b), and the bloodsucking bug *Triatoma infestans* (Luz *et al.*, 2012). Notably, a strain of *T. castaneum* has been documented to develop avoidance behavior towards desiccant dust (Rigaux *et al.*, 2001).

Addressing the potential behavioral effects of target species is crucial during the development of any new pest control agent. If DE acts as a repellent to stored-grain insects on treated grain surfaces, the worst-case scenario could result in treatment failure, as target insects may avoid exposure to DE particles altogether. Despite its well-documented insecticidal properties, research on the behavioral effects of DE at different grain surfaces remains limited. This study aimed to evaluate the repellency of a locally formulated DE product, Demite®, against three economically significant coleopteran stored-grain pests: the lesser grain borer (*R. dominica*), the

confused flour beetle (*T. confusum*) and the rice weevil (*S. oryzae*). The tests were conducted on both monolayer and bulk wheat surfaces to simulate real-world storage conditions.

Insect behavioral responses to DE formulations can vary depending on both the pest species and the method of application. Therefore, understanding species-specific and context-dependent responses is critical for the effective and optimized use of DE within integrated pest management (IPM) strategies. This study was expected to reveal significant differences in repellency among species, with more pronounced avoidance behavior particularly in *T. confusum* and *S. oryzae*, thereby suggesting that the Demite® formulation could be considered a promising alternative for the control of stored-product insect pests.

MATERIALS AND METHODS

Test insects: In the repellency experiments, the pests *S. oryzae*, *T. confusum*, and *R. dominica* were obtained from a stock culture maintained in the Entomology Laboratory of Kahramanmaraş Sütcü Imam University. *Sitophilus oryzae*, and *R. dominica* were reared on whole soft wheat with an 11% moisture content under controlled conditions: $26 \pm 1^\circ\text{C}$ with $65 \pm 5\%$ relative humidity (Rh.) for *S. oryzae*, and $30 \pm 1^\circ\text{C}$ with $65 \pm 5\%$ Rh. for *R. dominica*. Meanwhile, *T. confusum* was reared at $26 \pm 1^\circ\text{C}$ and $65 \pm 5\%$ Rh. on a diet consisting of wheat flour and yeast in a 17:1 ratio (w/w), following standard culture techniques. Seven- to ten-days old adults of *T. confusum*, *S. oryzae*, and *R. dominica* were used in the repellency experiments.

Diatomaceous Earth formulation: The local DE formulation, Demite®, was used in the biological tests. This micronized powder formulation, characterized by its fine particle size distribution, was produced by grinding DE deposits using a laboratory-scale ball mill (RAM1107; Rantek Test Solutions, Ankara). Demite® is a mixture derived from freshwater DE deposits obtained from three reserves in Central Anatolia, Turkey. It primarily consists of naturally occurring amorphous silica and has a white-grey color (Figure 1). The formulation was developed by Entoteam Rand Food Agriculture Co. for use as a pest control-grade insecticide. Scanning electron microscopy (SEM) images of the Demite® formulation (Figure 2) show numerous cylindrical and rod-shaped diatom skeletons.

Quantitative mineral analyses of the Demite®, were performed at the Accredited Mineralogy and Petrography Laboratory of the General Directorate of Mineral Research and Exploration of Turkey (MTA). An atomic absorption spectroscopy (AAS) device was used for element analysis after a melting and acid removal process. The composition of Demite® includes 82.2%

SiO₂, 4.70% CaO, 4.40% Al₂O₃, 1.55% Fe₂O₃, 0.60% MgO, 0.30% K₂O, 0.35% Na₂O, and less than 0.01% TiO₂ (wt/wt) (loss on ignition value: 5.80%). The particle diameter values of Demite®, corresponding to 10% (d(0.1)), 50% (d(0.5)), and 90% (d(0.9)) of the total

particle volume in the volumetric cumulative particle size distribution, are 3.72 μm, 14.09 μm, and 45.8 μm, respectively. The mean pH value of Demite® is 8.45 ± 0.03. The average loose and tapped bulk densities (g/L) of Demite® are 223.9 ± 1.6 and 294.2 ± 4.9, respectively.

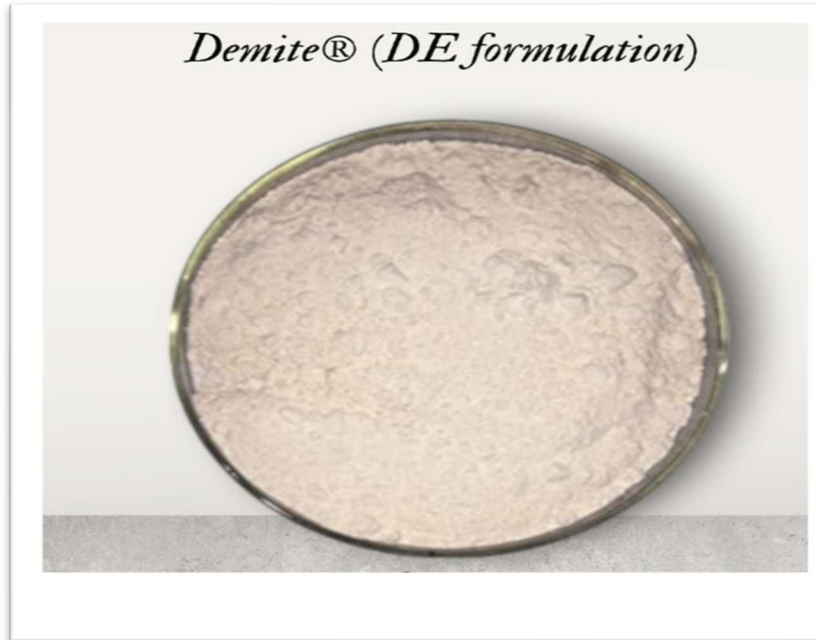


Figure 1. Micronize powder formulation of local diatomaceous earth (Demite®) used in biological tests

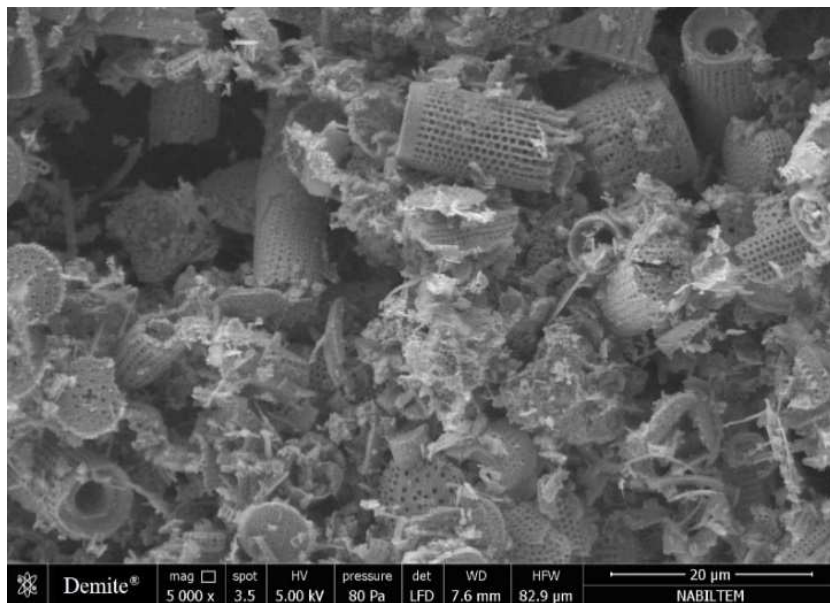


Figure 2. Scanning electron microscopy image of diatom frustules present in local DE formulation (Demite®) (20.000x magnification).

Wheat variety used for repellency test: A clean, soft wheat variety with very little dockage (0.8%), identified as (*Triticum aestivum* L., Poales: Poaceae) variety

Elbistan Yazlıđı, was used for repellency tests. The moisture content of the wheat used in the biological tests,

measured using a Dickey-John moisture meter, ranged from 11.0% to 11.4%.

Experimental arenas used for two-choice repellency test:

Two methods were developed to evaluate the repellency effects of DE treatments. These methods utilize two distinct experimental setups: one for mono-layer wheat in shallow plastic boxes and the other for bulk wheat in deeper plastic containers (Bullards and Shumake, 1979). The procedures for each method are outlined below:

- A. Two-choice tests on mono-layer wheat: Experimental areas for mono-layer wheat were prepared using rectangular plastic boxes with dimensions of $25 \times 13 \times 6$ cm (L \times W \times H). The preparation steps were as follows: 1) dry concrete mix was combined with water and poured into the plastic boxes to a depth of 2 cm., 2) the concrete surface was left to dry completely before the experimental zones were created. After the concrete had dried, three distinct areas were established: DE-treated wheat area: A section measuring 10×13 cm

was designated for wheat treated with DE. Transition zone: A neutral empty area, 5×13 cm, was left between the treated and untreated wheat areas to allow for free pest movement. Untreated wheat area: Another section, also 10×13 cm, was allocated for wheat without DE treatment. These zones are illustrated in Figure 3 (A).

- B. Two-choice test on bulk wheat: For bulk wheat tests, experimental areas consisted of plastic storage cups with dimensions of $10 \times 10 \times 7$ cm (L \times W \times H). The preparation steps were as follows: Each plastic container was divided into two equal parts using a 2 mm metal sieve. This divider allowed pests to move freely between the two areas while maintaining physical separation of the treated and untreated wheat. Two zones were created within the container: 1) DE-treated wheat area: one section, measuring $10 \times 5 \times 7$ cm (L \times W \times H), contained wheat with DE, 2) Untreated wheat area: the other section, also $10 \times 5 \times 7$ cm, contained wheat without DE treatment. These zones are depicted in Figure 3 (B).

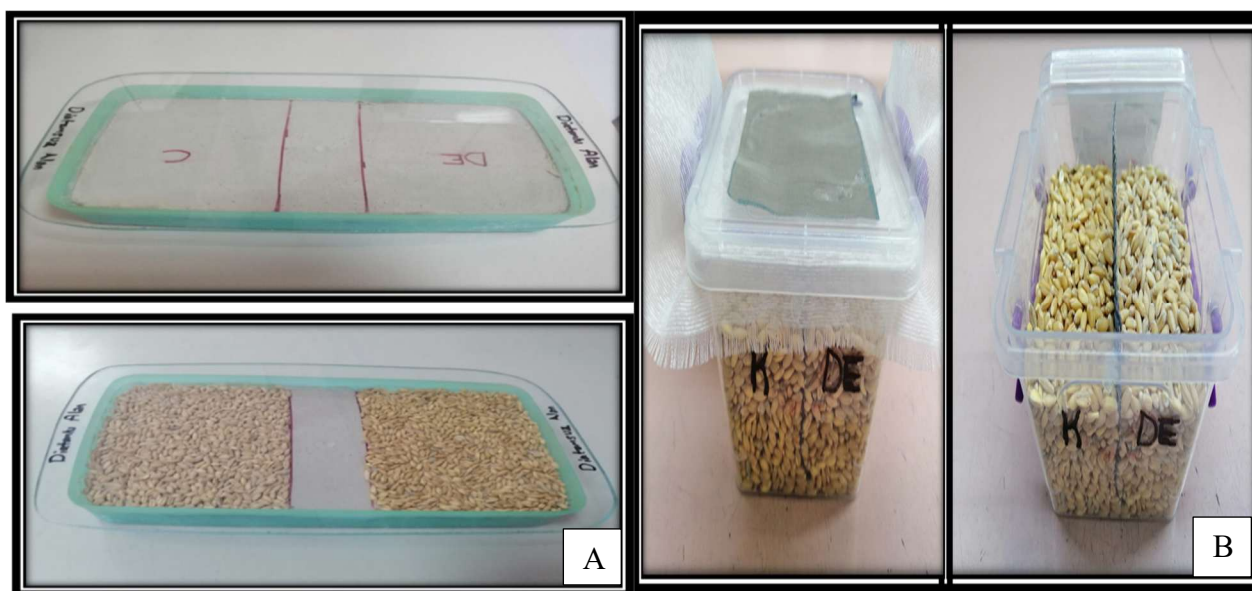


Figure 3. Containers used in two-choice tests conducted in mono-layer wheat (A) and bulk wheat (B).

The procedure of repellency test on the mono-layer wheat: Repellency tests were conducted following a randomized plot design with five replicates, using a 1000 ppm of Demite® (mg DE/kg wheat). The two-choice test method was employed to determine the probability of pests avoiding contact with DE-treated wheat. Wheat lots were treated with Demite® at a concentration of 1000 ppm. The treated wheat was manually shaken for approximately 15 minutes to ensure an even distribution of DE dust within the grain mass (Subramanyam and Roesli, 2000). For each test, 25 g of DE-treated wheat and 25 g of untreated (control) wheat were placed in a

mono-layer within a rectangular plastic arena. Rectangular plastic containers were used as the experimental arena. The edges of the containers were coated with Fluon (polytetrafluoroethylene, Sigma Aldrich, St. Louis, MO, USA) to prevent insects from climbing and escaping. A total of 30 adult insects, older than 14 days and of mixed sex, were released into an empty arena. Repellency tests were designed according to a completely randomized design and each treatment was replicated 5 times. The arena was then covered with a plexiglass lid containing small holes to allow ventilation while preventing escape. The total number of insects

found in the DE-treated and untreated wheat areas was recorded at the following time intervals: 0.5, 1, 3, 5, and 7 days. The counts provided data on the insects' movement and their tendency to avoid DE-treated wheat.

The procedure of repellency test in bulk wheat: Repellency tests were conducted using a randomized parcel design with five replicates at a 1000 ppm concentration of Detech® (mg DE/kg wheat). The procedures mirrored those used in two-choice tests on mono-layer product surfaces. For each treatment, 150 g of wheat was treated with Detech® at a concentration of 1000 ppm (mg DE/kg wheat). Untreated wheat (control) was also weighed to the same amount (150 g) for comparison. The DE-treated and untreated wheat were placed separately into designated areas within the experimental arena. Thirty adults of mixed sex and aged >14 days from each tested insect species were used. The insects were introduced into the area containing DE-treated wheat. The experimental arena was sealed with a plexiglass lid containing small holes to provide ventilation and prevent insects from escaping.

Observations and insect counts were conducted at 1, 3, 5, and 7 days after the start of the experiment. After each observation period, the wheat from both DE-treated and untreated areas was sieved separately using a 300 µm sieve (Retsch GmbH, Germany). The number of insects present in each area was recorded after sieving.

Data processing and analysis: For two-choice repellency experiments with *T. confusum*, *S. oryzae* and *R. dominica*, the proportion of the insects in areas for each count was analyzed using a two-sample Chi-square test. The test evaluated whether the mean proportions of insects differed significantly from an equal distribution of 50% in the experimental arenas. The percentage repellence (PR) of DE treatment for each insect species was also calculated after each observation time using the formula: $((N_c - N_t) / (N_c + N_t)) \times 100$, where N_c is the number of insects present in the untreated wheat arena and N_t is the number of insects present in DE-treated wheat arena. The average was then assigned to different classes (0 to V) using the scale given in Table 1 (Juliana and Su, 1983).

Table 1. Repellency effect classes are assigned based on the percentage repellency ranges.

Class	Repellency (%)	Class	Repellency (%)
0	0.01-0.10 (no repellency)	III	40.1-60.0 (Good repellency)
I	0.11-20.0 (Slight repellency)	IV	60.1-80.0 (Strong repellency)
II	20.1-40.0 (Moderate repellency)	V	80.1-100 (Very strong repellency)

RESULTS

Repellency experiment results on mono-layer wheat: Repellency experiments were conducted on mono-layer wheat surfaces placed in shallow plastic containers, featuring two-choice test areas. These experiments evaluated the differences in insect presence between Demite®-treated wheat (at a concentration of 1000 ppm) and untreated wheat. Statistical analysis revealed significant differences in the total number of *S. oryzae* and *T. confusum* insects treated versus untreated wheat at all observation times ($P < 0.0001$). For *R. dominica*, however, significant differences were only observed at specific time points. No significant differences were found on the 5th and 7th observation days ($P = 0.284$ and $P = 0.142$, respectively), while significant differences were noted at 0.5, 1, and 3 days ($P \leq 0.05$). The repellency tests demonstrated that *S. oryzae* and *T. confusum* adults consistently preferred untreated wheat over Demite®-treated wheat at all observation times. Conversely, *R. dominica* showed no clear preference between treated and untreated wheat, with insect distribution generally equal across the two-choice areas (Table 2). Overall, the Demite® mono-layer treatment proved highly effective against *S. oryzae* and *T. confusum*, as evidenced by a significant reduction in their numbers in the treated areas.

In contrast, this level of repellency was not observed for *R. dominica* adults.

Repellency experiment results on bulk wheat: Repellency effect experiments were conducted on bulk wheat using deep plastic containers with two-choice test areas. Statistical analysis showed that the differences in the total number of *S. oryzae* and *T. confusum* adults in Demite®-treated wheat (1000 ppm concentration) and untreated wheat areas were significant at all observation times ($P < 0.0001$). In contrast, the differences in the total number of *R. dominica* adults between treated and untreated areas at all observation times were not statistically significant ($P = 0.253$, $P = 0.870$, $P = 0.624$, $P = 0.414$, and $P = 0.414$ at 0.5, 1, 3, 4, 5, 6, and 7 days, respectively). The results indicated that *S. oryzae* and *T. confusum* consistently preferred untreated wheat areas at all observation times. However, *R. dominica* exhibited no significant preference between Demite®-treated and untreated areas, with adults distributing approximately equally between the two-choice areas (Table 3). Overall, a very high repellency effect was observed against *S. oryzae* and *T. confusum* in the treated areas, whereas the same level of repellency was not observed for *R. dominica* adults. A significant reduction in the number of *S. oryzae* and *T. confusum* adults was consistently noted in the treated areas.

Table 2. The total number of *Sitophilus oryzae*, *Tribolium confusum*, and *Rhyzopertha dominica* insects present in Demite®-treated (1000 ppm concentration) and untreated areas (two-choice tests) on mono-layer wheat

Observation times	Mono-layer wheat test	Total insects present in two-choice areas		
		<i>S. oryzae</i>	<i>T. confusum</i>	<i>R. dominica</i>
Day 0.5	DE-treated wheat area	33	27	88
	Untreated wheat area	117	122	58
	Chi-square (df)**, P value	47.04 (1), P<0.0001*	60.57 (1), P<0.0001*	6.16 (1), P=0.013*
Day 1	DE-treated wheat area	38	9	87
	Untreated wheat area	110	141	63
	Chi-square (df)**, P value	35.02 (1), P<0.0001*	116.50 (1), P<0.0001*	3.84 (1), P=0.05
Days 3	DE-treated wheat area	36	5	60
	Untreated wheat area	114	145	87
	Chi-square (df)**, P value	40.56 (1), P<0.0001*	130.66 (1), P<0.0001*	4.95 (1), P=0.026*
Days 5	DE-treated wheat area	44	6	67
	Untreated wheat area	106	144	80
	Chi-square (df)**, P value	25.62 (1), P<0.0001*	126.96 (1), P<0.0001*	1.14 (1), P=0.284
Days 7	DE-treated wheat area	42	4	66
	Untreated wheat area	108	146	84
	Chi-square (df)**, P value	29.04 (1), P<0.0001*	134.42 (1), P<0.0001*	2.16 (1), P=0.142

*Statistically significant difference at 5% significance level compared to the Chi-square test. **Degree of freedom.

Table 3. The total number of adult *Sitophilus oryzae*, *Tribolium confusum*, and *Rhyzopertha dominica* adults present in Demite®-treated (1000 ppm concentration) and untreated areas during bulk wheat deep-cup trials

Observation times	Bulk wheat tests	Total adults present in two-choice areas		
		<i>S. oryzae</i>	<i>T. confusum</i>	<i>R. dominica</i>
Day 0.5	DE-treated wheat area	43	41	82
	Untreated wheat area	110	109	68
	Chi-square (Df)**, P value	29.33 (1), P<0.0001*	30.82 (1), P<0.0001*	1.30 (1), P=0.253
Day 1	DE-treated wheat area	25	32	76
	Untreated wheat area	125	118	74
	Chi-square (Df)**, P value	66.66 (1), P<0.0001*	49.30 (1), P<0.0001*	0.026 (1), P=0.870
Days 3	DE-treated wheat area	29	46	68
	Untreated wheat area	121	104	82
	Chi-square (Df)**, P value	56.42 (1), P<0.0001*	22.42 (1), P<0.0001*	1.30 (1), P=0.253
Days 5	DE-treated wheat area	20	31	72
	Untreated wheat area	130	119	78
	Chi-square (Df)**, P value	80.66 (1), P<0.0001*	51.62 (1), P<0.0001*	0.24 (1), P=0.624
Days 7	DE-treated wheat area	15	16	70
	Untreated wheat area	135	134	80
	Chi-square (Df)**, P value	96(1), P<0.0001*	92.82 (1), P<0.0001*	0.66(1), P=0.414

*Statistically significant difference at 5% significance level compared to the Chi-square test. **Degree of freedom

The mean proportions of *S. oryzae* (A), *T. confusum* (B), and *R. dominica* (C) insects present in Demite®-treated wheat and untreated wheat areas at specific observation times in two-choice tests on mono-layer wheat are shown in Figure 4. According to Chi-square tests, the differences in percentages of *S. oryzae* and *T. confusum* between the treated and untreated areas were statistically significant (P<0.0001). For *R. dominica*, however, the differences between the two areas were not statistically significant (P=0.033, P=0.110, P=0.069, P=0.419, and P=0.230 on days 1, 3, 5, and 7, respectively). In the repellency tests,

it was observed that *S. oryzae* and *T. confusum* adults consistently preferred the untreated wheat DE areas at all observation times (Figure 1 A, B). Conversely, *R. dominica* exhibited no preference between treated and untreated areas across all observation times except at 0.5 days (Table 3). When examining the number of insects in the treated areas, the most significant decrease was observed in *T. confusum*, followed by *S. oryzae*, while no notable decrease was observed in the number of *R. dominica* insects.

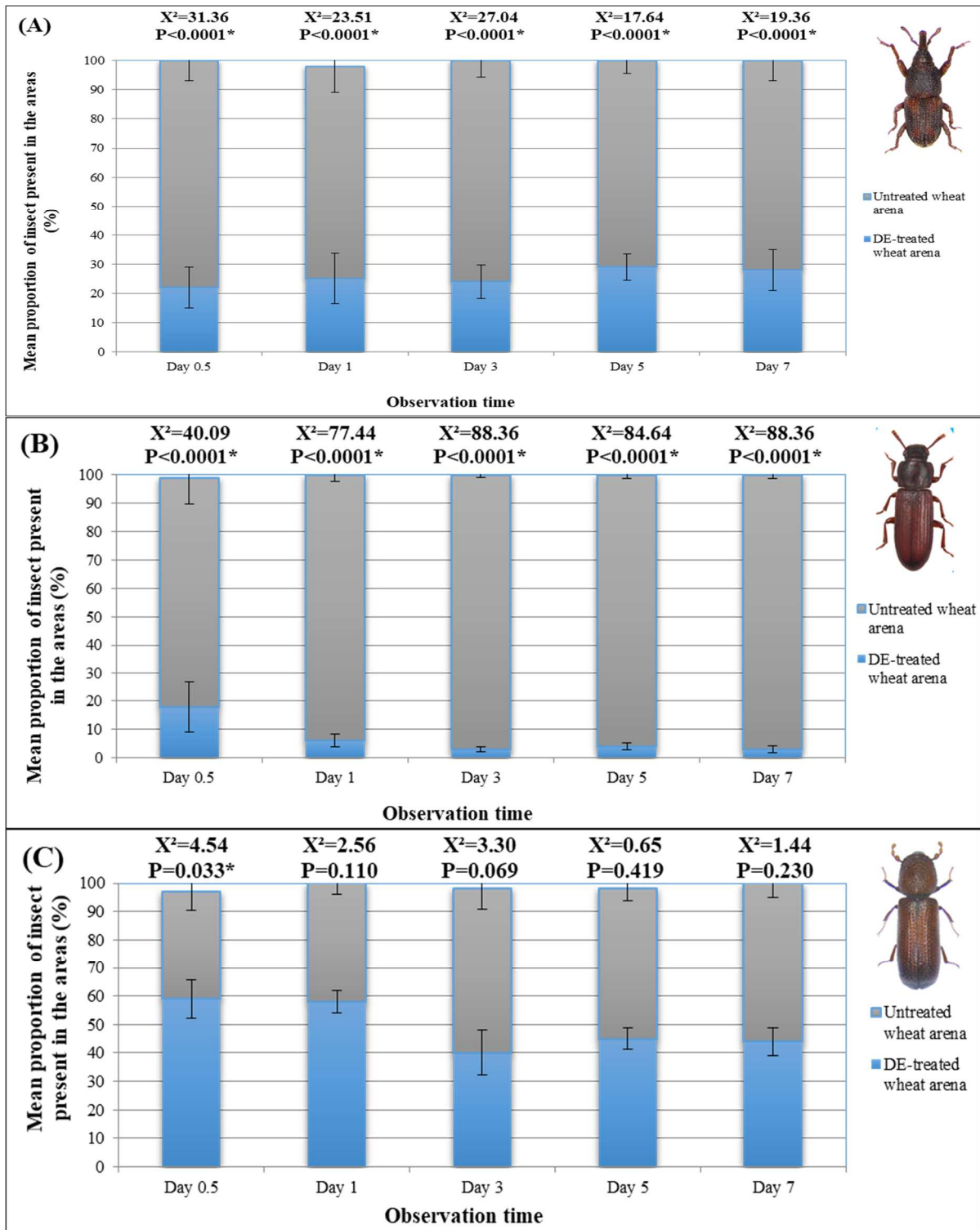


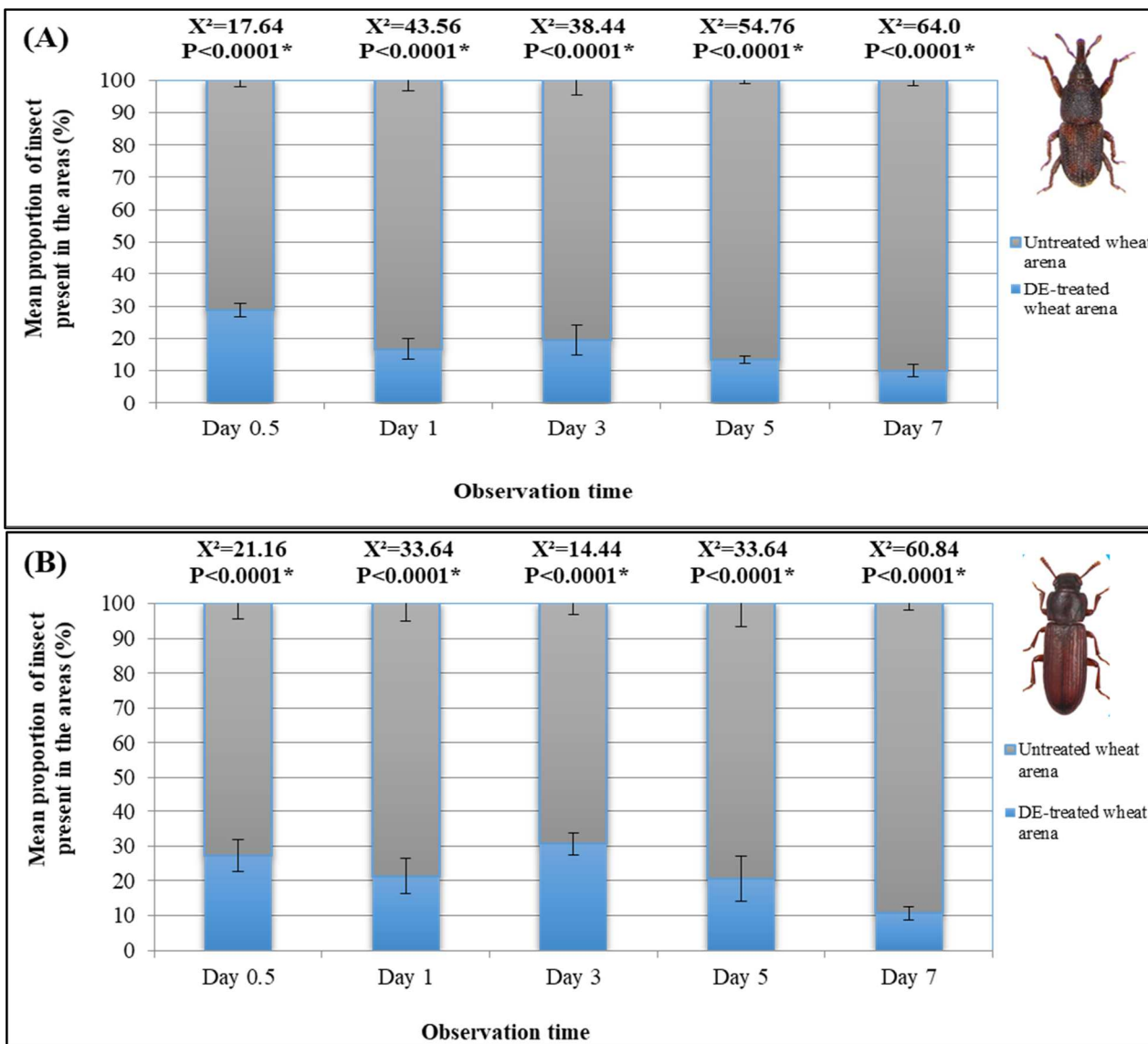
Figure 4. The mean proportions of *Sitophilus oryzae* (A), *Tribolium confusum* (A), and *Rhyzopertha dominica* (C) adults in two-choice areas—Demite®-treated (1,000 ppm concentration) and untreated mono-layer wheat—at specific observation times during repellency tests

The percentages of *S. oryzae*, *T. confusum*, and *R. dominica* in Demite®-treated and untreated wheat areas after specific observation times are presented in

Figure 5 (A, B, C). The tests were conducted on bulk wheat in deep cups. According to Chi-square tests, the differences between the percentages of *S. oryzae* and *T.*

confusum adults in treated and untreated areas were statistically significant ($P < 0.0001$). In contrast, the differences in the rates of presence for *R. dominica* between the two areas were not statistically significant ($P = 0.317$, $P = 0.841$, $P = 0.317$, $P = 0.689$, and $P = 0.549$ for 0.5, 1, 3, 5, and 7 days, respectively). The results indicated that *S. oryzae* and *T. confusum* exhibited high

repellency rates, consistently preferring untreated wheat areas at all observation times. When examining the number of adults in the treated areas, the largest decrease was observed in *T. confusum*, followed by *S. oryzae*. However, no significant decrease was noted in the number of *R. dominica* adults in the treated areas.



Comparison of repellency rates on mono-layer and bulk wheat: The repellency classifications for mono-layer wheat tests revealed that the percentage repellency for *T. confusum* ranged from 63.7% to 94.6%, corresponding to repellency classes IV and V. These values were significantly higher than those observed for *S. oryzae* and *R. dominica* (Table 4). In contrast, *S. oryzae* exhibited repellency percentages between 41.3% and 56% at certain observation times, falling into repellency class III. Meanwhile, *R. dominica* showed

much lower repellency, with percentages ranging from 20.5% to 18.3%, placing it in classes 0 to I. In bulk wheat tests, *S. oryzae* displayed repellency percentages of 43.3% to 80%, corresponding to repellency classes III and IV. These values were significantly higher than those for *T. confusum* and *R. dominica* (Table 4). The repellency of *T. confusum* ranged from 41.3% to 56%, placing it in classes II to IV during the observation period. By contrast, *R. dominica* continued to exhibit minimal repellency in bulk wheat tests, with percentages

ranging from -9.3% to 9.3%, falling into classes 0 to I. These findings suggest that the repellency of Demite® varied by insect species and application method (mono-layer vs. bulk wheat tests). In mono-layer DE treatments,

T. confusum adults demonstrated the highest repellency, whereas in bulk wheat treatments, *S. oryzae* adults showed the greatest repellency.

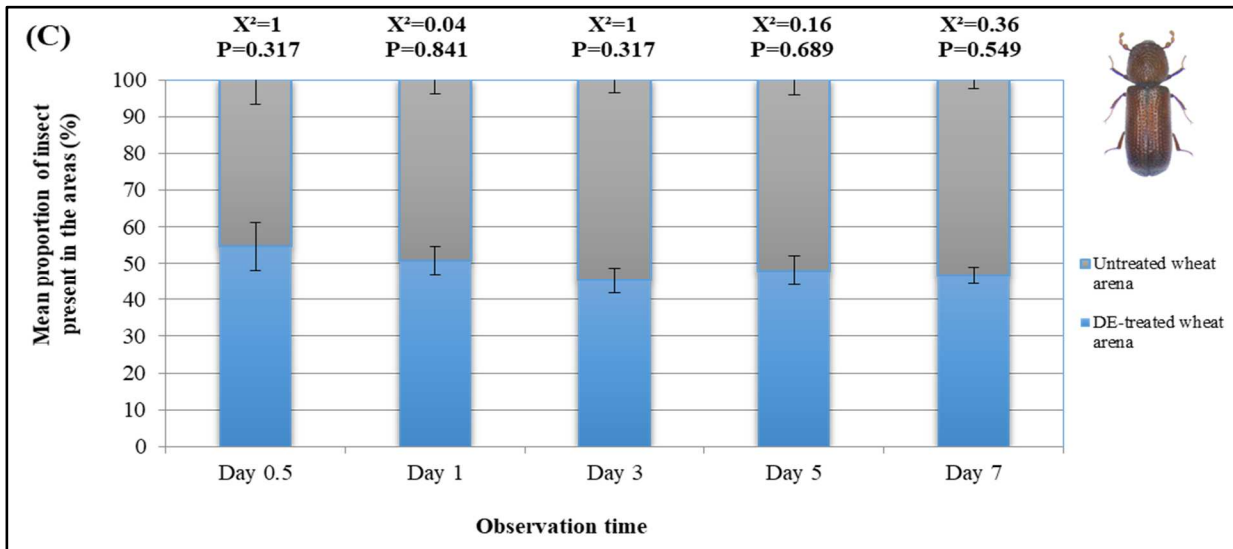


Figure 5. The mean proportions of *Sitophilus oryzae* (A), *Tribolium confusum* (A), and *Rhyzopertha dominica* (C) insects in two-choice areas—Demite®-treated (1,000 ppm concentration) and untreated bulk wheat—at specific observation times during repellency tests.

Table 4. Percentage repellency (PR) of *Sitophilus oryzae*, *Tribolium confusum*, and *Rhyzopertha dominica* adults on mono-layer and bulk wheat, and their corresponding repellency classes.

Observation times	Percentage repellency (class)					
	Mono-layer wheat			Bulk wheat		
	<i>S. oryzae</i>	<i>T. confusum</i>	<i>R. dominica</i>	<i>S. oryzae</i>	<i>T. confusum</i>	<i>R. dominica</i>
Day 0.5	56 (III)	63.7 (IV)	-20.5 (0)	43.3 (III)	45.3 (III)	-9.3 (0)
Day 1	48.6 (III)	88 (V)	-16 (0)	66.6 (IV)	57.3 (III)	-1.3 (0)
Days 3	52 (III)	93.3 (V)	18.3 (I)	61.3 (IV)	38.6 (II)	9.3 (0)
Days 5	41.3 (III)	92 (V)	8.8 (0)	73.3 (IV)	58.6 (III)	4 (0)
Days 7	44 (III)	94.6 (V)	12 (I)	80 (IV)	78.6 (IV)	6.6 (0)

*Numbers in parentheses indicate repellency classes.

DISCUSSION

Diatomaceous earth (DE) has demonstrated both insecticidal (Subramanyam *et al.*, 1994; Korunic, 1998; Subramanyam and Roesli, 2000; Wakil *et al.*, 2006; Athanassiou *et al.*, 2007; Kostyukovsky *et al.*, 2010; Saglam *et al.*, 2022) and repellent properties (Quarles, 1992; Rigaux *et al.*, 2001; Mohan and Fields, 2002) against various stored grain pests. While the mechanisms underlying these properties have been debated, the consensus is that DE functions primarily through the sorptive nature of its dust particles. This increases the adsorption of the insect's wax coating, leading to abrasion, desiccation, and eventual compromise of the insect epicuticle (Zacher and Kunicke, 1931; Ebeling, 1971; Rohitha Prasantha *et al.*, 2015). Additionally, DE

exerts a repellent effect due to its physical presence (White *et al.*, 1966; Ebeling, 1971; Fields, 1998; Mewis and Ulrichs, 2001; Prasantha, 2003; Saglam *et al.*, 2022), which helps deter insects from attacking or infesting treated grain.

The results of the two-choice tests in this study revealed varying levels of repellency among stored-product pests on DE-treated mono-layer wheat. *Tribolium confusum* displayed the strongest repellent response, consistently avoiding treated wheat in favor of untreated wheat across all observation periods. This suggests that DE could serve as an effective repellent for this species. Conversely, repellency tests with *S. oryzae* indicated a preference for untreated wheat, though the repellent effect was less pronounced than in *T. confusum*, reflecting moderate sensitivity to DE treatment. These

behavioral differences imply that DE's primary mode of action may function more as a deterrent than a lethal agent, particularly for *T. confusum* and *S. oryzae*. Similar findings have been documented in other studies. For example, Nwaubani and Farsoranti (2008) and Nwaubani *et al.* (2014) reported that *S. oryzae* avoided DE-treated wheat, resulting in significantly fewer adults in treated areas. Additionally, a local DE formulation (Detech®) exhibited repellent properties, reducing infestation risks by deterring *T. confusum* and *S. oryzae* from entering treated grain. Okonkwo *et al.* (2018) also observed repellency effects in *Callosobruchus maculatus*. Other research has demonstrated similar responses in different insect species: *B. germanica* avoided DE-treated areas (Faulde *et al.*, 2006a), as did the drywood termite *Incisitermes minor* (Ebeling and Wagner, 1959).

The results of two-choice tests in this study indicated that *R. dominica* was largely unaffected by DE, showing no strong preference between treated and untreated wheat. This suggests that DE is less effective as a repellent for this species. Similarly, several studies have reported that *R. dominica* adults did not avoid DE-treated wheat, exhibiting no significant preference between treated and untreated samples (Mohan and Fields, 2002; Vardeman *et al.*, 2007; Bayram *et al.*, 2020; Saglam *et al.*, 2022;). However, Nwaubani and Farsoranti (2008) and Nwaubani *et al.* (2014) observed that *R. dominica* avoided DE-treated wheat, resulting in significantly fewer adults in treated areas. The differences in observed behavior may stem from *R. dominica*'s lower mobility and its tendency to feed directly on the product, as noted by Vardeman *et al.* (2007).

According to the two-choice repellency test on mono-layer wheat, *T. confusum* adults consistently ranked in the highest repellency class (V) during nearly all observation periods among the tested insect species. In contrast, *S. oryzae* ranked in the highest repellency class (IV) on bulk wheat, while *R. dominica* consistently exhibited repellency classes of 0–I across both test methods. The repellency of Demite® against the tested insect species varied depending on the application method (mono-layer vs. bulk wheat tests). Specifically, in mono-layer DE treatments, *T. confusum* adults demonstrated the highest repellency, whereas in bulk wheat treatments, *S. oryzae* adults exhibited the greatest repellency. Previous studies have demonstrated that stored-product insects can be repelled by DE applied to bulk grain (White *et al.*, 1966; Mohan and Fields, 2002). However, in our study, repellency of the DE treatment was observed only for *S. oryzae* and *T. confusum* on bulk wheat. Similarly, Saglam *et al.* (2022) found that a local DE formulation, Detech®, at 1000 ppm, was highly repellent to *T. confusum* (class V), moderately repellent to *S. oryzae* (class III–V), and exhibited no or limited repellency to *R. dominica* adults. Based on the findings from the study, it is considered crucial to evaluate the

insecticidal efficacy and repellent effects of DE in an integrated manner, as this holds significant importance for pest management. The integrated approach is particularly relevant for real-world grain protection, where both the insecticidal and repellent effects can be harnessed to reduce pest populations effectively. To utilize these multifaceted effects more effectively within IPM strategies, a more comprehensive analysis that considers the synergistic potential of both effects is necessary.

Conclusion: In conclusion, the two-choice repellency tests confirmed that adults of *T. confusum* and *S. oryzae* avoided contact with DE-treated grains, while *R. dominica* showed minimal avoidance, likely due to its reduced mobility. This repellent effect could influence the efficacy of DE by decreasing the exposure time of *S. oryzae* and *T. confusum* as they attempt to escape the DE-treated wheat, thereby increasing their movement activity. A potential negative implication of this behavior is that stored grain beetle pests, particularly *T. confusum* and *S. oryzae*, may reduce the effectiveness of DEs in real storage conditions through their avoidance responses (Rigaux *et al.*, 2001). Overall, the local DE formulation, Demite®, demonstrated notable repellent properties, making it a valuable tool in pest control strategies. For optimal use, DE could be applied as a preliminary treatment to eliminate existing pests from empty grain storage facilities before grains are stored.

This study was conducted under laboratory conditions on three important coleopteran pests. Future research could test a wider range of pests to explore the repellent effects of DE across different species. Additionally, testing the developed commercial DE formulations under semi-commercial and commercial conditions will be crucial for future studies to evaluate their effectiveness and applicability in more realistic settings. Such studies will lay the foundation for integrating DE-based solutions into broader integrated pest control strategies.

Acknowledgements: This study was supported by KSU Scientific Research Projects Management Unit. (Project No: 2018/5-5 YLS)

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