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Original article

Insecticidal efficacy of native raw and commercial diatomaceous earths against *Tribolium confusum* DuVal (Coleoptera: Tenebrionidae) under different environmental conditions

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ABSTRACT

Background: Stored wheat grains are infested by several insect pests which lead to notable financial losses, compromised food security, and higher wastage. The confused flour beetle [*Tribolium confusum* DuVal (Coleoptera: Tenebrionidae)] is a widespread pest infesting stored flour and grains. Pest management in stored wheat requires ecofriendly option with lower toxicity to stored grains. Diatomaceous earths (DEs) are considered environment-friendly, green insecticides and often used to manage stored product pests.
Methods: This study determined the impacts of different temperatures, relative humidity levels, and doses on the efficacy of three DEs [i.e., two raw native (Ankara, and Aydin), and one commercial (Silico-Sec)]. Two temperatures (25 °C and 30 °C), two relative humidity levels (40% and 60%) and five doses (0, 250, 500, 750 and 1000 ppm) of the tested DEs were included in the study. Different DE doses were mixed with 500 g of wheat grains in plastic containers and 30 adults of *T. confusum* were released. The containers were kept under different temperature and relative humidity levels according to the treatments and mortality data was recorded at 7, 14 and 21 days after treatments (DAT).
Results: The mortality linearly increased with increasing time intervals and DEs doses. The highest and the lowest mortality was noted at 21 and 7 DAT, respectively. All DEs caused higher mortality under 30 °C temperature, 40% relative humidity and 1000 ppm dose. The native DE Aydin and commercial DE Silico-Sec caused comparable mortalities.
Conclusion: Overall, the highest mortality was recorded with 1000 ppm dose of all DEs under 30 °C temperature and 40% relative humidity. Therefore, the DEs must be applied at these environmental conditions for getting higher efficacy. Furthermore, the native DE Aydin could be utilized to manage *T. confusum* in the granaries. The farmer granaries in the region have similar temperature and relative humidity conditions; therefore, the DEs can be successfully used to lower the damages caused by *T. confusum* at farmers' level.

1. Introduction

Cereals, especially wheat (*Triticum aestivum* L.), is a strategic crop for Türkiye and produced all over the country. It is ranked first in the country among agronomic crops in terms of area under cultivation and production (TUIK 2022). Cereals make a significant contribution towards agricultural production of the country and stored for future use. However, stored grains are infested by

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numerous pests. A total 37 species infesting stored wheat grains have been reported from Türkiye. The infestation by Coleopteran species causes significant losses in grain weight, seed germination, and quality. These species include *Trogoderma granarium* Everts, *Sitophilus granarius* (L), *S. oryzae* (L.), *Rhizopertha dominica* F., *Tribolium confusum* DuVal, *T. castaneum* Herbst., *Tenebrio molitor* (L.), *Oryzaephilus surinamensis* L. and *Cryptolestes ferrugineus* Steph. (Ergül et al., 1972; Işıkber et al., 2005; Mutlu et al., 2019; Ozar & Yucel, 1982; Şen et al., 2019).

The confused flour beetle [*Tribolium confusum* DuVal (Coleoptera: Tenebrionidae)] is one of the most common and destructive pests of grains and other food products in granaries, silos, grocery stores and homes. Although confused flour beetle is known as a secondary pest, it can easily feed on damaged and broken grains, increases its population, and makes flour and other processed bakery products unusable. These insects are frequently observed in significant quantities within contaminated grains, where they consume broken grains, grain dust, and additional foodstuffs commonly found in households, including flour, rice, dried fruit, nuts, and beans (Baldwin and Fasulo, 2003).

The confused flour beetle has evolved resistant to numerous pesticides being used in storage granaries for pest control (Rossi et al., 2010). Hence, several studies have evaluated diatomaceous earths (DEs) for their potential to suppress the infestation of confused flour beetle in stored grains. The reaction of the beetle to the DEs is mediated by numerous factors, including temperature, relative humidity, and food availability (Arthur, 2000a, 2000b; Fields and Korunic, 2000; Morrill et al., 2001; Subramanyam and Hagstrum, 2012). Food availability and environmental conditions strongly alter the efficacy of DEs.

It is crucial to prevent the losses caused insect pests in stored grains. Insect pest infestation in Türkiye causes 10% losses in cereal grains during storage (Emekçi and Ferizli, 2000). Chemical management is probably the most popular approach for minimizing losses caused by stored grain pests in granaries. Fumigation with aluminum phosphide is the most used management method in Türkiye for pest infestation in stored grains (Mutlu et al., 2019). However, it has been noted that stored grain pests have developed broad resistance to the phosphine employed in fumigation (Benhalima et al., 2004; Mutlu et al., 2019; Pimentel et al., 2010). In addition, resistance to malathion (the pesticide used to protect stored products) has also been reported (Champ and Dyte, 1976; Mutlu et al., 2019). Unconscious pesticide use has resulted in pesticide resistance, residues, and negative effects on human health. Alternative control methods have gained popularity in recent years because of the drawbacks caused by chemical control (Mutlu et al., 2019). There has been a significant interest in the use of DEs, and much progress has been made in this regard (Alkan et al., 2019; Ciniviz and Mutlu, 2020; Ertürk, 2021; Kılıc and Mutlu, 2020; Korunic, 1998; Sousa et al., 2013).

The DEs cause physical damage to the cuticle of stored grain pests, resulting in water loss and eventually death. The DEs distort the epicuticle by absorbing lipids, making insects susceptible to dehydration once the waterproof layer is destroyed. The physical characteristics of DEs, target insects, habitat and food supply etc. have significant impact on the effectiveness of DEs (Golob, 1997; Korunic, 1998). Temperature and relative humidity significantly influence pest control efficiency of DEs (Arthur 2000a). The DEs absorb cuticular wax coat of insects which leads to desiccation and death.

High quality DEs resources are found different regions of Türkiye (i.e., Afyon, Ankara, Aydin, Balikesir, Bingöl, Çanakkale, Ç ankırı, Denizli, Eskişehir, Kayseri, Konya, Kütahya, Niğde, Sivas and Van). It predicted that there could be >200 million tons of DEs present in the country. These DEs can successfully halt product losses and serve as an alternative to the chemicals used in granaries. This study used native raw DEs collected from Ankara and Aydin to test; (a) insecticidal activity, (b) effect of DE doses, relative humidity, and temperature on mortality; and (c) the effect of exposure times on F1 fertility of confused flour beetle. It was hypothesized that different DEs will differ in their insecticidal activity under different temperature and relative humidity levels, and doses. It was further hypothesized that native DEs would cause equivalent mortality to commercial DEs. The results would help to select the optimum temperature, relative humidity, and dose for native and commercial DEs.

2. Materials and methods

2.1. Experimental site

The current study was conducted at Entomology Laboratory, Diyarbakır Plant Protection Research Institute, Diyarbakır Türkiye.

2.2. Diatomaceous earths (DEs)

The efficacy of different doses of three DEs [i.e., two raw native (Aydin, Ankara), and one commercial (Silico-Sec)] was tested on the mortality of confused flour beetle under various temperatures and relative humidity levels. Ankara DE was obtained from the mines of Ankara province. It contains ≈92.8% SiO_2, 4.2% Al_2O_3, 1.5% Fe_2O_3, 0.6% CaO, 0.3% MgO and 1–5% water. The average particle size is 8–12μ. Aydın DE was obtained from the mines of Aydın province. It contains ≈94.2% SiO_2, 4.6% Al_2O_3, 1.6% Fe_2O_3, 0.7% CaO, 0.3% MgO and 1–5% water. The average particle size is 8–12μ. Silico-Sec (Biofarma) is commercially available from Germany and contains 92% SiO_2, 3% Al_2O_3, 1% Fe_2O_3 and 1% Na_2O. The average particle size is 8–12μ.

2.3. Test insects

The test insects with no history of exposure to insecticides were obtained from the breeding stock of Plant Protection Research Institute, Diyarbakir, Türkiye. Test insects were reared on wheat flour in plastic jars (1000 ml) under controlled conditions (25 ± 1 °C and 65 ± 5% RH) and complete dark. The cultures were maintained at the same condition to obtain new generation adults. The emerging adults were collected with a suction tube and used in the experiments.

2.4. Laboratory bioassays

Five different doses, i.e., 0, 250, 500, 750, and 1000 ppm of each DE. Wheat grains ('Pehlivan' bread wheat variety) were sterilized at 55 °C for 48 h. Afterwards, 500 g of wheat grains was placed in a 1 kg plastic container and mixed manually for 1 min. The 100 g homogenized wheat grains obtained after mixing were taken as a control. The calculated amounts of DEs according to the desired doses as described above were added to the remaining 400 g grains. The plastic containers containing wheat grains and DEs were manually shaken for 2 min to thoroughly mix the grains and DEs. After mixing, 100 g grains were taken and placed in separate containers, whereas treatments were initiated by releasing 30 adults in each container.

Samples prepared in this way were kept at 25 and 30 °C in cabinets adjusted to 40% and 60% relative humidity. Dead and alive adults were counted at 7, 14 and 21 days after adults' release. During the trials, temperature and relative humidity were monitored with the testo 174H brand temperature/humidity data logger.

2.5. F1 productivity

After data collection on 21 days after adult release, alive adults (if any) were collected and kept under the same conditions for ∼2 months to determine F1 productivity.

2.6. Statistical analysis

The mortality (%) was computed by number of dead adults in each treatment. The mortality data were corrected by Abbot's formula relative to the mortality observed in the control treatment. Analysis of variance (ANOVA) was used to analyze the mortality data (Steel et al., 1997). The data of each diatom was analyzed separately. The normality in the data was tested prior to ANOVA, which indicated that the data were normally distributed. Therefore, original data were used in the statistical analysis. Three-way ANOVA was used to infer the difference between individual and interactive effects of DE dose, temperature, and relative humidity. Duncan's multiple comparison test was used to determine the differences between the means where ANOVA denoted significant differences. Statistical analyses were conducted by using SPSS statistical software version 21 (IBM, 2015).

3. Results

The mortality of confused flour beetle was significantly altered by individual and interactive effects of different doses of Ankara, DE, temperature, and relative humidity at different time intervals (Table 1). The individual and interactive effects of temperature, relative humidity and various Aydin DE doses had significant effects on the mortality at different times (Table 2). Similarly, different individual and interactive effects of temperature, relative humidity and Silico-Sec DE doses significantly altered the mortality at 7, 14 and 21 DAT (Table 3).

The mortality increased with increasing time after the initiation of treatments. Overall, the highest mortality was caused by all DEs at 21 DAT, whereas the lowest mortality was noted at 7 DAT. The mortality caused by Ankara, Aydin, and Silico-Sec DEs under different temperatures ranged between 27.79–54.76%, 34.60–67.21% and 33.06–64.68%, respectively (Table 4). Overall, the lowest mortality was recorded under 25 °C at 7 DAT for all DEs included in the study, whereas the highest mortality was noted at 21 DAT under 30 °C (Table 4). Similarly, the mortality caused by different DEs under different relative humidity levels was 26.90–55.41, 33.35–68.15 and 31.86–65.62% for Ankara, Aydin, and Silico-Sec, respectively. All DEs included in the study caused higher mortality under 40% relative humidity, whereas lower mortality was observed under 60% relative humidity. These results indicate that high temperature and low humidity are more feasible for initiating the insecticidal action of DEs.

Like time intervals, the mortality increased with increasing doses of DEs and the highest mortality was recorded in the insects treated with the highest dose, i.e., 1000 ppm of all DEs. The mortality caused by different dose of Ankara, Aydin, and Silico-Sec DEs was 21.92–61.71%, 27.39–76.10% and 26.11–73.37%, respectively. The highest mortality was noted with the highest dose of the tested DEs at 21 DAT (Table 4).

Temperature by relative humidity interaction indicated that all DEs caused the highest mortality at 30 °C temperature and 40% relative humidity at 21 DAT (Table 5). The lowest mortality was caused by all DEs with 25 °C temperature and 60% relative humidity at 7 DAT.

Temperature by dose interaction revealed that the highest dose of all DEs included in the study caused the highest mortality under 30 °C at 21 DAT. The lowest mortality was caused by all DEs at 7 DAT under 250 ppm dose and 25 °C. Similarly, relative humidity by dose interaction revealed that the highest mortality was caused by interaction between the 1000 ppm dose of all DEs and 40% relative humidity at 21 DAT. The lowest mortality of all DEs was caused at 250 pp dose and 60% relative humidity (Table 5).

Three-way interaction of temperature, relative humidity and DE doses indicated that the highest mortality occurred under 30 °C temperature, 40% relative humidity and 1000 ppm dose of all DEs included in the study (Table 6). The mortality linearly increased with the increase in time after treatment. The highest

Table 1
Analysis of variance for individual and interactive effects of different temperatures, relative humidity levels and Ankara diatom doses on mortality of *Tribolium confusum* at different time intervals.

Source	DF	SS	MS	*F* value	*P* value
7 DAT					
Temperature (T)	1	121.85	121.85	340.70	**<0.0001***
Relative humidity (R)	1	330.29	330.29	923.48	**<0.0001***
Dose (D)	3	2098.07	699.36	1955.41	**<0.0001***
T × R	1	4.58	4.58	12.82	**0.00***
T × D	3	4.18	1.39	3.90	**0.01***
R × D	3	6.45	2.15	6.01	**0.00***
T × R × D	3	0.06	0.02	0.06	**0.009***
14 DAT					
Temperature (T)	1	77.70	77.70	372.00	**<0.0001***
Relative humidity (R)	1	229.14	229.14	1096.99	**<0.0001***
Dose (D)	3	1655.50	551.83	2641.82	**<0.0001***
T × R	1	1.27	1.27	6.09	**0.02***
T × D	3	3.58	1.19	5.72	**0.00***
R × D	3	1.66	0.55	2.64	**0.006***
T × R × D	3	0.54	0.18	0.87	**0.0047***
21 DAT					
Temperature (T)	1	71.00	71.00	137.34	**<0.0001***
Relative humidity (R)	1	185.53	185.53	358.90	**<0.0001***
Dose (D)	3	1935.74	645.24	1248.18	**<0.0001***
T × R	1	3.99	3.99	7.72	**0.008***
T × D	3	5.32	1.77	3.43	**0.024***
R × D	3	2.18	0.72	1.40	**0.0025**
T × R × D	3	7.63	2.54	4.92	**0.005***

Here, DF = degree of freedom, SS = sum of squares, MS = mean squares, * = significant, DAT = days after treatment. The bold values in P value column indicate that the relative individual or interactive effects had significant impact on the mortality of *Tribolium confusum*.

Table 2
Analysis of variance for individual and interactive impacts of different temperatures, relative humidity levels and doses of Aydin diatom doses on mortality of *Tribolium confusum* at different time intervals.

Source	DF	SS	MS	*F* value	*P* value
7 DAT					
Temperature (T)	1	133.05	133.05	405.94	**<0.0001***
Relative humidity (R)	1	463.54	463.54	1414.24	**<0.0001***
Dose (D)	3	2971.55	990.51	3022.01	**<0.0001***
T × R	1	4.26	4.26	13.01	**0.001***
T × D	3	1.33	0.44	1.35	**0.007***
R × D	3	6.76	2.25	6.88	**0.001***
T × R × D	3	3.22	1.07	3.28	**0.029***
14 DAT					
Temperature (T)	1	102.16	102.16	478.60	**<0.0001***
Relative humidity (R)	1	321.39	321.39	1505.68	**<0.0001***
Dose (D)	3	2507.50	835.83	3915.74	**<0.0001***
T × R	1	4.47	4.47	20.95	**<0.0001***
T × D	3	2.54	0.84	3.96	**0.013***
R × D	3	6.61	2.20	10.33	**<0.0001***
T × R × D	3	0.24	0.08	0.38	**0.005**
21 DAT					
Temperature (T)	1	90.20	90.20	130.98	**<0.0001***
Relative humidity (R)	1	292.06	292.06	424.12	**<0.0001***
Dose (D)	3	3007.76	1002.58	1455.89	**<0.0001***
T × R	1	3.60	3.60	5.22	**0.027***
T × D	3	4.01	1.33	1.94	**0.005**
R × D	3	2.87	0.95	1.39	**0.007**
T × R × D	3	20.52	6.84	9.93	**<0.0001***

Here, DF = degree of freedom, SS = sum of squares, MS = mean squares, * = significant, DAT = days after treatment. The bold values in P value column indicate that the relative individual or interactive effects had significant impact on the mortality of *Tribolium confusum*.

Table 3
Analysis of variance for individual and interactive impacts of different temperatures, relative humidity levels and doses of Silico-Sec diatom doses on mortality of *Tribolium confusum* at different time intervals.

Source	DF	SS	MS	*F* value	*P* value
7 DAT					
Temperature (T)	1	125.10	125.10	304.68	**<0.0001***
Relative humidity ®	1	431.80	431.80	1051.63	**<0.0001***
Dose (D)	3	2740.74	913.58	2224.94	**<0.0001***
T × R	1	3.90	3.90	9.50	**0.003***
T × D	3	3.85	1.28	3.12	**0.034***
R × D	3	9.57	3.19	7.77	**0.000***
T × R × D	3	0.36	0.12	0.29	**0.0082***
14 DAT					
Temperature (T)	1	103.17	103.17	126.21	**<0.0001***
Relative humidity (R)	1	302.32	302.32	369.84	**<0.0001***
Dose (D)	3	2566.60	855.53	1046.60	**<0.0001***
T × R	1	8.97	8.97	10.97	**0.002***
T × D	3	3.32	1.10	1.35	**0.00268**
R × D	3	0.97	0.32	0.39	**0.00756**
T × R × D	3	1.33	0.44	0.54	**0.00655**
21 DAT					
Temperature (T)	1	77.96	77.9	170.02	**<0.0001***
Relative humidity (R)	1	267.89	267.89	584.20	**<0.0001***
Dose (D)	3	2825.98	941.99	2054.22	**<0.0001***
T × R	1	3.20	3.2	6.98	**0.011***
T × D	3	2.12	0.70	1.54	**0.0216**
R × D	3	6.64	2.2	4.83	**0.005***
T × R × D	3	6.48	2.16	4.71	**0.006***

Here, DF = degree of freedom, SS = sum of squares, MS = mean squares, * = significant, DAT = days after treatment. The bold values in P value column indicate that the relative individual or interactive effects had significant impact on the mortality of *Tribolium confusum*.

mortality caused by Ankara, Aydin, and Silico-Sec DEs was 64.44%, 78.77% and 76.39% respectively. The results of three-way interaction revealed that the native DE caused higher mortality than commercially available DE Silico-Sec. Therefore, the native DE could be utilized to manage the target pest used in the current study.

Temperature and relative humidity indicated interesting interactions where high temperature and low relative humidity caused higher mortality. Similarly, three-way interactions revealed that the highest dose, high temperature and low relative humidity combination proved more effective in bringing higher mortality. Although higher doses, temperatures and relative humidity levels were not tested in the current study, these need to be tested in future studies.

3.1. F1 productivity

No F1 progeny were recorded in all DEs under all tested doses, temperature, and relative humidity levels. Therefore, the tested DEs have higher efficacy to stop the emergence of F1 productivity. Although mortality did not reach 100% in the tested DE doses, the

Table 4
The impact of individual effects of different temperatures, relative humidity levels, and doses of different diatomaceous earths on the mortality of *Tribolium confusum* at different time intervals.

Treatments	Mortality (%)								
	Ankara DE			Aydın DE			Silico-Sec DE		
	7 days	14 days	21 days	7 days	14 days	21 days	7 days	14 days	21 days
Temperature (°C)									
30	30.55a	39.45a	54.76a	37.49a	48.57a	67.21a	35.86a	46.46a	64.68a
25	27.79b	37.25b	52.66b	34.60b	46.05b	64.83b	33.06b	43.92b	62.47b
LSD 0.05	0.30	0.23	0.38	0.28	0.23	0.41	0.32	0.45	0.34
Relative humidity (%)									
40	31.44a	40.24a	55.41a	38.74a	49.55a	68.15a	37.05a	47.36a	65.62a
60	26.90b	36.46b	52.01b	33.35b	45.07b	63.88b	31.86b	43.02b	61.53b
LSD 0.05	0.31	0.25	0.36	0.30	0.24	0.42	0.33	0.45	0.34
Diatom doses (ppm)									
1000	36.80a	45.23a	61.71a	45.03a	55.81a	76.10a	43.16a	53.81a	73.37a
750	32.16b	40.82b	54.36b	39.76b	50.24b	66.62b	37.87b	48.09b	64.13b
500	25.80c	35.44c	52.49c	32.00c	43.87c	64.48c	30.69c	41.81c	62.07c
250	21.92d	31.90d	46.28d	27.39d	39.31d	56.88d	26.11d	37.05d	54.74d
LSD 0.05	0.42	0.32	0.51	0.40	0.32	0.59	0.45	0.64	0.48

Any two means sharing a letter in common are statistically non-significant ($p > 0.05$).

Table 5
The impact of two-way interactions of different temperatures, relative humidity levels and doses of three diatomaceous doses on the mortality of *Tribolium confusum* at different time intervals.

Treatments	Mortality (%)								
	Ankara DE			Aydin DE			Silico-Sec		
	7 days	14 days	21 days	7 days	14 days	21 days	7 days	14 days	21 days
	Temperature × Relative humidity								
T_1R_1	29.80b	39.00b	54.11b	37.04b	48.02b	66.73b	35.41b	45.72b	64.30b
T_1R_2	25.79d	35.49d	51.20d	32.17d	44.07d	62.93d	30.71d	42.12d	60.65d
T_2R_1	33.09a	41.48a	56.72a	40.44a	51.08a	69.58a	38.70a	49.01a	66.95a
T_2R_2	28.01c	37.42c	52.81c	34.54c	46.07c	64.83c	33.01c	43.91c	62.41c
LSD 0.05	0.44	0.34	0.51	0.40	0.32	0.59	0.45	0.64	0.48
	Temperature × Diatom doses								
T_1D_1	20.61h	30.65h	44.92g	26.10h	38.04h	55.32g	24.83h	35.46h	53.36g
T_1D_2	24.58f	34.19f	51.85e	30.50f	42.61f	63.50e	29.40f	40.58f	61.20e
T_1D_3	30.99d	40.12d	53.43d	38.44d	49.27d	65.34d	36.66d	47.15d	63.03d
T_1D_4	34.99b	44.03b	60.43b	43.38b	54.28b	75.16b	41.34b	52.49b	72.30b
T_2D_1	23.23g	33.16g	47.65f	28.68g	40.59g	58.44f	27.38g	38.63g	56.12f
T_2D_2	27.02e	36.70e	53.13d	33.51e	45.13e	65.45d	31.98e	43.05e	62.94d
T_2D_3	33.34c	41.51c	55.30c	41.08c	51.22c	67.89c	39.08c	49.04c	65.23c
T_2D_4	38.62a	46.43a	62.98a	46.68a	57.35a	77.05a	44.98a	55.13a	74.44a
LSD 0.05	0.58	0.45	0.53	0.57	0.46	0.83	0.64	0.90	0.68
	Relative humidity × Diatom doses								
R_1D_1	24.15e	34.06f	48.28g	30.14e	42.11f	59.22g	28.80e	39.32f	57.22g
R_1D_2	27.99d	37.27e	54.09d	34.50d	45.95e	66.70d	33.00d	44.02e	63.93d
R_1D_3	34.06b	42.55c	55.87c	42.09b	52.35c	68.40c	40.07b	50.06c	65.77c
R_1D_4	39.58a	47.08a	63.42a	48.22a	57.80a	78.30a	46.35a	56.05a	75.58a
R_2D_1	19.70f	29.74g	44.29h	24.64g	36.52g	54.54h	23.41f	34.77g	52.26h
R_2D_2	23.61e	33.62f	50.89f	29.50f	41.78f	62.25f	28.38e	39.60f	60.21f
R_2D_3	30.27c	39.08d	52.86e	37.43c	48.14d	64.83e	35.67c	46.13d	62.49e
R_2D_4	34.03b	43.38b	59.99b	41.84b	53.83b	73.91b	39.97b	51.56b	71.16b
LSD 0.05	0.60	0.47	0.72	0.58	0.46	0.84	0.64	0.90	0.68

Here, T_1 = 25 °C, T_2 = 30 °C, R_1 = 40% relative humidity, R_2 = 60% relative humidity, D_1 = 250 ppm, D_2 = 500 ppm, D_3 = 750 ppm, D_4 = 1000 ppm. Any two means sharing a letter in common are statistically non-significant ($p > 0.05$).

F1 emergence was completely retarded. This indicates that the application of DEs would not cause mortality to existing individuals but also retard the emergence of F1 progeny.

4. Discussion

Insecticidal activity of two native (Ankara, Aydin) and a commercial (Silico-Sec) DEs was tested against confused flour beetle adults in stored wheat at two different temperatures and relative humidity levels and five different doses. The activities of all DEs decreased with increased humidity, whereas increased with the increase in temperature. This result demonstrates that insecticidal efficacy of DEs depends on the circumstances under which the grains are stored (Sousa et al., 2013). The mortality rate significantly increased with increasing temperature and was greater at 30 °C than at 25 °C. The native DE Aydin caused higher mortality than Ankara and Silico-Sec DEs. Aydin DE showed higher insecticidal effect at 30 °C than rest of the DEs. According to prior research on the effect of temperature on the effectiveness of various locally produced and commercially available DEs against storage pests (*S. oryzae*, *T. confusum*, and *R. dominica*), increasing temperature increased the insecticidal efficiency against *S. oryzae* (Arthur, 2002; Athanassiou et al., 2005; Fields and Korunic, 2000; Rojht et al., 2010; Şen et al., 2019; Vassilakos et al., 2006).

Table 6
The impact of three-way interactions of different temperatures, relative humidity, and doses of diatomaceous earths on the mortality of *Tribolium confusum* at different time intervals.

Treatments	Mortality (%)								
	Ankara DE			**Aydin DE**			**Silico-Sec DE**		
	7 days	14 days	21 days	7 days	14 days	21 days	7 days	14 days	21 days
$T_1R_1D_1$	22.60i	32.60k	46.28j	28.70i	40.54l	56.89j	27.37j	37.13j	55.28j
$T_1R_1D_2$	26.46g	36.00i	53.32f	32.35g	44.34i	65.89f	31.35g	42.57g	62.92g
$T_1R_1D_3$	32.61d	41.61f	54.44e	40.62d	51.18f	66.31f	38.60d	48.74e	64.21f
$T_1R_1D_4$	37.53b	45.78b	62.41b	46.48b	56.04b	77.84a	44.31b	54.44b	74.77b
$T_1R_2D_1$	18.63k	28.70m	43.56l	23.50k	35.54n	53.76l	22.29l	33.80l	51.44l
$T_1R_2D_2$	22.70i	32.37k	50.39hi	28.64i	40.87l	61.12i	27.46j	38.60i	59.49i
$T_1R_2D_3$	29.37f	38.63h	52.42fg	36.26f	47.35h	64.36gh	34.72f	45.56f	61.85h
$T_1R_2D_4$	32.46d	42.28e	58.46c	40.28d	52.52e	72.49c	38.37d	50.54d	69.83d
$T_2R_1D_1$	25.70g	35.53i	50.28i	31.58g	43.68j	61.56i	30.23h	41.51gh	59.15i
$T_2R_1D_2$	29.53f	38.53h	54.86e	36.66f	47.56h	67.52e	34.65f	45.48f	64.94f
$T_2R_1D_3$	35.51c	43.49d	57.30d	43.56c	53.51d	70.48d	41.53c	51.37cd	67.32e
$T_2R_1D_4$	41.63a	48.39a	64.44a	49.96a	59.57a	78.77a	48.39a	57.67a	76.39a
$T_2R_2D_1$	20.77j	30.79l	45.02k	25.79j	37.51m	55.32k	24.54k	35.75k	53.08k
$T_2R_2D_2$	24.52h	34.87j	51.40gh	30.36h	42.70k	63.38h	29.31i	40.61h	60.94h
$T_2R_2D_3$	31.17e	39.54g	53.31f	38.61e	48.93g	65.31fg	36.62e	46.70f	63.13g
$T_2R_2D_4$	35.61c	44.48c	61.52b	43.39c	55.14c	75.33b	41.58c	52.59c	72.50c
LSD	0.85	0.65	1.02	0.81	0.65	1.18	0.91	1.28	0.96

Here, T_1 = 25 °C, T_2 = 30 °C, R_1 = 40% relative humidity, R_2 = 60% relative humidity, D_1 = 250 ppm, D_2 = 500 ppm, D_3 = 750 ppm, D_4 = 1000 ppm. Any two means sharing a letter in common are statistically non-significant ($p > 0.05$).

The current study revealed that the toxicity of Aydin DE increased with increasing temperature. In fact, higher mortality was noted just after 7 days of exposure, at 30 °C than 25 °C. This may be explained by the fact that in warmer temperatures insects are often more movable and have a greater likelihood of exposure to dust particles (Arthur, 2000b, 2000a; Fields and Korunic, 2000; Rigaux et al., 2001). Additionally, higher temperatures are likely to result in greater water loss (Arthur, 2000b, 2000a; Fields and Korunic, 2000; Rigaux et al., 2001). Similar to this Arthur (2000b) discovered that Protect-It DE increased mortality of *T. confusum* and *T. castaneum* when temperature increased from 22 °C to 27 °C and 32 °C.

The current study indicated that exposure duration, dose, and DE formulation had significant impact on the effectiveness of all DEs. The highest insecticidal efficacy was noted with the highest dose, i.e., 1000 ppm. Previous studies have found similar findings (Alkan et al., 2019; Arnaud et al., 2005; Arthur, 2000c; Kılıc and Mutlu, 2020; Korunic, 1998; Şen et al., 2019; Shams, 2011; Vayias et al., 2006).

Increasing dose and time after DEs' application significantly increased mortality rate. Mortality was strongly influenced by dose, temperature, and exposure interval. Mortality was higher at longer exposure intervals. The efficacy of all evaluated combinations of temperature, humidity and DE dose reduced with increase in relative humidity. The quick water loss in adults with a broken cuticle layer brought on by DEs at low humidity is assumed to be the cause of this scenario. It is believed that improved efficacy with increasing temperature is because pests are exposed to more DEs due to their increased movement with rising temperatures and more water loss (Arthur, 2000a, 2000c; Fields and Korunic, 2000; Rigaux et al., 2001). Additionally, higher temperatures are likely to result in greater water loss (Arthur, 2000a, 2000b; Fields and Korunic, 2000).

Although tested DEs had varying particle sizes, they exhibited varying insecticidal efficacies. The Aydin DE caused the highest mortality followed by Silico-Sec and Ankara DEs. The findings showed that the effectiveness of DEs varies depending on the origin. The fact that DEs from various geological regions have varying efficacies and those from maritime areas are less effective has also been described by Golob (1997) and KoruniĆ (1997). In addition, many studies reported significant insecticidal changes between DEs extracted from different geographical regions and mines. Korunic, (1998) investigated insecticidal efficacy and bulk density of 25 different DEs in stored products and reported that different DEs had varying insecticidal activity and bulk density. It seems that variations in the morphological and physical properties of numerous DE formulations are likely to have diverse effects (Fields and Korunic, 2000; Subramanyam and Hagstrum, 2012). Likewise, Kavallieratos et al. (2007) stated that the effectiveness of DEs was significantly altered by formulation type and temperature.

In the current study, no new generation adult emergence was recorded from the wheat grains treated with tested DEs. The confused flour beetle individuals are secondary pests, they need damaged or broken grains for their feeding. The inability to obtain a new generation adult is thought to be due to this reason. Like this study, Kavallieratos et al. (2007), tested DEs formulations (Insecto and Silico-Sec) at 500, 1000 and 1500 ppm doses against *S. oryzae*, *R. dominica* and *T. confusum* under different insect density, wheat amount and broken grain ratio. Significant differences were noted for mortality among DEs formulations, insect density, wheat content, and broken grain ratio. Similarly, F1 generation adults were unable to emerge. Kabir (2013), on the other hand, found 16% reproductive efficiency of *T. castaneum* in the control group for F1 yield after 40-day storage period at 26 and 32 °C and 48–65% relative humidity.

The fact that DEs are natural substances harmless to human and environmental health, does not cause residue problems in the product, making its use as an insecticide very attractive. The results presented in this study suggest that 1500 ppm dose of Aydin DE with high temperatures can be recommended to control *T. confusum* with 7 days exposure time.

5. Conclusion

All DEs used in the current study caused greater mortality under 30 °C temperature, 40% relative humidity and 1000 ppm dose. The local DE Aydin and commercial DE Silico-Sec caused almost equivalent mortalities. Overall, the highest mortality was observed with 1000 ppm dosage of all DEs under 30 °C temperature and 40% relative humidity. Therefore, the DEs must be used under these environmental circumstances for gaining increased effectiveness. Furthermore, the local DE Aydin might be applied to control confused flour beetle in the warehouses and used commercially. The structural changes in the insects were not explored in the current study; therefore, these should be inferred in the future studies.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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