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Residual Toxicity of Iranian Diatomaceous Earth against *Rhyzopertha dominica* and *Tribolium confusum* on Concrete, Galvanized Steel, and Mosaic Surfaces

N. Delgarm[1], and M. Ziaee[1*]

ABSTRACT

Diatomaceous Earths (DEs) have a long history for on-farm and commercial grain storage, hygiene, and structural treatment. Structural treatments by DEs have shown to be useful for eradication of residual insect infestations in storage facilities. In this study, the residual toxicity of different DE formulations was examined against adults of *Rhyzopertha dominica* (F.) (Coleoptera: Bostrychidae) and *Tribolium confusum* Jacquelin du Val (Coleoptera: Tenebrionidae) on different surfaces including concrete, galvanized steel, and mosaic. The surfaces were treated with 0.2 mg cm^{-2} concentration of DE formulations that included SilicoSec®, Protect-It, and an Iranian DE formulated from a Mamaghan Mine, Iran, supplemented with an amorphous silica gel product to enhance efficacy. The residual toxicity of DEs was assessed at 7, 15, 30, 45, and 60 days post-treatment. The mortality was determined after 1, 3, 5, and 7 days of insects' exposure to each surface. According to the results, the most effective product proved to be SilicoSec® when compared to Protect-It and the Iranian Mamaghan DE. The toxicity and persistence of DE formulations were higher on the galvanized steel compared to that achieved on the concrete and mosaic surfaces. Nevertheless, the results demonstrated that an Iranian DE containing 10% locally available amorphous silica is capable of controlling *R. dominica* and *T. confusum* in warehouses and other storage facilities. However, additional studies are needed to confirm these findings.

Keywords: Grain storage facilities, Mamaghan Mine, Surface treatment, Warehouse hygiene

INTRODUCTION

Stored-product insects' damage to cereal grains in storage severely reduces the quantity, quality, and consumption value of grains (Hill, 2002). In addition, the insects' feces, exuviae, and body parts also contaminate food and reduce the products marketability (Lord, 2004). Two of the most important insect species commonly found within stored cereal grains worldwide are *Rhyzopertha dominica* (F.) (Coleoptera: Bostrychidae) and *Tribolium confusum*, Jacquelin du Val. (Coleoptera: Tenebrionidae). *Rhyzopertha dominica* is a significant insect pest with both adult and larvae being voracious feeders. This pest attacks cereals like wheat, corn, and rice. Their infestations diminish stored grain quality and quantity (Mahroof and Hagstrum, 2012a). *Tribolium confusum* is also a worldwide insect species that is a secondary pest of stored food and feed grains. Both larvae and adults are destructive and infest broken damaged grains and milled products (Mahroof and Hagstrum, 2012b).

Hygiene in grain silos and other bulk storage facilities is an essential and first stage in insect pest control for storage facilities. Hygiene involves physical cleaning followed

[1] Department of Plant Protection, Faculty of Agriculture, Shahid Chamran University of Ahvaz, Ahvaz, Islamic Republic of Iran.
*Corresponding author; e-mail: m.ziaee@scu.ac.ir

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up by the application of insecticidal sprays or dusts to storage structures and surfaces. Due to the side effects of synthetic insecticides, the use of safe and natural compounds seems to be of high importance (Jessica *et al*., 2019). Diatomaceous earths is an appropriate treatment that maintain their efficacy for significantly longer terms than chemical insecticides for this application (Opit *et al*., 2012). Diatomaceous earth is an inert dust; it contains fossilized diatoms, single-celled algae. Essentially, it is amorphous silicon dioxide with an internal structure that is very effective in the absorption of insects' protective cuticle wax layer, leading to insects' death through desiccation and, to much lesser extent, by abrasion (Korunic, 2013). Several DE formulations have been registered for surface treatments and the effectiveness of DEs has been documented against different stored products insect pests (Shah and Khan, 2014). The effectiveness of DE formulations was enhanced by combination with different additives including silica aerogel in Protect-it (Korunic and Fields, 1998), Fossilshield® (Mewis and Ulrichs, 2001), and Probe-A® DE formulations (Badii *et al*., 2014), and food grade in Insecto® (Subramanyam *et al*., 1994). Plant extracts have also been employed in DEBBM (Athanassiou and Korunic, 2007), and F_1H and F_2H DE formulations (Liška *et al*., 2018), and chemical insecticides including abamectin in DEA (Athanassiou and Korunic, 2007), and deltamethrin in Mamaghan DE formulation (Delgarm *et al*., 2020).

Several studies have demonstrated the insecticidal potential of local DEs from different geographic locations including Africa (Machekano *et al*., 2017; Mvumi *et al*., 2006), South-Eastern Europe (Vayias *et al*., 2009), Serbia (Andrić *et al*., 2012; Kljajić *et al*., 2010), Greece and Romania (Athanassiou *et al*., 2016), Croatia (Liška *et al*., 2018), Argentina (Dal Bello *et al*., 2018), Egypt (Abd El-Aziz and Abd El-Ghany, 2018), and Turkey (Akçali *et al*., 2018; Gultekin *et al*., 2018; Mortazavi and Ferizil, 2018) for controlling stored-product insects. Iran has large deposits of diatomaceous earth that can be exploited for protection of bulk-grain storage, and structural treatment of empty storages, silos, and handling grain storage systems (Delgarm *et al*., 2020; Ziaee *et al*., 2018; Ziaee and Moharramipour, 2012).

Previous studies on natural DE deposits from Iran have indicated that local DE from Mamaghan Mine was very effective against stored grain beetles in different grain commodities (Delgarm *et al*., 2020; Ziaee *et al*., 2016; Ziaee *et al*., 2018; Ziaee and Moharramipour, 2012; Ziaee *et al*., 2013). However, no study has examined the efficacy of local DEs from Iran as surface treatment. Treating grain silos and other stored-grain facilities with a DE before filling with grains could control and prevent the damage of stored products insect-pests (Shah and Khan, 2014). Thus, we aimed to better understand the activity of a DE formulation based on Mamaghan DE deposit as structural treatment, the residual activity of Mamaghan DE deposit supplemented with amorphous silica gel and compare it with two established commercial DE formulations, namely, SilicoSec® and Protect-It, to control *R. dominica* and *T. confusum*.

MATERIALS AND METHODS

Insects

Laboratory population of *R. dominica* and *T. confusum* were taken from a culture in Entomology Laboratory of Shahid Chamran University, Ahvaz, Khuzestan, Iran, for 3 years with no history of exposure to insecticides. *Rhyzopertha dominica* was reared on whole wheat (Chamran variety) and *T. confusum* was reared on wheat flour plus 5% brewer's yeast (by weight). The insects' culture was kept in incubator at 27°C and 60% RH and held in continuous darkness. Adults with 7-14 day-old and of mixed sexes were used in the experiments.

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Diatomaceous Earth Formulations

Three diatomaceous earths were used in the experiments. SilicoSec®, a freshwater DE formulation was obtained from Biofa GmbH, Munsingen, Germany. SilicoSec® contains 92% SiO_2, 3% Al_2O_3, 1% Fe_2O_3 and 1% Na_2O (Ziaee and Khashaveh, 2007).

Protect-It™, a freshwater DE formulation was obtained from Hedley Technologies Inc., Canada. Protect-It™ is composed of 83.7% DE, 10% silica aerogel, 5.6% Al_2O_3, 2.3% Fe_2O_3, 0.9% CaO, 0.3% MgO, and 1.9% other oxides (e.g. TiO_3, P_2O_3), with 3–5% moisture content (m.c.) (Korunic and Fields, 1998).

Mamaghan DE, a marine DE, was collected from Mamaghan Diatomite Mine, Mamaghan, Iran. It contains 89.9% amorphous silicon dioxide, 1.1% Al_2O_3, 0.85% Fe_2O_3, 0.4% CaO, 0.4% Na_2O, 0.3% MgO, and 6.5% m.c. (Ziaee and Moharramipour, 2012). The proportion of 10% silica aerogel was added to Mamaghan DE and this DE was used in all the experiments.

Surfaces

The concrete, galvanized steel, and mosaic surfaces were used for the experiments. One day before the experiments, the bottoms of the plastic trays (30 cm length and width) were covered with the concrete (Cemex Holdings Philippines, Inc., Makati, Philippines). Liquid slurry was prepared by adding water to concrete, and poured into each plastic tray to a thickness of 10 mm. The trays were kept dry for one day. Galvanized steel and mosaic surfaces were also provided with 30 cm length and 30 cm width.

Bioassay

DE formulations were added by a camel hairbrush (No. 2) at 0.2 mg cm^{-2} on concrete, galvanized steel, and mosaic surfaces. For any type of surface, three surfaces each with three glass rings as sub-replications were used for the experiments. For each post-treatment time, separate surfaces were used in the experiments. The internal walls of rings were covered with paraffin (Parschemical Co., Iran) to prevent insects escape. Before releasing insects in each ring, 2 g of wheat kernels (Chamran variety) was placed inside each glass ring as food source. Ten adults of *R. dominica* or *T. confusum* were released in each glass rings as a replicate. Untreated surfaces were considered as control. Residual toxicity were assessed at 7, 15, 30, 45, and 60 days post-treatment at 27°C, 60% RH, and continuous darkness. For each post-treatment bioassay, separate experiments were carried out. The mortality was counted after 1, 3, 5, and 7 days of exposure on the treated surfaces. Mortality was determined as described by Machekano *et al*. (2017); when none of adults' appendages moved after being pricked three times with a needle, the insects were consider dead.

Statistical Analysis

No mortality was recorded in the control surfaces, so, no correction was performed. The normality test was made using Shapiro-Wilk test. Since the same glass rings were checked for mortality at exposure days, the mortality data were analyzed using a repeated measures ANOVA with exposure time as the repeated measures variable, and DE formulation, post-treatment time, as the main effects. The response variable was insect mortality. Means were compared using Tukey-Kramer's test (HSD) at P= 0.05 significance level (IBMCorp., 2007).

RESULTS

MANOVA parameters indicated that all main effects and their associated interactions were significant for mortality levels of *R. dominica* and *T. confusum* on concrete, galvanized steel, and mosaic surfaces (Table 1).

The mortality of *R. dominica* exposed on concrete treated with 0.2 mg cm^{-2} of different DE formulations is presented in Table 2. In 7-day post-treatment time, the mortality was 71.1%, 62.2%, and 47.7% when adults were exposed for 1 day on concrete treated with SilicoSec®, Protect-It®, and Mamaghan, respectively, and the mortality exceeded to ~ 100% after 3 days exposure time. While the percentage of mortality decreased with increasing post-treatment time, at the 60-day post-treatment period, the adult mortality decreased to 7.7, 6.6, and 3.3%, 1 day after exposure to the concrete surfaces treated with SilicoSec®, Protect-It®, and Mamaghan, respectively (Table 2).

Results obtained for *R. dominica* exposed on galvanized steel treated with 0.2 mg cm^{-2} of different DE formulations are presented in Table 3. At 7-day post-treatment time, the highest adult mortality of 83.3% was found for SilicoSec® after 1 day exposure to steel while complete mortality (100%) was observed after 3 days of exposure. The lowest mortality (31.1%) of *R. dominica* was recorded at 60-day post-treatment time, 1 day after adult's exposure to the galvanized steel treated with 0.2 mg cm^{-2} of Mamaghan DE (Table 3).

In the case of *R. dominica* exposed on mosaic surface, all main effects and associated interactions for mortality levels were significant. The adult mortality 1 day after exposure to mosaic treated with SilicoSec®, Protect-It®, and Mamaghan was 77.7, 70.0 and 58.8% at 7-day post-treatment time, respectively. At 60-day post-treatment time, the lowest *R. dominica* mortality (24.4%) was recorded when adults were exposed for 1 day on mosaic treated with Mamaghan DE. However, the efficacy of Mamaghan DE on *R. dominica* was significantly increased with the period of exposure, and 85.5% mortality was found 7 days after exposure (Table 4).

Results obtained for *T. confusum* exposed on concrete treated with 0.2 mg cm^{-2} of DEs are presented in Table 5. At 7-day post-treatment time, SilicoSec® (53.3%) gave higher mortality levels in comparison with Protect-It® (22.2%), and Mamaghan DE (20%) after 3 days of exposure, while the

Table 1. Repeated measured parameters of MANOVA for the main effects and associated interactions of mortality percentage of *Rhyzopertha dominica* and *Tribolium confusum* (Between exposures error df= 32, Within exposures error df= 256).

Source of variances		*Rhyzopertha dominica*			*Tribolium confusum*		
		Concrete	Steel	Mosaic	Concrete	Steel	Mosaic
	df	F, P	F, P	F, P	F, P	F, P	F, P
Post-treatment time	4	1072.5, < 0.001	500.5, < 0.001	571.4, < 0.001	514.3, < 0.001	297.1, < 0.001	396.9, < 0.001
Formulation	2	229.6, < 0.001	273.8, < 0.001	128.2, < 0.001	95.7, < 0.001	219.0, < 0.001	63.1, < 0.001
Exposure time	3	2604.9, < 0.001	1535.5, < 0.001	2930.5, < 0.001	6379.6, < 0.001	4411.4, < 0.001	7438.9, < 0.001
Post-treatment time× Formulation	8	11.2, < 0.001	8.5, < 0.001	7.7, < 0.001	2.4, < 0.001	12.6, < .001	4.3, < 0.001
Post-treatment time× Exposure time	12	94.2, , < 0.001	80.1, < 0.001	90.2, < 0.001	82.9, < 0.001	41.4, < 0.001	73.7, < 0.001
Formulation× Exposure time	6	9.1, < 0.001	33.1, < 0.001	31.6, < 0.001	12.4, < 0.001	26.9, < 0.001	7.6, < 0.001
Post-treatment time× Formulation× Exposure time	24	7.2, < 0.001	4.2, < 0.001	7.3, < 0.001	3.9, < 0.001	6.3, < 0.001	4.3, < 0.001

Table 2. Mean mortality percentage (±SE) of *Rhyzopertha dominica* exposed on concrete treated with 0.2 mg cm^{-2} of different DE formulations. [a]

Exposure time (day) Formulation	Post-treatment time (Day) 7	15	30	45	60
1 Day of exposure					
SilicoSec®	71.1±2.6aA	51.1±2.0aB	34.4±1.7aC	21.1±2.6D	7.7±3.2E
Protect-It®	62.2±2.7aA	45.5±1.7aB	28.8±2.0bC	18.8±2.0D	6.6±2.8E
Mamaghan	47.7±2.7bA	35.5±1.7bB	23.3±1.6cB	15.5±1.7C	3.3±1.6D
3 Days of exposure					
SilicoSec®	100.0±0.0A	88.8±2.6aB	65.5±1.7aC	57.7±2.7aC	45.5±2.4aD
Protect-It®	100.0±0.0A	81.1±2.6abB	48.8±2.0bC	38.8±2.0bD	26.6±1.6bE
Mamaghan	97.7±1.4A	74.4±2.9bB	42.2±2.2bC	30.0±2.3cD	24.4±1.7bD
5 Days of exposure					
SilicoSec®	100.0±0.0 aA	100.0±0.0 aA	97.7±1.4aA	87.7±2.7aB	77.7±2.2aC
Protect-It®	100.0±0.0 aA	100.0±0.0 aA	93.3±2.3abB	68.8±2.0bC	56.6±1.6bD
Mamaghan	100.0±0.0 aA	100.0±0.0 aA	86.6±2.3 bB	61.1±3.5bC	51.1±2.6bD
7 Days of exposure					
SilicoSec®	100.0±0.0	100.0±0.0	100.0±0.0	100.0±0.0	100.0±0.0
Protect-It®	100.0±0.0	100.0±0.0	100.0±0.0	100.0±0.0	100.0±0.0
Mamaghan	100.0±0.0	100.0±0.0	100.0±0.0	100.0±0.0	100.0±0.0

[a] (A-E and **a-c)** For each exposure time separately, means within each columns followed by the same lower case letter and upper case letter in each row are not significantly different using Tukey-Kramer (HSD) test at P> 0.05. Where no letters exist, no significant differences were noted.

Table 3. Mean mortality percentage (±SE) of *Rhyzopertha dominica* exposed on galvanized steel treated with 0.2 mg cm^{-2} of different DE formulations.

Exposure time (Day) Formulation	Post-treatment time (Day) 7	15	30	45	60
1 Day of exposure					
SilicoSec®	83.3±1.6aA	74.4±1.7aB	65.5±1.7aC	54.4±1.7aD	45.5±1.7aE
Protect-It®	75.5±1.7bA	67.7±2.2aA	55.5±1.7bB	46.6±2.3bC	38.8±2.0aC
Mamaghan	64.4±1.7cA	55.5±1.7bB	44.4±1.7cC	37.7±1.4cCD	31.1±2.0bD
3 Days of exposure					
SilicoSec®	100.0±0.0A	96.6±1.6AB	88.8±2.6aB	70.0±2.8aC	61.1±2.6aC
Protect-It®	100.0±0.0A	92.2±2.7A	71.1±2.6bB	60.0±2.8bC	52.2±2.2bC
Mamaghan	100.0±0.0A	87.7±4.0B	62.2±2.7bC	46.6±2.3cD	35.5±1.7cE
5 Days of exposure					
SilicoSec®	100.0±0.0A	100.0±0.0A	100.0±0.0A	95.5±1.7aAB	91.1±2.6aB
Protect-It®	100.0±0.0A	100.0±0.0A	97.7±1.4A	87.7±3.6abB	80.0±2.3bB
Mamaghan	100.0±0.0A	100.0±0.0A	97.7±1.4A	78.8±2.6bB	66.6±3.3cC
7 Days of exposure					
SilicoSec®	100.0±0.0	100.0±0.0	100.0±0.0	100.0±0.0	100.0±0.0 a
Protect-It®	100.0±0.0	100.0±0.0	100.0±0.0	100.0±0.0	100.0±0.0a
Mamaghan	100.0±0.0A	100.0±0.0A	100.0±0.0A	98.8±1.1AB	94.4±2.4bB

(A-E and a-c) For each exposure time separately, means within each columns followed by the same lower-case letter and upper-case letter in each row are not significantly different using Tukey-Kramer (HSD) test at P> 0.05.

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Table 4. Mean mortality percentage (±SE) of *Rhyzopertha dominica* exposed on mosaic treated with 0.2 mg cm^{-2} of different DE formulations.

Exposure time (Day)	Post-treatment time (Day)				
Formulation	7	15	30	45	60
1 Day of exposure					
SilicoSec®	77.7±2.2aA	63.3±1.6aB	54.4±1.7aC	44.4±1.7aD	34.4±1.7aE
Protect-It®	70.0±2.3aA	62.2±2.2aA	50.0±3.3abB	36.6±1.6bC	25.5±1.7bD
Mamaghan	58.8±2.6bA	53.3±1.6bA	44.4±1.7 bB	35.5±1.7bC	24.4±1.7bD
3 Days of exposure					
SilicoSec®	100.0±0.0A	94.4±2.4AB	86.6±2.3aB	71.1±3.0aC	52.2±2.2aD
Protect-It®	100.0±0.0A	91.1±3.0B	76.6±1.6bC	52.2±2.2bD	43.3±1.6bE
Mamaghan	100.0±0.0A	87.7±2.7B	58.8±2.6cC	40.0±2.8cD	28.8±2.6cE
5 Days of exposure					
SilicoSec®	100.0±0.0A	100.0±0.0aA	100.0±0.0aA	97.7±1.4 aA	85.5±1.7aB
Protect-It®	100.0±0.0A	100.0±0.0aA	100.0±0.0aA	82.2±2.2bB	72.2±1.4bC
Mamaghan	100.0±0.0A	100.0±0.0aA	94.4±2.4bA	67.7±3.2cB	58.8±3.5cB
7 Days of exposure					
SilicoSec®	100.0±0.0	100.0±0.0	100.0±0.0	100.0±0.0a	100.0±0.0a
Protect-It®	100.0±0.0	100.0±0.0	100.0±0.0	100.0±0.0a	97.7±1.4a
Mamaghan	100.0±0.0A	100.0±0.0A	100.0±0.0A	94.4±2.4bA	85.5±2.9 bB

[a](A-E and a-c) For each exposure time separately, means within each columns followed by the same lower case letter and upper case letter in each row are not significantly different using Tukey-Kramer (HSD) test at P> 0.05.

mortality reached 100% of all three DE formulations after 7 days of exposure. However, the efficacy declined with post-treatment time, and at 60-day post-treatment, there were no *T. confusum* mortality after 1 day of exposure, and mortality did not exceed 73.3% for SilicoSec®, 62.2, and 58.9% for Protect-It®, and Mamaghan, respectively (Table 5).

The lowest mortality was found 1 day after *T. confusum* exposure to galvanized steel treated with DE formulations, which did not

Table 5. Mean mortality percentage (±SE) of *Tribolium confusum* exposed on concrete treated with 0.2 mg cm^{-2} of different DE formulations.[a]

Exposure time (Day)	Post-treatment time (Day)				
Formulation	7	15	30	45	60
1 Day of exposure					
SilicoSec®	3.3±1.7	2.2±1.5	0.0±0.0	0.0±0.0	0.0±0.0
Protect-It®	2.2±1.5	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0
Mamaghan	2.2±1.5	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0
3 Days of exposure					
SilicoSec®	53.3±2.4aA	26.7±2.4aB	18.9±2.6aCD	12.2±2.2D	11.1±2.6D
Protect-It®	22.2±2.2bA	14.4±1.7bAB	12.2±2.2abB	8.9±2.6B	6.7±2.4B
Mamaghan	20.0±2.3bA	14.4±1.7bAB	10.0±2.4bBC	7.8±2.2 BC	5.5±1.7C
5 Days of exposure					
SilicoSec®	95.5±1.7aA	73.3±2.4aB	65.6±2.9aB	45.5±1.7C	43.3±1.7aC
Protect-It®	84.4±2.9bA	62.2±1.5bB	58.9±2.6abB	40.0±2.9C	35.6±1.7bC
Mamaghan	81.1±2.6bA	58.9±2.6bB	53.3±1.7bB	37.8±2.2C	32.2±2.2bC
7 Days of exposure					
SilicoSec®	100.0±0.0A	100.0±0.0A	97.7±1.5A	74.4±1.7aB	73.3±1.7aB
Protect-It®	100.0±0.0A	97.7±1.5A	94.4±2.4 A	67.8±4.0abB	62.2±2.8bB
Mamaghan	100.0±0.0A	95.5±1.7A	93.3±2.4A	64.4±1.7bB	58.9±2.6bB

[a] (A-D and a-b) For each exposure time separately, means within each columns followed by the same lower case letter and upper case letter in each row are not significantly different using Tukey-Kramer (HSD) test at P> 0.05.

exceed 6% in all three DE formulations. After 7 days of exposure, all three tested DEs caused significantly higher mortality of *T. confusum.* For galvanized steel treated with SilicoSec®, complete mortality was recorded during the 45-d post-treatment period. However, the efficacy of all tested DE formulations declined slightly with post-treatment time, such that at 60-day post-treatment time 97.8, 72.2, and 68.9% mortality was observed after 7 days of exposure to SilicoSec®, Protect-It®, and Mamaghan DE, respectively (Table 6).

SilicoSec® applied on mosaic surfaces was more effective against *T. confusum* adults. Similar to that recorded for concrete and galvanized steel, the residual toxicity of all three tested DEs on mosaic surfaces declined during the post-treatment period. At 60-day post-treatment time, the insecticidal efficacy of SilicoSec®, Protect-It®, and Mamaghan DE was reduced by 83.3, 65.5, and 63.3% after 7 days of exposure, respectively (Table 7).

DISCUSSION

As in previous experiments using four Iranian DE deposits to control three stored product insects, Mamaghan DE proved to be the most effective Iranian DE against insect species including *Sitophilus oryzae* (L.) (Coleoptera: Curculionidae), *Tribolium castaneum* (Herbst) (Coleoptera: Tenebrionidae), and *T. confusum* (Ziaee *et al*., 2018). Also, in agreement with previous studies of commercial DE formulations, SilicoSec® (Collins and Cook, 2006; Delgarm *et al*., 2020; Mortazavi and Ferizil, 2018; Schöller and Reichmuth, 2010) and Protect-It (Kavallieratos *et al*., 2012; Perišić *et al*., 2018; Timlick and Fields, 2010) are recognized as being two of the most effective DE formulations. The results of this study confirm SilicoSec® as being the most efficacious DE for the control of insect species on all three treated test surfaces. According to Athanassiou *et al*. (2011), SilicoSec® applied as a surface treatment on glass Petri dishes at application rates of 5, 10 and 20 g m^{-2} was more effective than DEs originating from central and southeastern Europe against *S. oryzae, R. dominica* and *T. confusum.* Bohinc *et al*. (2018) reported that the highest mortality levels of *Sitophilus zeamais* Motschulsky (Coleoptera:

Table 6. Mean mortality percentage (±SE) of *Tribolium confusum* exposed on galvanized steel treated with 0.2 mg cm^{-2} of different DE formulations.

Exposure time (Day)	Post-treatment time (Day)				
Formulation	7	15	30	45	60
1 Day of exposure					
SilicoSec®	5.5±1.7A	3.3±1.7AB	2.2±1.5AB	0.0±0.0B	0.0±0.0B
Protect-It®	4.4±1.5A	1.1±1.1AB	0.0±0.0B	0.0±0.0B	0.0±0.0B
Mamaghan	3.3±1.7A	0.0±0.0B	0.0±0.0B	0.0±0.0B	0.0±0.0B
3 Days of exposure					
SilicoSec®	53.3±2.3aA	45.5±2.4aAB	40.0±2.3aBC	32.2±2.2aCD	27.7±2.2aD
Protect-It®	48.9±2.6aA	28.9±3.0bB	21.1±2.0bBC	17.8±2.2bC	13.3±2.3bC
Mamaghan	32.2±2.2bA	21.1±2.6bB	16.7±3.3bBC	11.1±2.6bBC	8.9 ± 2.6bC
5 Days of exposure					
SilicoSec®	100.0±0.0aA	93.3±2.9aAB	90.0±2.3aB	76.6±1.7aC	68.9±2.6aC
Protect-It®	97.7±1.5abA	77.7±3.2bB	65.5±3.4bC	47.8±2.2bD	42.2±2.2bD
Mamaghan	93.3±2.4bA	71.1±2.6bB	62.2±3.6bB	45.5±2.9bC	37.7±2.2bC
7 Days of exposure					
SilicoSec®	100.0±0.0	100.0±0.0	100.0±0.0a	100.0±0.0a	97.8±1.5a
Protect-It®	100.0±0.0A	100.0±0.0A	97.7±1.5A	76.6±2.3bB	72.2±2.2bB
Mamaghan	100.0±0.0A	97.7±1.5A	95.5±1.7A	75.5±2.9bB	68.9±2.6bB

[a](A-D and a-b) For each exposure time separately, means within each columns followed by the same lower case letter and upper case letter in each row are not significantly different using Tukey-Kramer (HSD) test at P> 0.05. Where no letters exist, no significant differences were noted.

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Table 7. Mean mortality percentage (±SE) of *Tribolium confusum* exposed on mosaic treated with 0.2 mg cm^{-2} of different DE formulations. [a]

Exposure time (Day)	Post-treatment time (Day)				
Formulation	7	15	30	45	60
1 Day of exposure					
SilicoSec®	4.4±1.7A	0.0±0.0B	0.0±0.0B	0.0±0.0B	0.0±0.0B
Protect-It®	3.3±1.6A	0.0±0.0B	0.0±0.0B	0.0±0.0B	0.0±0.0B
Mamaghan	2.2±1.4	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0
3 Days of exposure					
SilicoSec®	35.5±1.7aA	27.7±2.2aAB	22.2±2.2aB	18.8±2.6aBC	12.2±2.2aC
Protect-It®	32.2±2.2aA	16.6±1.6bB	16.6±2.8abB	13.3±2.3abB	11.1±2.6B
Mamaghan	22.2±3.2bA	17.7±2.2bAB	12.2±2.2bBC	10.0±2.3bBC	6.6±1.6C
5 Days of exposure					
SilicoSec®	100.0±0.0aA	83.3±2.8aB	67.7±2.2aC	55.5±2.4aD	47.7±2.2aD
Protect-It®	95.5±1.7aA	67.7±2.7bB	64.4±3.3B	43.3±1.6bC	41.1±2.6aC
Mamaghan	86.6±3.3bA	64.4±2.9bB	61.1±2.6B	40.0±2.3bC	32.2±2.2bC
7 Days of exposure					
SilicoSec®	100.0±0.0A	100.0±0.0A	100.0±0.0aA	91.1±2.6aB	83.3±1.6aC
Protect-It®	100.0±0.0A	97.7±1.4A	96.6±1.6A	67.7±2.2bB	65.5±2.4bB
Mamaghan	100.0±0.0A	96.6±1.6A	95.5±1.7A	65.5±2.4bB	63.3±1.6bB

[a](A-D and a-b) For each exposure time separately, means within each columns followed by the same lower case letter and upper case letter in each row are not significantly different using Tukey-Kramer (HSD) test at P> 0.05.

Curculionidae) were noted in plastic Petri dishes treated with SilicoSec® compared to those achieved with three different wood ashes, and this could be in accordance to SiO_2 content of SilicoSec®. Athanassiou *et al*. (2018) reported that adults and larvae of *T. confusum* showed favorable preference to Insecto and SilicoSec® DE-treated surfaces. Therefore, the insects' attracted to the SilicoSec® treated surface increases the insects' exposure time, resulting in more DE particles adhesion to their cuticle and higher mortality.

The susceptibility levels of the insect species varied depending upon the DE formulations to which they were exposed. This should be taken into account when selecting an appropriate DE formulation that is effective against the dominant insect species occurring in the grain storage facilities. This study demonstrated that *T. confusum* was less susceptible to DE- treated surfaces than *R. dominica*. Fields and Korunic (2000) stated that *Cryptolestes ferrugineus* (Stephens) (Coleoptera: Laemophloeidae) was the most susceptible stored-product beetle to various DE formulations including Protect-It, Dryacide®, and Insecto®. Subsequently, *Oryzaephilus surinamensis* (L.) (Coleoptera: Silvanidae) and *S. oryzae* were more resistant to *C. ferrugineus*. Although, *R. dominica* and *Tribolium* spp. showed the highest tolerance to the DE formulations, the resistance of *R. dominica* to DEs could be due to the behavior of this insect that feeds and acts within the seed. In the case of *T. castaneum*, the tolerance of the species could be due to the cuticle properties of the insect, which has less DE particles adhesion to its cuticle. Our results were consistent with Toews *et al*. (2003), where the tolerance of *T. castaneum* to spinosad on concrete was greater among eight stored-product beetles. Collins and Cook (2006) evaluated the insecticidal efficacy of DE SilicoSec® and Diasecticide™ on the wooden surfaces and reported that *O. surinamensis* was very susceptible, *Sitophilus granarius* (L.) (Coleoptera: Curculionidae) was moderately susceptible,

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while *T. castaneum* was the least susceptible.

Apart from insect species, the type, characterization and composition of surface influence the effectiveness of the insecticides applied against stored product beetles (Arthur *et al*., 2018). In our study, the mortality of both tested species observed on galvanized steel and concrete was higher than the mosaic. It has been noted that residual toxicity of DE formulations and synthetic insecticides is affected by physical properties of the surfaces (Arthur *et al*., 2018; Gowers and le Patourel, 1984; Schöller and Reichmuth, 2010; Vassilakos *et al*., 2014). Gowers and le Patourel (1984) noted that the insects pick-up more dust particles in flat surfaces. However, insects' behavior can also be useful in preventing them from contacting DE particles on some surfaces. It appears that on wooden surfaces, insects are more easily able to stand as soon as they fall onto the surface, which reduces the number of adhering particles to the body (Collins and Cook, 2006). Arthur (2008) found that nonporous surfaces like metal, tile and glass petri dishes are more persistent to chlorfenapyr compared with porous surfaces such as concrete against adult *T. castaneum* and *T. confusum.* Vassilakos *et al*. (2014) reported that activity of spinetoram against *T. confusum* adults was higher on concrete and galvanized steel than the other tested surfaces, namely, ceramic tile and plywood; indicating that activity depends on the physical properties of the surfaces. The higher insect mortality on concrete can be due to the insect's inability to recover on this surface (Toews *et al*., 2003). In addition, most synthetic insecticides degrade over storage time and lose their insecticidal activity when applied to different surfaces such as concrete (Wijayaratne *et al*., 2012). However, DEs are physically stable with long-lasting effect for controlling stored products insects (Korunic, 2013). The high stability of DE formulations protects different surfaces for longer terms and reduces the need for re-treatment, making them more cost-effective than the more susceptible chemical treatments (Collins and Cook, 2006).

It was apparent that the effectiveness of DE formulations declined at a 60-d post-treatment period, and it was more evident in the case of concrete surface. Similar results were observed when methoprene was applied on the concrete surface against larvae of *T. castaneum.* This may be a result of the high porosity of the concrete that adsorbs the insecticidal agent or, perhaps, a chemical reaction between the concrete and the chemical reagent that results in the decline of residual toxicity of the insecticide with time (Wijayaratne *et al*., 2012). The interactions of the insecticide particles with concrete surface lead to more absorbance of the residues form the surface (Arthur, 2008). Our findings are in accordance with their results and the decrease in DE effectiveness on concrete over time can be related to the characteristics and composition of the materials used in concrete surface. The results of this study show that Mamaghan DE formulation at 0.2 mg cm^{-2} gave satisfactory control on concrete and steel surfaces that are commonly used in grain storage silos and warehouses. Further studies under farm scale conditions is required to confirm the results.

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سمیت باقیمانده خاک دیاتومه ایرانی علیه سوسک کشیش، *Rhyzopertha domica* و شپشه آرد، *Tribolium confusum* روی سطح‌های بتن، استیل گالوانیزه و موزائیک

ن. دلگرم، و م. ضیایی

چکیده

استفاده از خاک‌دیاتومه برای بهداشت انبار های داخل مزرعه و انبارهای تجاری دانه غلات از قدیم رایج بوده است. چنین نشان داده شده که برای از بین بردن آلودگی حشرات در انبارها، تیمار سازه‌های انبارها توسط خاک‌دیاتومه مفید است. در این مطالعه، سمیت باقی‌مانده فرمولاسیون مختلف خاک-دیاتومه علیه حشرات بالغ سوسک کشیش *Rhyzopertha dominica* (F.) (Coleoptera: Bostrychidae) و شپشه آرد *Tribolium confusum* Jacquelin du Val. (Coleoptera: Tenebrionidae) روی سطح‌های مختلف شامل بتن، استیل گالوانیزه و موزائیک مورد بررسی قرار گرفت. سطح‌ها با غلظت ۰/۲ میلی‌گرم بر سانتی‌مترمربع از فرمولاسیون‌های خاک‌دیاتومه شامل SilicoSec®، Protect-It و فرمولاسیون ایرانی خاک دیاتومه از معدن ممقان، ایران، که با سیلیکاژل بی‌شکل برای افزایش اثر بخشی ترکیب شده بود، تیمار شدند. سمیت باقیمانده خاک‌دیاتومه در ۷، ۱۵، ۳۰، ۴۵ و ۶۰ روز پس از تیمار بررسی شد. تلفات ۱ ، ۳ ، ۵ و ۷ روز پس از رهاسازی حشرات در هر سطح شمارش شد. درصد تلفات با افزایش غلظت خاک‌دیاتومه و زمان قرار گیری حشرات در معرض آن افزایش یافت. با توجه به نتایج، SilicoSec® در مقایسه با خاک‌دیاتومه Protect-It و خاک دیاتومه ایرانی ممقان موثرترین ترکیب بود. سمیت باقی‌مانده و دوام فرمولاسیون‌های خاک‌دیاتومه در استیل گالوانیزه از بیشتر بتن و موزائیک بود. اگرچه، نتایج نشان داد که خاک دیاتومه ایرانی مورد استفاده حاوی ۱۰ درصد سیلیکای بی‌شکل قادر به کنترل *R. dominica* و *T. confusum* در انبارها و سایر امکانات ذخیره‌سازی است. هر چند مطالعات بیشتری برای تأیید این یافته‌ها نیاز است.

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