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Effect of particle size of two Iranian diatomaceous earth deposits and a commercial product on *Sitophilus granarius* (Col.: Dryophthoridae)

M. Ziaee[1], S. Moharramipour[1&*] and K. Dadkhahipour[2]
1. Department of Entomology, Faculty of Agriculture, Tarbiat Modares University, Tehran, Iran, 2. Iranian Research Institute of Plant Protection, Tehran, Iran.
*Corresponding author, E-mail: moharami@modares.ac.ir

Abstract

Laboratory bioassays were carried out to study the insecticidal efficacy of two Iranian diatomaceous earths (DEs) and SilicoSec® formulation against *Sitophilus granarius* (L.). Four different particle sizes of DEs were applied at five concentrations. The mortality was counted 2, 5 and 7 days after exposure. The experiment was carried out at 27 ± 1°C and 55 ± 5% relative humidity in continuous darkness. For SilicoSec® and Mamaghan with less than 37 µm particle size, 500 ppm was sufficient to cause 100% mortality after 5 days of exposure. While in case of Maragheh deposit with the same particle size, complete mortality was recorded at 1000 ppm after 5 days. LC_{50} values indicated that SilicoSec® and Mamaghan with < 37 µm particle size were the most efficient DE samples against *S. granarius*. In most cases, the efficacy of DEs increased with decreasing particle size. The visual observation of scanning electron microscopy (SEM) images showed that SilicoSec® has more attachment to insects' body. However, the adhesiveness of DE particles cannot be the main indicative of their insecticidal effectiveness.

Key words: diatomaceous earth, insecticidal efficacy, particle size, *Sitophilus granarius*, wheat

چکیده

تأثیر اندازه‌ی ذرات دو خاک دیاتومه‌ی ایرانی و فرمولاسیون تجاری روی *Sitophilus granarius* (Col.: Dryophthoridae)

معصومه ضیایی، سعید محرمی‌پور و کاظم دادخواهی‌پور

آزمایشات زیست‌سنجی به‌منظور بررسی اثر حشره‌کشی دو خاک دیاتومه‌ی ایرانی و فرمولاسیون تجارتی SilicoSec® علیه *Sitophilus granarius* (L.) انجام شد. خاک‌های دیاتومه در چهار اندازه‌ی ذره‌ی مختلف و در پنج غلظت به کار گرفته شد. مرگ و میر حشرات کامل ۲، ۵ و ۷ روز پس از قرارگیری در معرض خاک دیاتومه شمارش شد. آزمایش‌ها در دمای ۱ ± ۲۷ درجه‌ی سلسیوس، رطوبت نسبی ۵ ± ۵ درصد و تاریکی انجام شد. در مورد فرمولاسیون SilicoSec® و نمونه‌ی ممقان، غلظت ۵۰۰ پی‌پی‌ام از خاک دیاتومه با اندازه‌ی ذره‌ی کم‌تر از ۳۷ میکرون برای ایجاد ۱۰۰ درصد تلفات پس از ۵ روز کافی بود، درحالی‌که در مورد نمونه‌ی مراغه با همان اندازه‌ی ذره، تلفات کامل در غلظت ۱۰۰۰ پی‌پی‌ام پس از ۵ روز به‌دست آمد. مقادیر LC_{50} نشان داد که SilicoSec® و خاک دیاتومه‌ی ممقان با اندازه‌ی ذره‌ی کم‌تر از ۳۷ میکرون، کارآمدترین نمونه‌ی دیاتومه در کنترل *S. granarius* بودند. در اغلب موارد، اثر خاک دیاتومه با کاهش اندازه‌ی ذرات افزایش یافت. مشاهده‌ی تصاویر میکروسکوپ الکترونی (SEM) نشان داد که SilicoSec ® چسبندگی بیش‌تری به بدن حشرات دارد. با این حال، چسبندگی ذرات خاک دیاتومه نمی‌تواند معیار اصلی اثر حشره‌کشی آن‌ها باشد.

واژگان کلیدی: خاک دیاتومه، اثر حشره‌کشی، اندازه‌ی ذره، *Sitophilus granarius*، گندم

Introduction

Diatoms are unicellular algae from the Eocene/Miocene epochs. There are more than twenty five thousand species of diatoms with no two having the same morphology (Round *et al.*, 1990). Tiny shells of dead diatoms sink over the centuries and form thick layers of deposits which fossilize and compress into a soft, chalky rock called diatomaceous earth (DE) (Korunic, 1998). DE is prepared for commercial use by quarrying, drying, and milling. This process reduce the moisture content to about 3-6% and mean aggregate particle size to 0.5 to over 100 microns (Korunic, 1997). Diatomaceous earth is probably one of the most effective naturally occurring protective powders for controlling stored-product insect pests. DEs adhere to the insect body and absorb the protective wax layer of cuticle resulting in death due to desiccation and, to a less degree, abrasion (Ebeling, 1971).

The efficacy of DEs against different stored-product insect species has been studied by several authors. To give some examples, Fields & Korunic (2000) ranked the following species based on their susceptibility to DE: *Cryptolestes* spp., *Oryzaephilus* spp., *Sitophilus* spp., *Rhyzopertha dominica* F. and *Tribolium* spp. Athanassiou *et al*. (2009) investigated the efficacy of DEs against three species, *Sitophilus zeamais* (Motsch.), *Tribolium castaneum* (Herbst) and *Cryptolestes ferrugineus* (Stephens), and found that *C.*

ferrugineus and *T. castaneum* were the most susceptible and resistant species, respectively. The authors speculated that *S. zeamais* was moderately susceptible to DEs.

Geographical location of DEs may play an important role on its insecticidal activity. Korunic (1997) evaluated the efficacy of DEs from different geographical locations (U.S.A., Mexico, Chile, Canada, Australia, Japan, China, and Macedonia) against *Sitophilus oryzae* (L.) and *T. castaneum*. He stated that in many cases a great difference was observed in insecticidal activity. However, according to his findings the efficacy of DE depends greatly on physical, chemical, and morphological properties of the DEs rather than on its origin. Also, Korunic (1997) speculated that DEs particle size may be an important factor influencing the insecticidal activity. DEs with a small and uniform particle size (less than 10 µm in diameter) in many cases were more effective than those with larger particle size.

The granary weevil, *Sitophilus granarius* (L.), is a key pest of stored products in the world. This species is usually found in grain storage facilities and damage stored grains. All larval stages and the pupal stage occur within the grain. The larvae feed inside the grain until pupation, after which they bore a hole out of the grain and emerge (Rees, 1996). The objective of the present study was to determine the effect of concentration levels, time of exposure, DEs geometry and particle size on their insecticidal activity against *S. granarius*.

Materials and methods

Sitophilus granarius were obtained from cultures maintained in the Entomology laboratory of Tarbiat Modares Univesity, Tehran, for at least three years with no history of exposure to insecticides. Beetles were reared at 27 ± 1°C and 55 ± 5% relative humidity in continuous darkness. 'Pishtaz Madary', a variety of wheat, which was purchased from Agricultural Support Services Company (Iran), used for rearing insects and running experiments. All adults used in the experiments were 7-14 days old of mixed sex. Wheat kernels were stored at -24°C, for at least 48 h. Before running the experiments, wheat kernels were kept for a week in an incubator set at 27 ± 1°C and 55 ± 5% R.H. to achieve the moisture content related to environmental relative humidity. The moisture content (m.c.) of wheat was measured by milling and then drying 10 g of wheat in a ventilated oven set at 110°C. The m.c. was about 12%, i.e. equilibrium to 55% R.H. (Pixton & Warburton, 1971).

Diatomaceous earth deposits

Experiments were carried out with two Iranian deposits of DEs collected from mines in North West of Iran with marine origin. One of the deposits was collected from a mine in Mamaghan region (37°50'18.04" N, 46°2'25.70" E) composed of 89.87% SiO_2, 1.13% Al_2O_3, and 0.85% Fe_2O_3. The other one from Maragheh region (37°22'41.39" N, 46°19'28.16" E) which was composed of 75.35% SiO_2, 7.72% Al_2O_3 and 2.33% Fe2O3 (Ziaee & Moharramipour, 2012). Also, the efficacy of SilicoSec®, a commercial DE formulation, was evaluated for comparison. SilicoSec® is a freshwater formulation composed of 92% SiO_2, 3% Al_2O_3, 1% Fe_2O_3, and 1% Na_2O with median particle size of 8-12 µm (Material Safety Data Sheet, Biofa GmbH, Münsingen, Germany).

Diatomaceous earth preparation

The method of preparing Iranian deposits was the same as Korunic (1997) and Vayias *et al.* (2009). Iranian DEs were milled, dried in an oven set at 60°C for 24 h to achieve about 3-6% moisture content. Subsequently, samples were sifted using a lab sieve with 149 µm apertures size (100 mesh) to remove all particles larger than 150 µm. Ideally, these fractions contain sand, rocks, clay, and other impurities. The second step was to take a larger part of sample containing particles < 149 µm and sieve them using a sieve with 74 µm apertures size (200 mesh). Then, another part of the samples with less than 74 µm particle sizes were sifted with a 400-mesh sieve to obtain particles < 37 µm. Therefore, four fractions with particle size of (1) < 149 µm, (2) 75-149 µm, (3) < 74 µm, and (4) < 37 µm were prepared for bioassay experiments. In case of SilicoSec®, more than 92% of the particles were < 37 µm, so pure SilicoSec® was used in this study.

Bioassays

Exposure studies were carried out at 27 ± 1°C and 55 ± 5% relative humidity in continuous darkness. The experiment was conducted in order to determine the efficacy of concentration level and time of exposure on insecticidal activity of the DE samples. Glass vials of 280 ml volume were used as exposure chambers. They were filled with 50 g wheat kernels and treated with 100, 250, 500, 1000, and 1500 ppm of DE samples. Each concentration was replicated four times and untreated wheat served as a control. The vials were shaken for five min. to achieve equal distribution of the dust in the entire grain mass. Thereafter, 25 adults of *S. granarius* were introduced to each vial and samples were covered with a muslin cloth for sufficient ventilation. All vials were placed in incubators set at the above conditions. The mortality was counted two, five, and seven days after exposure. When no leg or antennal movements were observed, insects were considered dead.

Another experiment was carried out to assess the LC_{50} of SilicoSec® formulation and Iranian DEs. Conditions of this experiment were the same as previous one unless different concentrations were applied due to the DEs activity. For each DE sample, preliminary concentration setting experiment was carried out to determine a range of concentrations that cause between 20-80% mortality via logarithmic scales (Robertson *et al*., 2007) (see table 1 for concentration levels of each DE). The mortality was measured two days after exposure to DEs.

Scanning electron microscopy (SEM)

According to Fields & Korunic (2000), 600 ppm is an appropriate concentration for comparing attachment of different DEs to the cuticle of several stored-product beetles. Therefore, concentration of 600 ppm was selected for comparing DEs attachment to the *S. granarius*. Four glass vials of 280 ml were filled with wheat, of which three vials treated with 600 ppm of SilicoSec®, Maragheh and Mamaghan deposits with the size of < 37 µm, and the last vial remained untreated as a control. Subsequently, adults of *S. granarius* were introduced to each vial and were kept in an incubator at 27 ± 1°C and 55 ± 5% R.H. in continuous darkness. Insects were removed two days after exposure and glued to SEM studs. If adults were not dead at that time, they were frozen at -24°C in 10 min. Then studs were kept in a desiccator at 0% relative humidity for one week. In addition, DE samples were placed and secured on aluminum studs. Adults analyzed at 15 kV acceleration voltage after gold sputtering using an Auto sputter coater (BIORAD Polaron Division). The beetles were photographed from ventral abdominal and head surfaces with the second detector.

Data analysis

In the first experiment, mortality counts were corrected using Abbott's (1925) formula. Percent mortality was transformed to square root of arcsine to normalize the data. Non-transformed data are presented in tables 2-4. The data were analyzed using factorial analysis of variance with complete randomized design (first variable: concentration; second variable: time) and Tukey's test was used to determine significant differences among concentrations and the effects of exposure time at each concentration (SPSS, 2007) at $P < 0.05$. Data obtained from the second experiment were subjected to Probit analysis (Finney, 1971) to estimate LC_{50} values and 95% confidence limits using SAS 6.12 software (SAS Institute, 1997).

Results

Two days after exposure to 1000 ppm of SilicoSec®, the mortality level of insects was recorded 100%. The main effects of SilicoSec® concentration

($F_{4, 59}$ = 149.37; P < 0.001) and exposure time ($F_{2, 59}$ = 204.08; P < 0.001) were significant. The interaction between concentration and time was also significant ($F_{8, 59}$ = 92.36; P < 0.001). Following seven days of exposure, in all concentrations a complete (100%) mortality was observed (table 2). Iranian deposits showed a great potential as grain protectants. For Maragheh deposits (< 149, 75-149, < 74 and < 37 µm particle size) the main effects of concentration ($F_{4, 59}$ = 195.91, 370.51, 169.92, 164.76, respectively), time ($F_{2, 59}$ = 461.79, 789.93, 478.0, 436.87, respectively), and interaction of time × concentration ($F_{8, 59}$ = 9.75, 17.61, 24.92, 77.46, respectively) were significant (P < 0.001). In case of Mamaghan deposits with the same particle sizes, all main effects and interactions were also significant (concentration: $F_{4, 59}$ = 187.16, 156.56, 256.08, 554.87; time: $F_{2, 59}$ = 509.45, 776.83, 304.69, 1232.35; interaction: $F_{8, 59}$ = 6.48, 8.29, 30.86, 189.04, respectively). Insecticidal efficacy increased by increasing the concentration of DEs and exposure time (tables 3, 4). For Maragheh fractions with < 149 and 75-149 µm, the highest concentration of 1500 ppm resulted in complete mortality seven days after exposure. While five days of exposure to this concentration was sufficient to achieve 100% mortality in insects exposed to fractions with < 74 µm (table 3). In case of Mamaghan deposits, almost five days of exposure caused 100% mortality at 1500 ppm (table 4). For the < 37 µm particle size, complete mortality was recorded after five days of adults' introduction to 1000 ppm of Maragheh samples (table 3) and 500 ppm of Mamaghan deposits (table 4).

Based on LC_{50} values and their 95% confidence limits, SilicoSec® and Mamaghan samples with < 37 µm were the most effective among the DEs tested with the value of 144.57 and 167.92 ppm, respectively (table 5). The following ranking was derived in order of decreasing efficacy: Maragheh 0-37 ≥ Mamaghan 0-74 ≥ Maragheh 0-74 ≥ Mamaghan 0-149 ≥ Maragheh 0-149 ≥ Mamaghan 75-149 ≥ Maragheh 75-149 µm. The "≥" mark show that DEs insecticidal activity is more than the next one but there is an overlap in 95% confidence interval (table 5).

The SEM photographs indicated the adherence of DE particles on the cuticle of *S. granarius*. The coating effect of the dust on insect body was observed when compared with untreated ones (fig. 1).

Table 1. Concentrations of SilicoSec® formulation, Maragheh and Mamaghan deposits of diatomaceous earth (DE) to estimate LC_{50} values for adults of *Sitophilus granarius***.**

DE	Particle size (µm)	Concentration (ppm)				
SilicoSec®	-	50	125	200	275	350
Maragheh	< 149	200	600	1000	1400	1800
Maragheh	75-149	200	800	1400	2000	2600
Maragheh	< 74	150	450	750	1050	1350
Maragheh	< 37	100	250	400	550	700
Mamaghan	< 149	200	800	1400	2000	2600
Mamaghan	75-149	200	1000	1800	2600	3400
Mamaghan	< 74	100	300	500	700	900
Mamaghan	< 37	50	200	350	500	650

Table 2. Percent mortality of *Sitophilus granarius* exposed to different concentrations of SilicoSec® formulation.

Concentration (ppm)	Time (days)	Mortality ± SE (%)
100	2	36 ± 4.32d
	5	89 ± 2.51b
	7	100a
250	2	81 ± 1.91c
	5	97 ± 1.91a
	7	100a
500	2	94 ± 1.15ab
	5	100a
	7	100a
1000	2	100a
	5	100a
	7	100a
1500	2	100a
	5	100a
	7	100a

Means followed by the same letter were not significantly different; Tukey's Test at P < 0.05.

Discussion

In the current study, the interaction between concentration and exposure time was significant. So, the mortality of insects increased with increasing concentration level and time of exposure from two to seven days. These results are in a good agreement with previous reports (Athanassiou *et al*., 2005; Korunic & Fields, 2006; Ziaee *et al*., 2007; Vayias *et al*., 2009)

Table 3. Percent mortality of *Sitophilus granarius* exposed to different concentrations of Maragheh deposits of diatomaceous earth.

Concentration (ppm)	Time (days)	Mortality ± SE (%)			
		Particle size (µm)			
		> 149	75-149	> 74	> 37
	2	7 ± 1.9g	5 ± 1.0h	15 ± 3.0h	23 ± 1.9e
100	5	34 ± 2.6f	30 ± 2.6g	60 ± 1.6fg	87 ± 1.9b
	7	67 ± 2.5c	60 ± 1.6e	76 ± 2.8de	93 ± 1.0ab
	2	34 ± 4.2f	27 ± 3.4g	25 ± 3.4h	49 ± 3.4d
250	5	53 ± 3.4de	42 ± 2.6f	84 ± 3.3cde	92 ± 2.8ab
	7	93 ± 1.0ab	88 ± 1.6cd	96 ± 1.6ab	96 ± 1.6ab
	2	43 ± 3.4ef	45 ± 1.9f	54 ± 3.8g	71 ± 3.4c
500	5	66 ± 2.6c	57 ± 1.9e	92 ± 0.0ab	96 ± 1.6ab
	7	95 ± 1.0ab	96 ± 1.6abc	97 ± 1.0ab	97 ± 1.0a
	2	60 ± 1.6cd	57 ± 1.0e	72 ± 1.6ef	92 ± 1.6ab
1000	5	85 ± 3.4b	84 ± 1.6d	99 ± 1.0a	100a
	7	99 ± 1.0a	98 ± 1.2ab	100a	100a
	2	69 ± 3.4c	65 ± 1.9e	85 ± 4.7bcd	100a
1500	5	94 ± 1.2ab	90 ± 2.6bcd	100a	100a
	7	100a	100a	100a	100a

Means followed by the same letter in each column were not significantly different using Tukey's Test at $P < 0.05$.

Table 4. Percent mortality of *Sitophilus granarius* exposed to different concentrations of Mamaghan deposits of diatomaceous earth.

Concentration (ppm)	Time (days)	Mortality ± SE (%)			
		Particle size (µm)			
		> 149	75-149	> 74	> 37
	2	16 ± 2.8g	10 ± 1.2i	20 ± 3.3h	39 ± 1.0e
100	5	47 ± 1.9f	38 ± 2.6gh	67 ± 1.0f	73 ± 1.0c
	7	67 ± 1.9de	79 ± 3.4bc	75 ± 1.0ef	98 ± 1.2a
	2	26 ± 2.6g	28 ± 3.7h	56 ± 1.6g	55 ± 1.0d
250	5	64 ± 1.6e	50 ± 1.2f	76 ± 1.6def	89 ± 1.9b
	7	84 ± 3.7bc	84 ± 1.6b	85 ± 1.0cd	99 ± 1.0a
	2	44 ± 3.7f	44 ± 1.6fg	68 ± 1.6f	75 ± 1.0c
500	5	78 ± 2.6cd	63 ± 1.9de	83 ± 1.0cde	100a
	7	93 ± 1.0ab	95 ± 1.9a	95 ± 1.9ab	100a
	2	60 ± 1.6e	54 ± 2.6ef	83 ± 1.9cde	93 ± 1.0b
1000	5	83 ± 2.5bc	69 ± 1.9cd	90 ± 1.2bc	100a
	7	100a	99 ± 1.0a	100a	100a
	2	71 ± 1.9de	63 ± 1.9de	91 ± 3.0abc	98 ± 1.2a
1500	5	94 ± 2.6ab	75 ± 1.9bc	96 ± 2.8ab	100a
	7	100a	100a	100a	100a

Means followed by the same letter in each column were not significantly different using Tukey's Test at $P < 0.05$.

Table 5. The lethal concentration for 50% (LC_{50}) of SilicoSec® formulation, Maragheh and Mamaghan deposits of diatomaceous earth (DE) against adults of *Sitophilus granarius* two days after exposure.

DE samples	Particle size (µm)	n	LC_{50} (ppm)	95% Confidence limits		Chi-square (df = 3)	*P* value
				Lower	Upper		
SilicoSec®	-	500	144.57	130.70	158.47	1.906	0.592
Maragheh	> 149	500	655.70	536.83	781.88	0.194	0.978
Maragheh	75-149	500	787.14	643.29	938.40	0.132	0.987
Maragheh	> 74	500	479.53	403.72	558.03	0.128	0.988
Maragheh	> 37	500	243.48	208.60	277.94	0.875	0.831
Mamaghan	> 149	500	611.01	473.40	750.13	0.045	0.997
Mamaghan	75-149	500	751.45	572.04	938.50	0.278	0.964
Mamaghan	> 74	500	249.78	203.01	295.57	0.403	0.939
Mamaghan	> 37	500	167.92	134.79	201.78	0.353	0.949

indicating that the efficacy of DEs requires extended exposure interval and elevated concentration levels. Athanassiou *et al*. (2005) investigated the insecticidal efficacy of SilicoSec® against *Tribolium confusum* du

Val and *S. oryzae*. They stated that mortality was increased with increasing concentration level and adequate exposure interval is required for the effectiveness of DE. Also, Korunic & Fields (2006) evaluated the susceptibility of *Sitophilus* spp. to two natural DEs. They noted that mortality increased with increasing concentration levels and time of exposing to each concentration. However, for SilicoSec® formulation, overall mortalities obtained at seven days were not significant among different concentrations.

Stored-product insect pests show a wide range of susceptibility to DEs. Among the beetles occurring in storage facilities, *Sitophilus* spp. are one of the sensitive insects to DEs (Korunic, 1998). According to Korunic & Fields (2006), *S. zeamais* was the most sensitive species to DEs in this genus, followed by the *S. oryzae* and *S. granarius*. Therefore, the concentration recommended for *S. granarius* might be desirable for other species of this genus.

The insecticidal efficacy of several DE formulations has been investigated on *S. granarius* by several researchers (Aldryhim, 1990; McLaughlin, 1994; Korunic & Fields, 2006; Saez & Fuentes Mora, 2007). Findings indicated that efficacy of DEs would be different against the same insect species. DEs differ in species of diatoms (shape), origin (marine or freshwater), particle size distribution, SiO_2 content and so on. Hence, these properties of DEs influence their insecticidal activities (Korunic, 1997). For example, the major and distinguished diatom species such as *Rhopalodia minuscule* O. Muller, *Melosira varians* C. A. Agardh, *Cymbella aspera* (Ehrenberg) Cleve, *Pinnularia maior* (Kutzing) Cleve, *Pinnularia gibba* Ehrenberg, *Diploneis elliptica* (Kutzing) Cleve, *Ellerbeckia arenaria* (Moore ex Ralfs) R. M. Crawford and *Navicula* spp. have been reported from Maragheh and Mamaghan DE samples (Ziaee *et al.* 2011a, 2011b).

The geometrical factors and spatial forms of diatoms vary from species to species, so that each species may has its own mode of action. For example, *M. varians* has 38 µm length, 14 µm width with 10-12 uniform striae in 10 µm. The diatom *C. aspera* has punctuate striae that are slightly radiate (especially at mid valve) to parallel; puncta are distinct and more or less ovoid containing 11-15 striae in 10 µm; 70-200 µm length and 20-30 µm width. The diatom *D. elliptica* is 17 µm long, 10 µm wide and 10-13 striae in 10 µm. The variety of species in a small sample of DE represents different insecticidal activity of each species. It seems wherever the number and size of pores and distribution of striae per unit area of species is greater; the insecticidal ability and capacity to absorb insects' cuticular waxes would be high, which permits excess water loss from the body. In addition, they may be abrasive to the cuticle which further increases water loss. Consequently, mortality is due to desiccation, which is a result of damage to the wax layer in the cuticle. Such micro scale dimensions with rigid silica pattern of valves have more effects in contact and/or at the moving state of pests, causing a decline in insect movement, until death occurs (Robinson, 2005).

Different results were achieved showing the influence of origin of DEs on their insecticidal activity. Snetsinger (1988) pointed out that DEs from marine origin are less efficacious than fresh water ones as an insecticide. While, according to Saez & Fuentes Mora (2007) no significant differences was obtained among the mortality of *C. ferrugineus*, *R. dominica*, *S. oryzae* and *S. granarius* with marine or freshwater origin of DEs. In the present study, SilicoSec® with marine origin had the highest efficacy, and the efficacy of Mamaghan sample with 0-37 µm particle size is more or less the same as SilicoSec® formulation. It seems that the origin of DEs doesn't have any considerable influence on its activity.

It could be concluded that particle size may play an important role in the mortality level of adults. Results of the current study confirmed the findings of Vayias *et al.* (2009) that the efficacy of DEs is inversely related to their particle size. In the case of Iranian deposits, the insecticidal efficacy increased with decreasing the size of particles. In some cases the

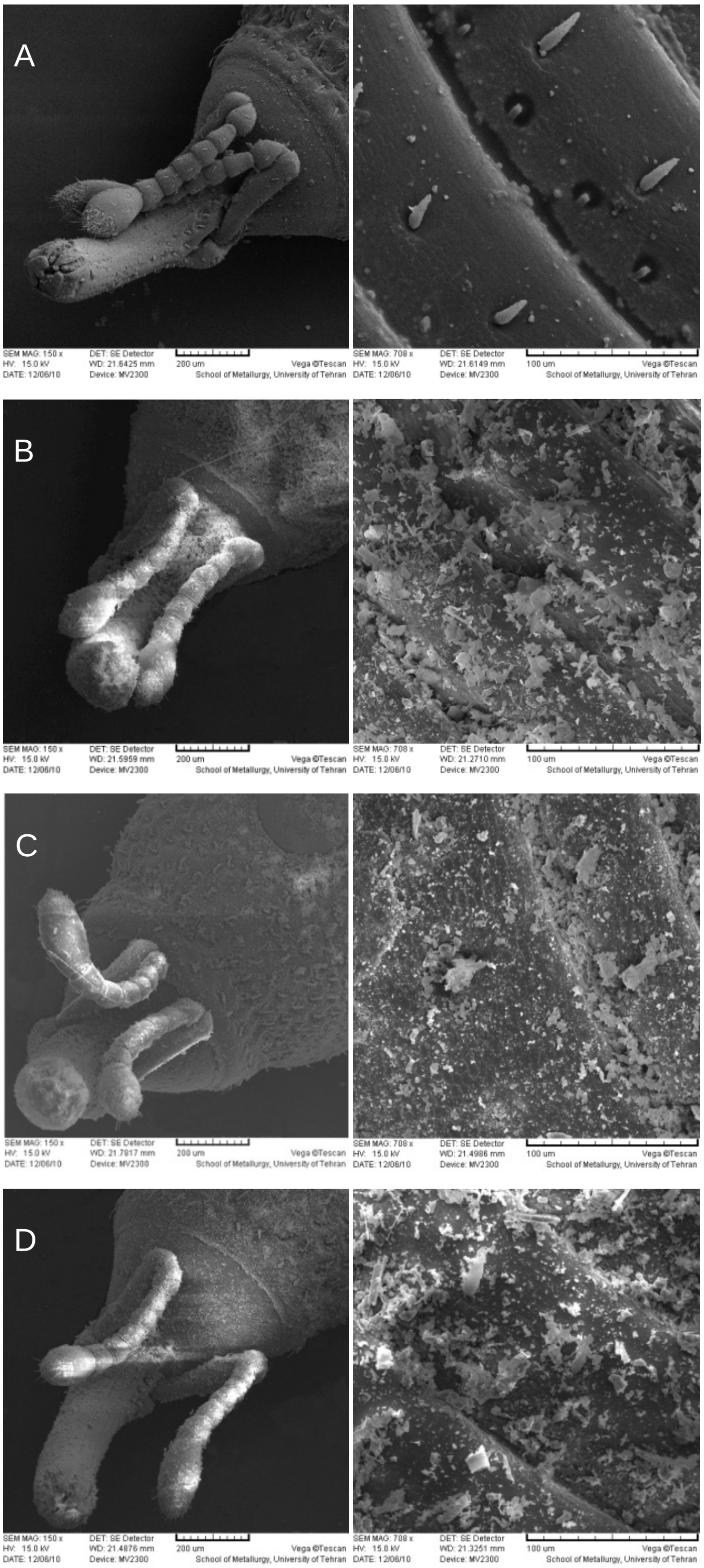

Fig. 1. Scanning electron micrographs of the ventral view of *Sitophilus granarius* head, ×150 (left) and ventral surface of abdomen, ×708 (right) indicating the adherence of diatomaceous earth (DE) particles on the insect's cuticle compared with the control. (A) Control, (B) SilicoSec® formulation, (C) Maragheh deposits of DE, (D) Mamaghan deposits of DE.

insecticidal efficacy of Iranian DEs with < 149 µm was more than DEs with 75-149 µm. It could be due to the fact that the part of particles with < 149 µm is less than 74 µm; while DEs with the particles of 75-149 µm are not smaller than 75 µm. Vayias *et al.* (2009) speculated that smaller particles have a great surface to volume ratio, so the contact area between DE particles and insects body increases. Also, high toxicity of smaller particles may be attributed to the high number of DE particles in the concentration used.

SEM photographs (fig. 1) showed that SilicoSec® particles had a good ability to attach to the treated beetles. Photos taken from ventral surface of the head proved that the adherence of DE particles to snout and antenna is somewhat more than other parts of the head. However, particles attached uniformly to the ventral surface of abdomen which could be attributed to the flat surface of the abdomen.

In conclusion, Iranian DEs have insecticidal potential and appropriate application rates of these DE deposits can be advised to the local farmers and managers as effective grain protectants. However, field tests should be carried out to confirm laboratory findings and to make natural DE deposits exploitable for commercial use as an integrated protection agent.

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