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Impact of Diatomaceous Earth Modifications for Controlling the Granary Weevil, *Sitophilus granarius* (Linnaeus) (Coleoptera: Curculionidae)

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ABSTRACT

During different exposure intervals (one, two, four, seven, and fourteen days), the efficacy of different concentrations of original Diatomaceous Earth (DE) and DEs-modifications against the granary weevil, *Sitophilus granarius* (Linnaeus) adults, were evaluated. The efficacy of DEs was evaluated by recording adult mortality and other parameters including effect on the progeny, grain weight loss, and DE-coherence on wheat kernels. The modified DEs showed higher efficacy than the original-DE. The most effective DE–modification was Al-DE followed by Ca-DE and Na-DE. At 14 days interval, the highest adult mortality reached 98% for Al-DE and Ca-DE modifications in comparison to the control untreated (10%) that indicates highly significant difference between them and control. At 1.5% concentration, Al-DE treatment almost suppressed the progeny (5.0±2.0) in comparison with the untreated treatment (83.333±4.041). The least weight loss was recorded for Al-DE treatment (7.0%) followed by Ca-DE (10.0%), Na-DE (13.0%) and the original-DE (25.0%) compared with the control (38%). Ultrastructural investigations of the antennae, mouth parts and posterior end of control and treated adults were performed using scanning electron microscope. DEs particles damaged sensory organs and parts of the integument throughout the body. Clear destruction among different types of the club segment sensilla was recorded. Moreover, integument cracking and cutting of the mouth parts and the genitalia were recorded. Both Al-DE and Ca-DE treatments strongly cohered very well to the wheat kernels with (1.493±0.002 and 1.492±0.002) at 1.5% concentration, respectively. The DEs coherence on wheat kernels matched directly to the insecticidal efficacy of the DE modifications against *S. granarius* adults.

Keywords: Insecticidal efficacy, Coherence, SEM, Ultrastructural investigations, Wheat kernel.

INTRODUCTION

Sitophilus granarius (Linnaeus), the granary weevil, is one of the most destructive stored grain insect pests, especially in bulk storages, grain mills and elevators. The granary weevil is a cosmopolitan pest of wheat, *Triticum aestivum* L. and barley, *Hordeum vulgare* L. (Abd El Ghany and Abd El-Aziz, 2017), and also attacks a variety of dried stored grain and bean products. The main infestation effects of *S. granarius* L. (adults and immature stages) are damaging the grains and producing the hot spots. The adults may feed upon milled products for a short time, but the larvae require hard masses of food at least as large as a wheat kernel for the completion of their development. The granary weevil females make small holes in the wheat kernel in which the eggs are laid. The larvae hatch and spend their lives within

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the kernels as legless grubs and eat out the interior of the kernels. Only a shell remains when the insects reach maturity (Shepard, 1947). Also, weevils' feeding reduces the quality of the grains by the frass and dust (Longstaff, 1981; Anonymous, 2008).

Diatomaceous Earth (DE) is a promising alternative healthy method for controlling stored products insect pests, which are formed from the fossils of diatoms. DEs are mainly composed of amorphous hydrated silica (Vayias and Athanassiou, 2004). Diatomaceous Earths (DEs) are considered as natural insecticides of low mammalian toxicity and were used against various insect species (Subramanyam and Roesli, 2000). Varied DE and DE formulations have been evaluated against various stored product pests **(**Korunic, 1997; Athanassiou and Kavallieratos, 2005; Athanassiou *et al*., 2008; Abd-El-Aziz and Sherief, 2010; Bohinc *et al*., 2013; Shah and Khan, 2014; Sabbour and Abd-El-Aziz, 2014, 2015; Ziaee *et al*., 2016). Using inert dusts in grain storages can provide constant protection against insect infestations and does not impact the baking of wheat (Abd-El-Aziz, 2011). The mode of action of DEs is to desiccate the cuticular layers of the insect by scraping and absorbs lipids in the waxy layer of the epicuticle (Ebeling, 1971; Mewis and Ulrichs, 2001). The insecticidal efficacy of DEs vary according to the physical, chemical, and morphological features of diatoms types that compose the DE, temperature, humidity, and characteristics of target pests **(**Abd-El-Aziz and Sherief, 2010; Rojht *et al*., 2010; Shah and Khan, 2014). Moreover, the structure of the pericarp of the grain impacts adherence of DE dust to kernels (Korunic, 1997; Subramanyam and Roesli, 2000; Kavallieratos *et al*., 2005).

The subjected study was designed to evaluate the efficacy of different concentrations of the original DE and DEs modifications against the granary weevil**,** *S. granarius* L. adults during different exposure intervals on adult mortality. In addition, the adult emergence, the grain weight loss and coherence of tested DEs were to be assessed.

MATERIALS AND METHODS

Insect Culture

The granary weevil, *S. granarius* L. (Coleoptera: Curculionidae), was maintained in glass containers covered by a plastic mesh. Insects were reared on a diet of whole wheat grains and kept at 26±2˚C and 70-80% Relative Humidity (RH). Every 3 days, adults having a fair color, compared to the dark brown or black of the older ones (48 hours) were removed and transferred to another box with new seeds in order to obtain the next generations of similar age. The adults of *S. granarius* L. were selected at age of 14 days, which was required for the sexual maturation, and used in the experiments.

Bioassay of Tested Materials

Diatomaceous Earths (DEs)

Original diatomaceous earth and three DEs-modifications were used. The origin of the natural diatomaceous earth was taken from Kom Oshim in east of Fayoum governorate, Egypt. This diatomaceous earth was formed in a separate fresh water lake rather than a part of the ancient Fayoum lake. The natural Diatomaceous Earth (DE) was chemically modified by different mono-, di-, tri-valent Metal HydrOxides (MOH, M= Na, Ca, Al) as strong bases with purity of 99.9% (Merck company) in order to improve its properties according to Mewis and Ulrichs (2001). Modification was performed as follows: 15 g of DE was mixed with 100 mL of 6M metal hydroxide solution at 100±5°C with continuous stirring and refluxing for 2 hours. The solutions were filtered in a vacuum filter flask and washed sequentially with deionized water to

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remove the salt ions and other residues. The resulting solid pellet was dried at 105°C for 24 hours, and keeps in a desiccators after drying (Khraisheh *et al*., 2004).

Insecticidal Efficacy of Tested DEs

The insecticidal efficacies of DEs were tested against adults of *S. granarius* L. The evaluation method was modified from Abd-El-Aziz and Sherief (2010). The tested DEs were treated at application rates of 1.5, 1.0, 0.75 and 0.375 g kg^{-1} wheat grain. For each case, three glass jars were used as replicates. Each replicate was treated individually with the respective DE quantity and then shaken manually for one minute to achieve equal distribution of the DE. Subsequently, ten mixed-sex adults were introduced into each glass jar and were covered with muslin for sufficient ventilation. Six replicates of glass jars containing untreated wheat served as untreated control. Jars were incubated at 26±2˚C and 70-80% RH. The efficacy of tested DEs was evaluated by recording adult mortality after five intervals (one, two, four, seven, and fourteen days). At the end of each exposure interval, the live and dead adults were recorded. After 14 days, the live adults were removed and the container was incubated at room temperature until fully grown larvae were investigated. Moreover, the effects of DEs on the progeny obtained from treated alive grain weevil adults after exposure times were recorded by counting the numbers of newly emerged *S. granarius* L. adults for each tested concentration. The percentage of grain weight lost was calculated from the differences between the original and the final weight in each jar using the following formulae:

% WL (% Weight Loss)= [(OW–FW)/OW]×100

Where, *OW*= Original Weight without inert dust and *FW*= Final Weight without inert dust.

Coherence of DEs to Wheat Grains

The method described by Korunic (1997) was followed to evaluate the degree of DEs coherence to the wheat grains at the four tested doses.

Scan Electron Microscope (SEM)

Adults of *S. granarius* L. after incubation with treated wheat grains were isolated and prepared for investigation under Scanning Electron Microscope (SEM) according to Abd El Ghany and Abd El-Aziz (2017). The samples were fixed immediately after isolated from the laboratory colony in glutaraldehyde sodium cocodylate buffer for 4-6 hours, then removed to be washed three times in buffer for 10 minutes. The samples were then kept in buffer for 24 hours at 4ºC. Osmium tetraoxide buffer was added to the samples after removal of glutaraldehyde buffer and kept for 2 hours till they became dark in color, and then washed again three times with glutaraldehyde buffer and left in it for 24 hours at 4ºC. Dehydration was made using different concentrations of ethyl alcohol (30, 50, 70, 80, 90 and 100%); two times for 5 minutes in each concentration. The samples were then dried using critical point drying to preserve the morphological characters. The samples were then stuck to aluminum stumps and covered by gold film using Sputter Cotter Edward. Samples were photographed using Quanta SEM field emission gun 250 (Koninklijke Philips N. V., Netherlands).

Statistical Analysis

The percentage of observed adults mortalities were recorded after different exposure intervals. Data were statistically compared using Analysis Of Variance (ANOVA) at the 5% probability level using SPSS computer software program to determine the effects of DEs deposits at different exposure intervals at each concentration level on adult’s mortality.

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RESULTS AND DISCUSSION

Unfortunately, the applied residual pesticides in stored products have negative side-effects: they can be toxic to mammals, residues can be accumulated in the treated products, and unfortunately many pest species could become resistant to the protectants. During recent years, inert dusts have received increased attention as grain protectants, and are considered among the most promising alternatives to residual insecticides. One of these is Diatomaceous Earth (DE), which consists of the fossilized remains of diatoms. Furthermore, laboratory bioassays were conducted to determine the effect of natural DE and three chemically modified DEs against *S. granarius* L. adult weevils. The effectiveness of different concentrations (1.5, 1.0, 0.75 and 0.375 g kg^{-1} grain) of each tested DEs at five exposure intervals (one, two, four, seven and

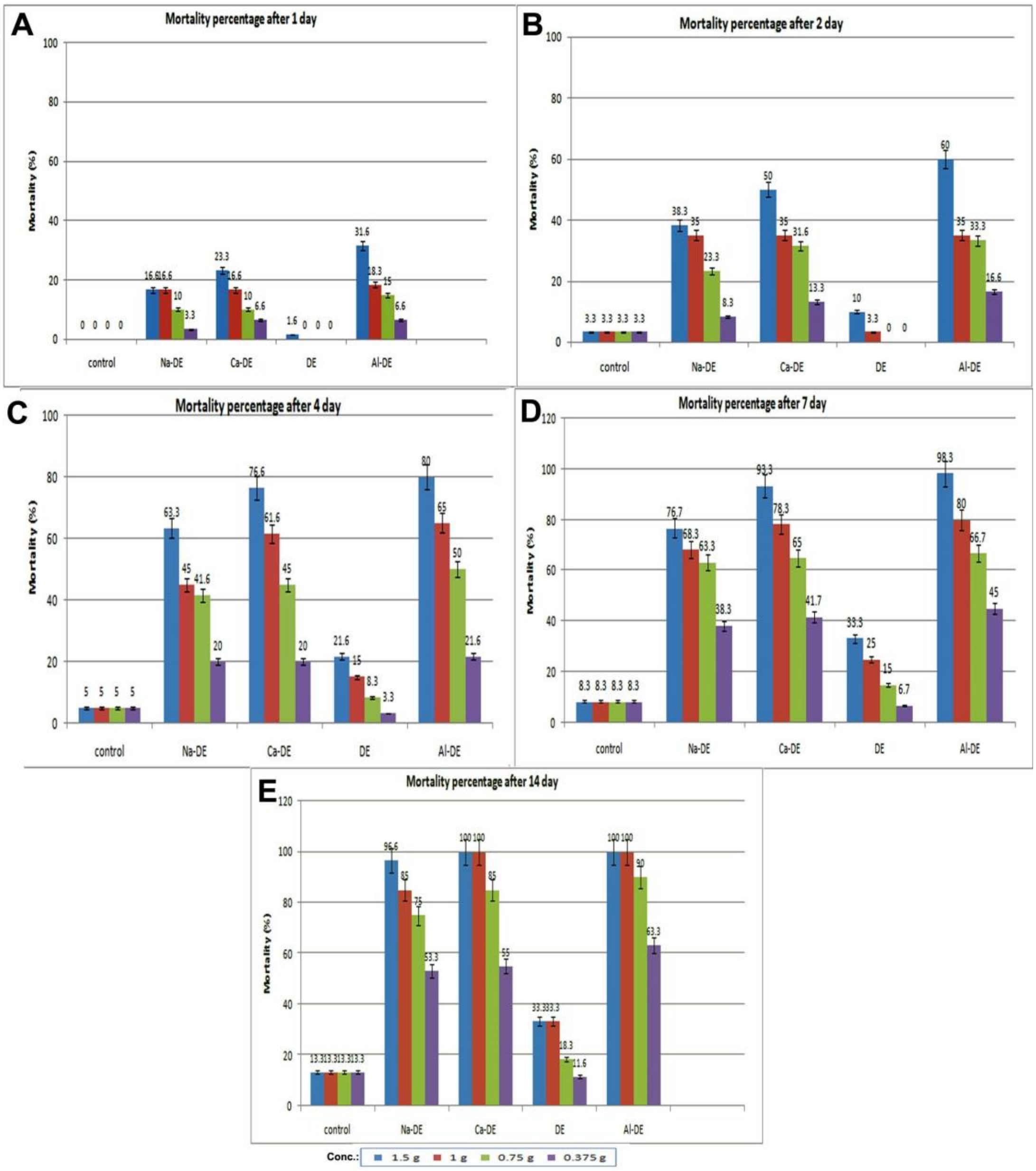

Figure 1. Mortality percentages of adult weevils treated with tested DEs (4 concs. each) during five intervals.

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fourteen days) were evaluated on adult mortality of *S. granarius* L. (Figure 1). Adult mortality percentages increased steadily with tested concentrations during exposure intervals. At 1.5% concentration, the initial mortality (one day interval) reached 31.6, 23.3 and 16.6% in case of Al-DE, Ca-DE, and Na-DE, respectively, in comparison to control (zero%). Gradually increasing in mortality percentages were noticed by increasing time of intervals. The same trend was obvious in the other tested intervals (two, four, seven and fourteen days). The most effective DE–modification was Al-DE followed by Ca-DE and Na-DE treatments. The original DE was the least effective treatment against adult weevils with tested concentrations at different intervals. This result was not in agreement with Abd-El-Aziz and Sherief (2010) who mentioned that the treatment of Ca-DE caused the complete mortality of *Callosobruchus maculates* Fabricius (100%) compared to the other tested DEs after 7 and 14 days interval.

At 14 days interval, the highest adult mortality reached 98% in case of Al-DE and Ca-DE modifications in comparison to the control (10%), and there was a highly significant difference between them and untreated control. This may be related to the insecticidal properties of both Al-DE and Ca-DE modifications. Our study revealed that both Al-DE and Ca-DE modifications can be used against *S. granarius* L., but some variables affected their efficacy, such as concentrations and exposure intervals. These results are in agreement with Korunic and Mackay (2000) who reported that wheat, *Triticum aestivum* (L.), treated with 0.5 and 0.75 g of diatomaceous earth Protect-It® per kg of wheat, reduced the population of *S. oryzae* Linnaeus, *Tribolium castaneum* Herbst and *Rhyzopertha dominica* Fabricius by 98 to 100% compared to the controls. This is due to the repellent properties of diatomaceous earth, which, probably, has very good spread capacity in the grain mass. DE–dose rate is decisive not only for activity but also for chemical properties of the DE modifications (Abd El-Aziz and Sherief, 2010). Also, Ebeling (1971) mentioned that all kinds of fossil diatoms are porous and this specific surface area (square meters per gram) gives them their insecticidal activity. Sabbour *et al*. (2012) found that modified diatoms with calcium hydroxide (Ca-DE) and modified diatoms with sodium hydroxide (Na-DE) achieved the highlight mortality percentages against tested insects, *Plodia interpunctella* Hübner, *Ephestia cautella* Walker and *E. kuehniella* Zeller.

The efficacy of treatments on the progeny (F1) of *S. granarius* L. is illustrated in Table 1. At 1.5% concentration, the treatment (Al-DE) on wheat grains almost completely suppressed the weevil progeny (5.0±2.0) in comparison with (83.333±4.041) in untreated control. There was highly significant difference ($P< 0.05$) between DEs treatments and the tested concentrations. The Al-DE and Ca-DE treatments had repellent activity that affected feeding and egg production by female weevils and reduced the progeny (F1). Behavior of egg laying of *C. maculatus* F. was activated by the DE treatments, resulting in reduced egg production. This may be attributed to the pressure of the inert dust or to the decrease of both physical and chemical (tactile) stimuli (Abd El-Aziz and Sherief, 2010). DEs treatments changed the surface structure of wheat grains and led to less cohesion between eggs and the surface of the grains, resulting in reduction of the progeny of *S. granarius* L. (F1). The red flour beetle, *T. castaneum* H., may be partially taken away from the treated layer with diatomaceous earth to untreated layers due to the repellent properties of diatomaceous earth and probably has very good spread power in the grain mass (White *et al.*, 1966).

The efficacy of the original DE and the three modified DEs on the weight loss of wheat grains as a result of weevils feeding on treated wheat grains is clarified in Figure 2. All tested DEs were significantly ($P< 0.05$) better than the untreated control. The least weight loss was recorded in case of Al-DE treatment (7.0%) followed by Ca-DE (10.0%), Na-DE (13.0%), and DE (25.0%) compared with the control (38%). Minimum levels of wheat grain damage were recorded

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Table 1. The efficacy of treatments on *S. granarius* progeny (F1).[a]

Parameters	Materials	Conc (g kg^{-1})	Mean±*SD*	*P* value
Mean number of the progeny (F1)	Control	0.0	83.333 ± 4.041	1.000
	Na-DE	1.5	18.333 ± 2.082 [cd]	0.000
		1	23.000 ± 2.646 [d]	
		0.75	25.667 ± 2.517 [ad]	
		0.375	43.000 ± 4.583 [abc]	
	Ca-DE	1.5	07.667 ± 1.155 [cd]	0.000
		1	12.000 ± 2.646 [cd]	
		0.75	19.667 ± 1.528 [abd]	
		0.375	60.667 ± 7.024 [abc]	
	DE	1.5	29.000 ± 2.000 [cd]	0.000
		1	32.667 ± 4.509 [cd]	
		0.75	43.000 ± 2.646 [abd]	
		0.375	77.333 ± 2.517 [abc]	
	Al-DE	1.5	05.000 ± 2.000 [bcd]	0.000
		1	11.667 ± 2.082 [acd]	
		0.75	19.000 ± 4.000 [abd]	
		0.375	26.333 ± 2.082 [abc]	

[a] Means within an entire column followed by the same letter are not significantly different (P> 0.05).

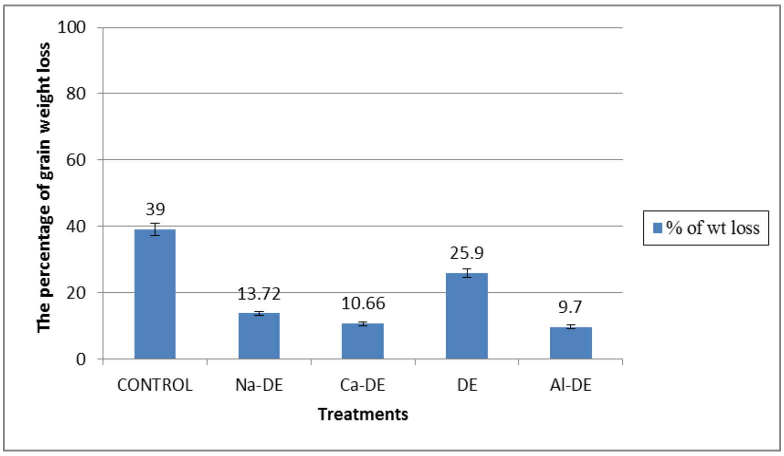

Figure 2. The efficacy of natural DE and the three modified DEs on the weight loss of wheat grains.

in case of Al-DE and Ca-DE as a result of weevil behavior to avoid contact with treated wheat grains. Insect feeding on grains is leading to quantitative deterioration of grain weight (Steffan, 1963; Golebiowska, 1969; Sabbour and Abd El-Aziz, 2007). Grain losses by stored product insects have been tested in laboratory experiments (Moino *et al*., 1998; Padina *et al*., 2002; Arya and Tiwari, 2013). After six months of storage, the efficacy of some native bio-products against *S. oryzae* L. on wheat grains was tested. The most effective treatments (neem, jatropha seed, mustard and cow dung ash) had no weight loss of seeds; while cow dung powder recorded the least weight loss (4.80%) in comparison to untreated control (34.80%) (Arya and Tiwari, 2013). After 28 days of treated maize or wheat with serial doses of Kensil F, Dryacide or Wood Ash (DEs), weight loss by *S. zeamais* Motschulsky infestation

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recorded 4.5%, followed by *Prostephanus truncates* Horn 4.2% and *R. dominica* F. 3.5% (Mugo *et al*., 2015).

The control and treated granary weevil adults with DEs were investigated using Scanning Electron Microscopy (SEM) in order to investigate the ultrastructural changes in different body parts. Results showed general damage through all parts of the granary weevil integument (Figure 3). The rostrum of granary weevil carries mouth parts on its apex and elbowed antennae laterally at the base end. Obvious damages among different types of sensilla on the club segment are illustrated in Figure 4. Clear d**e**struction was recorded in the mouth parts of granary weevil adult treated with DE in comparison to control one **(**Figures 5-A and -B). The destruction included labium, mandible, and labium palp as shown in Figure 5 (D and E). Multi-branched sensilla arranged longitudinally along two-third length on the dorso-lateral side of rostrum were highly affected and damaged (Figure 5-C). Moreover, the multiporous peg sensilla distributed on the frontal part of the rostrum showed obvious damages and the integument surrounding it was cracked (Figures 5-E, -F and -G), the DEs particles cement on the sensilla and/or block the pores of the sensilla. So, the weevil could not stimulate the food and failed to attack the grains. On the posterior end of the granary weevil body, the multi-branched sensilla were destructed and traces of DE- powder were noticed on the genitalia, as shown in Figure 6. These led to reduction in egg laying and adult weevil emergence (F1). Our findings are consistent with results obtained by Ebeling (1971) and Malia *et al.* (2016), who said that particles of DEs damage insect sensilla and close up the sensilla pores in epicuticular layer of insect cuticle, which may overlap with insect behavior. Also, DE corrupt sensory organs of olfaction and gustation, water balance, and gas exchange.

DEs Coherence

In the present study, both Al-DE and Ca-DE treatments strongly cohered very well to

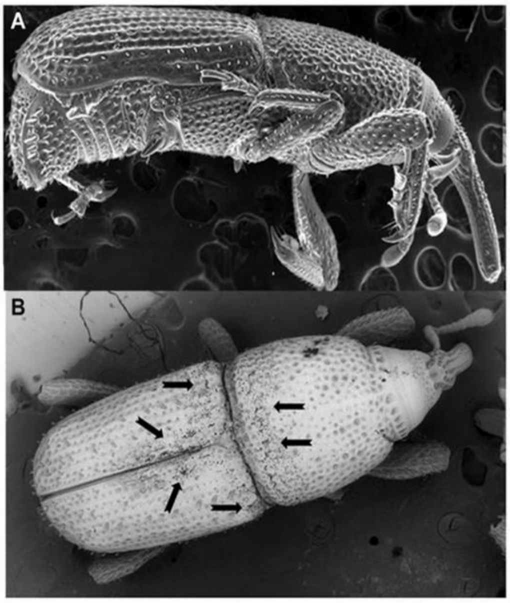

Figure 3. Electron micrograph shows lateral view of untreated control weevil (A), and DEs particles aggregated in thick layers on the dorsal parts of adult weevil body (B).

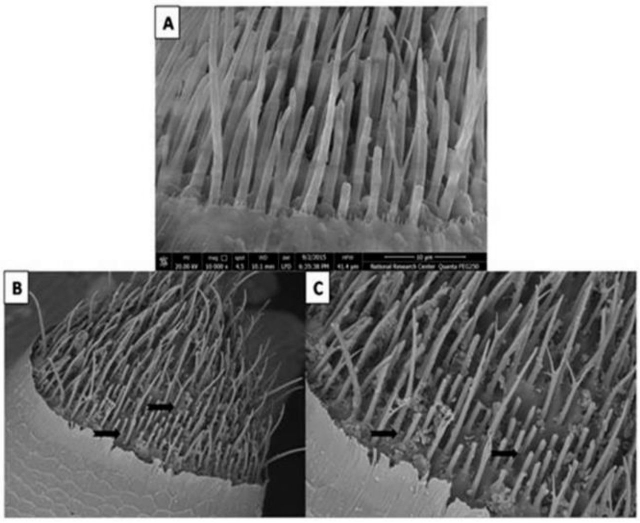

Figure 4. Electron micrograph shows different types of club segment sensilla were damaged with DEs particles.

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the wheat kernels with (1.493±0.002 g and 1.492±0.002 g) at 1.5% concentration, respectively, as shown in Table 2. There were no significant differences in the degree of coherence between Al-DE and Ca-DE

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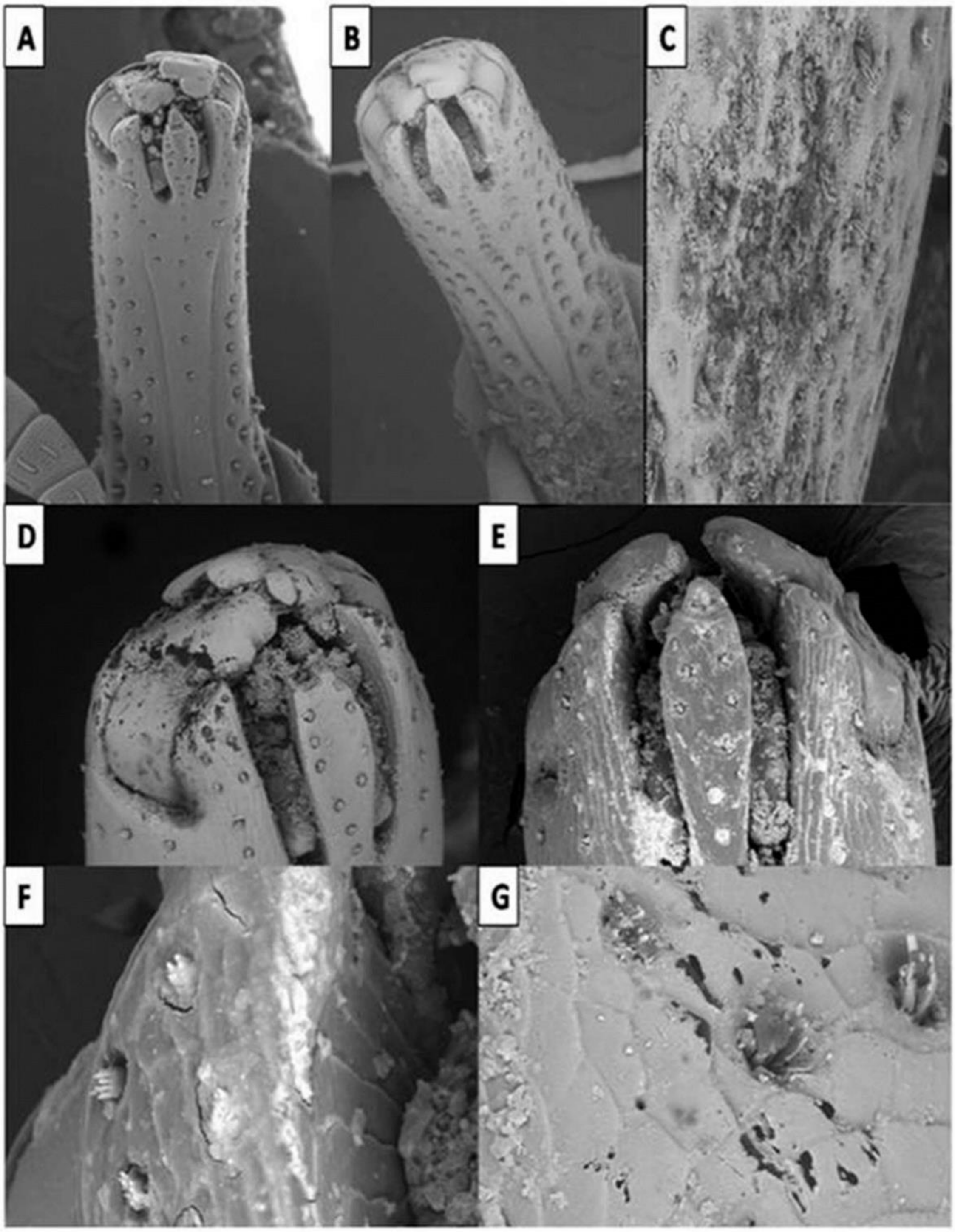

Figure 5. Electron micrograph shows untreated rostrum and mouthparts (A), a clear damage of different sensilla throughout the treated rostrum (B and C), and the mouth parts with DEs (D, E, F and G).

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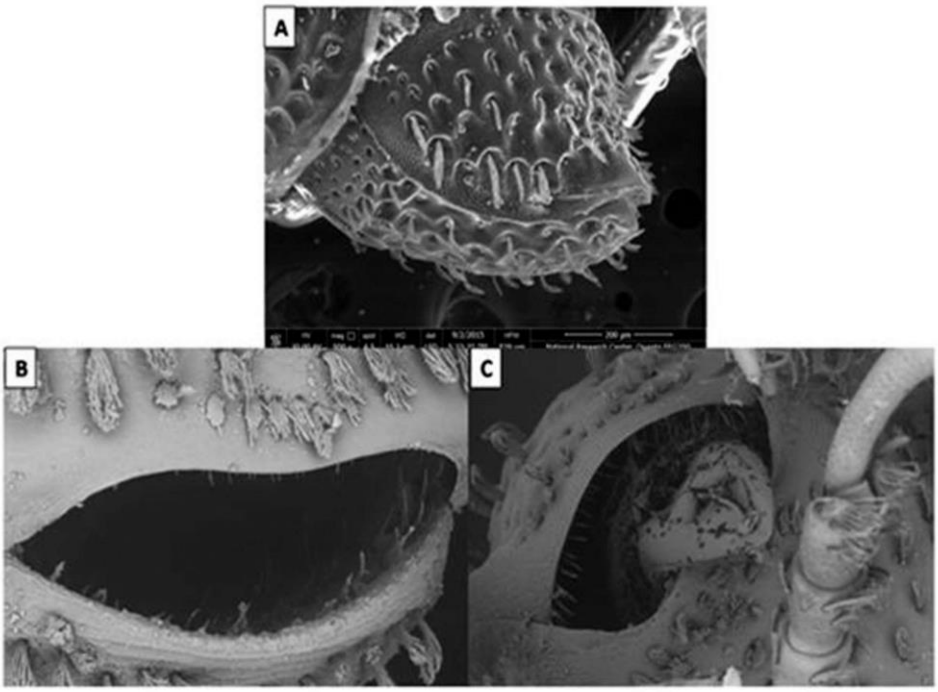

Figure 6. Electron micrograph shows untreated posterior end of the granary weevil (A), and the posterior ends of the granary weevil body the multi-branched sensilla were destructed and traces of DEs- powder were noticed on the genitalia (B and C).

Table 2. The tested DEs coherence to treated wheat grains.[a]

Conc (g kg^{-1})	Materials	Mean±*SD*	% Of coating	*P* value
1.5	Na-DE	1.478 ± 0.002 ^{bd}	98.53	0.000
	Ca-DE	1.492 ± 0.002 ^{ac}	99.47	
	DE	1.478 ± 0.004 ^{bd}	98.53	
	Al-DE	1.493 ± 0.002 ^{ac}	99.53	
1.0	Na-DE	0.990 ± 0.001 ^{bcd}	99.00	0.000
	Ca-DE	0.994 ± 0.002 ^{ac}	99.40	
	DE	0.987 ± 0.003 ^{abd}	98.70	
	Al-DE	0.995 ± 0.002 ^{ac}	99.50	
0.75	Na-DE	0.736 ± 0.002 ^{bd}	98.13	0.000
	Ca-DE	0.745 ± 0.002 ^{acd}	99.33	
	DE	0.736 ± 0.002 ^{bd}	98.13	
	Al-DE	0.747 ± 0.001 ^{abc}	99.60	
	Na-DE	0.355 ± 0.002 ^{c}	94.67	0.000
0.375	Ca-DE	0.368 ± 0.002 ^{c}	98.13	
	DE	0.319 ± 0.050 ^{abd}	85.07	
	Al-DE	0.370 ± 0.002 ^{c}	98.67	

[a] Means within an entire column followed by the same letter are not significantly different (P> 0.05).

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treatments at all tested concentrations. Both Na-DE and the original DE treatments had moderate coherence (1.478±0.002 and 1.478±0.002) at 1.5% concentration, respectively, and there were no significant differences in the degree of coherence between them. The DEs coherence on wheat kernels matched directly to the insecticidal efficacy of the DE modifications against *S. granarius* L. adults. These data are in agreement with those of Korunic (1997) and Abd El-Aziz and Sherief (2010) who mentioned that the coherence correlated well with the insecticidal activity of DEs. In contrast, the different dust coherences recorded among grain types or between dust coherences did not match directly to the insecticidal efficacy of the DEs against *R. dominica* F. adults (Kavallieratos, *et al.* 2005; Athanassiou *et al.*, 2008). The degree of coherence to the grains (kernel) limits the insecticidal efficacy of DE and is affected by the feature of the DE and physical characters of the kernel (Korunic, 1997; Subramanyam and Roesli, 2000).

CONCLUSIONS

A glance on data indicated that the modified DEs showed higher efficacy than the natural-DE. Results reveal that both Al-DE and Ca-DE applications are the most efficient tested DEs and have potential use for controlling the granary weevil, *S. granarius* (L.). They have insecticidal, repellent, and ovicidal effects against *S. granarius* (L.). Moreover, the modification of DEs treatments caused less cohesion between eggs and the surface of the treated grains leading to reduction in the progeny of *S. granarius* L. (F1). For instance, Al-DE treatment completely suppressed the weevil progeny in comparison with untreated control. The DEs coherence on wheat kernels matched directly to the insecticidal efficacy of the DE modifications against *S. granarius* adults. DEs particles damaged sensory organs and parts of the integument throughout the body of the granary weevil.

Wheat grains damage by the target insect was achieved with Al-DE and Ca-DE treatments. However, additional experiments are needed to determine the usage of these modified DEs in the control of the granary weevil, *S. granarius* (L.) at small scale storage.

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اثر تغییرات در خاک دیاتومه برای کنترل شپشه گندم *Sitophilus granarius* (Linnaeus) (Coleoptera: Curculionidae)

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چکیده

در این پژوهش، کارآیی غلظت های مختلف خاک دیاتومه اصلی (DE) و تغییر یافته (DEs) برای کنترل شپشه بالغ گندم *Sitophilus granarius* (Linnaeus) در طی دوره های مختلف در معرض قرار گرفتن (شامل ۱، ۲، ۴، ۷، و ۱۴ روز) ارزیابی شد. موثر بودن DEs با ثبت میرایی حشره بالغ و پارامترهای دیگر شامل تاثیر روی فرزندان، تلفات وزن دانه، و چسبندگی (coherance) DE روی دانه گندم ارزیابی شد.DEs تاثیر بیشتری از DE نشان داد. Al-DE موثرترین شکل تغییر یافته DE بود و بعد از آن Ca-DEو Na-DE بود. در دوره ۱۴ روزه، بیشترین مرگ ومیر حشره بالغ در مورد Al-DEو Ca-DEبه ۹۸٪ رسید که در مقایسه، این عدد در تیمار شاهد ۱۰٪ بود و تفاوتی شدیدا معنادار را نشان می داد. در غلظت ۱/۵٪، تیمار Al-DEتقریبا از زاد و ولد جلوگیری کرد (5.0±2.0) در مقایسه با تیمار شاهد که (83.333±4.041) بود. کمترین تلفات وزن دانه برای تیمار Al-DEثبت شد (۰/۷٪) و بعد از آن Ca-DE(۱۰٪)، و Na-DE (۱۳٪) و DE اولیه (۲۵٪) قرار داشتند در حالیکه تیمار شاهد (۳۸٪) بود. بررسی های فراساختمانی (Ultrastructural) با استفاده از میکروسکوپ الکترونی روبشی (SEM) روی شاخک، اجزای دهان، و قسمتهای انتهای عقبی حشره

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بالغ در تیمارهای دیاتومه و تیمار شاهد انجام شد. ذرات DEs در سراسر بدن حشره اندام های حسی و قسمت هایی از پوست را صدمه زد. مشاهدات از تخریب انواع مختلف بخش هایی از اندام حسی (club segment sensilla) ثبت شد. افزون بر این، شکاف هایی در پوست و بریدگی در قسمتهای دهانی و اندام زادآوری نیز به چشم می خورد. در غلظت ۱/۵٪، Al-DE و Ca-DE هر دو به خوبی به دانه گندم چسبیده بودند (به ترتیب 1.493±0.002 و 1.492±0.002). چسبندگی DEs روی دانه گندم مستقیما با کارآیی حشره کشی آن بر علیه حشره بالغ *S. granarius* همخوانی داشت.

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