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ORIGINAL ARTICLE

Efficacy of Nano-diatomaceous Earth against red flour beetle, *Tribolium castaneum* and confused Flour beetle, *Tribolium confusum* (Coleoptera: Tenebrionidae) under Laboratory and Storage conditions

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ABSTRACT
Tribolium confusum(Jacquelin) and Tribolium castaneum (Herbst), are widespread insects that attacks stored grains and foods in several commodities . The potential of Nano- Diatomaceous earth (Nano-DE) in comparison with natural Diatomaceous earth (DE) against the red flour beetle T. castaneum and the confused flour beetle, T. confusum under laboratory and stored conditions was evaluated .Percentage of larval mortality of tested insects increased with increase in the treatment concentrations. Larvae of T. confusum was more susceptible to the treatments than T. castaneum larvae. Nano-DE was more effective than natural-DE. The fecundity of tested insects was highly affected with both DE and nano-DE. The egg production was highly suppressed by nano-DE under stored conditions. The mean number of deposited eggs per female (the fertility) and % of adult emergence(F1) of each tested insects were greatly affected by natural DE and nano-DE in comparison to untreated with highly significant differences . Nano-DE strongly suppressed the number of deposited eggs of T. confusum more than T. castaneum (3.8±1.5 , 17.8±7.5 ,26.6±3.5 eggs/female and (13.8±1.5 , 37.8±7.5 , 46.6±3.5 eggs/female) after 20,90 and 120 storage interval days ,respectively .The persistent effect of nanoparticles displayed several different modes of action by reducing oviposition, adult emergence (F1) and infestation percentages of tested insects. The results showed that DE-nanoparticles can be used as a valuable tool in pest management programs of T. confusum and T. castaneum .
Keywords : *Nano- diatomaceous earth , SEM, Tribolium castaneum, Tribolium confusum , storage pests*

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INTRODUCTION
Tribolium confusum Jacquelin du Val, and *Tribolium castaneum* (Herbst), are widespread insects that attacks stored grains and foods in several commodities . In the store, beetles and larvae feed on broken and fine-grind grains and this makes them a more complicated problem. These pests may give rise to considerable economical losses if not adequately controlled because it has a very high rate of population increase [1].
The natural insecticides of low mammalian toxicity like diatomaceous earths (DEs) are used against a wide range of insect species. So, it can be inserted into Integrated Stored Grain Insect Management programs [2]. (DEs) contact directly with insect bodies resulting in absorbing the oily or waxy outer cuticle layer and then the insect loses water and dies [3]. Various DE formulations had been estimated against different stored product pests [4,5,6,7,8,9,10]. They are not toxic, can be easily removed from the grains and possibly recycled in storage bins.
Nanotechnology is a new promising field of research. It shows a wide range in various fields like insecticides, agriculture, pharmaceuticals and electronics. The possibility uses of nanotechnology are massive. The physical, biological and chemical properties of nanoparticles are associated with their atomic strength [11,12]. Silica nanoparticle does not affect the looseness and bulk density of grain mass like DE even with the highest dose used [13]. Under laboratory and store conditions, the nano particles of alamemium oxide and titanium oxide highly reduced the infestations of *S. oryzae* [14]. Nano particles (Cab-O-Sil-750 and Cab-O-Sil-500) were highly effective in controlling *S. oryzae* [15a]. Also, the nano-particle

(Zinc oxide ZnO) was more effective in decreasing the infestation of *S. oryzae* under laboratory and store condition [15b]. The nano Destruxin was tested against the locust and grasshopper which proved that the nano materials decreased the infestation number of both pests [16a, b].

The objective of our study was to evaluate the potential of Nano- Diatomaceous earth (Nano-DE) in comparison with natural Diatomaceous earth (DE) against the red flour beetle *T. castaneum* (Herbst) and the confused flour beetle, *T. confusum* (Jacquelin du Val) under laboratory and storage conditions.

MATERIAL AND METHODS

Tested Insects

Larvae and adults of *T. castaneum* and *T. confusum* were used in the experiments. The target insects were reared under laboratory conditions on semi-artificial diet (fine wheat with some adherent endosperm) with 20% glycin and 5% yeast powder. All cultures and experiments were held at 26 ± 2 °C and 70-80% R.H. with 16 hours light and 8 hours dark.

Diatomaceous earth (DE)

The Natural Diatomaceous earth (DE) formed in fresh water lake was used in the experiments.

Preparation of nano- DE

The Natural Diatomaceous earth (DE) was used for preparing nano- DE. DE Nano-particles were synthesized by hydrolyzing titanium tetra isopropoxide in a mixture of 1:1 anhydrous ethanol and water. 9 ml of titanium tetra isopr o-poxide is mixed with 41ml of anhydrous ethanol (A). 1:1 ethanol and water mixture is prepared. (B) Solution A is added in drop wise to solute ion B and stirred vigorously for 2hrs. At room temperature hydrolysis and condensation are performed, using 1M sulphuric acid and stirred for 2 hrs. Then the ageing was undertaken for 12hrs. The gel was transferred into an autoclave and tightly closed, and the mixture was subjected to hydrothermal treatment at 353K for 24hrs. After filtration the solid residue was washed thoroughly with water and ethanol mixture, dried at 373K in an oven and calcined at 773K .

Scanning Electron Microscope (SEM)

The examination, measurements and photographing of natural DE and DE-Nano particles were done through a Quanta Scanning Electron Microscope (FEG 250) equipped with image recording and processing system (SEM A fore).

Bioassays

The insecticidal efficacy of natural DE and nano-DE against tested insects

The insecticidal efficacy of natural DE and nano-DE were tested at three dose rates, 0.25, 0.50 and 1 g/kg wheat against the 3rd instar larvae of *T. castaneum and T. confusum* (Coleoptera: Tenebrionidae). For each case, four glass jars as replicates were used. Each replicate was treated individually with the respective treatments and then shaken manually for one minute to achieve equal distribution of the DEs. Subsequently, ten 3rd instar larvae of the two tested species were introduced into each glass jar and covered with muslin for sufficient ventilation. Twelve replicates glass jars containing untreated wheat served as control. Mortality was assessed after 7 d of exposure in the treated and untreated jars. All tests were conducted at 27 ± 2 °C and 65 ± 5% relative humidity (RH). All the experiments were repeated three times.

Ovipostional deterrent effect of tested natural DE and nano-DE against the target insects

The ovipositional deterrent effects of natural-DE and nano- DE were also tested. The natural-DE and nano-DE were used at the rate of 0.5 g/kg wheat. Four replicates of 100 g wheat for each treatment were used. Each replicate was treated individually with the treatments for 1 min and put inside glass jars. Four replicates in jars containing untreated wheat served as control. Subsequently, one paired of newly emerged adults (7–14day old) of mixed sex were introduced into each jar. The number of deposited eggs on treated or untreated wheat/female was counted.

Effect of different treatments against tested insects under store conditions

Experiment was designed to test the persistent effect of natural DE and nano-DE at rate (1.0 g/kg) of grains on tested insects at 20 day intervals over 120 days. All gunny sacks (20x20 cm each) were full of heat sterilized wheat grains (100 g each), fastened, each with a string. Four replicates of 100 g wheat grains for each treatment were used. Each replicate was treated individually with treatments and then shaken manually for 1 min to achieve equal distribution of the dust in the entire quantity and was placed in gunny sack. Four replicates jar containing untreated grain served as control. Following exposing to those treatments, two pairs of newly emerged beetles (7–14day old) of mixed sex were placed in a jar (2L capacity with four gunny sacks) and observed for egg laying. The number of deposited eggs on treated or untreated grains/female,% of adult emergence(F1) and % of infestation during storage intervals in the treated and untreated jars were calculated . Each experiment was repeated five times [17].

Statistical analysis

The data was analyzed using analysis of variance (ANOVA), where significant differences between the treatments were observed. Mean values were significantly separated by using the least significant difference (LSD) test at 5% level [18].

RESULTS AND DISCUSSIONS

The insecticidal efficacy of natural DE and nano-DE against tested insects

In all treatments, percentage of larval mortality of tested insects (Tables 1) increased with increase in the treatment concentrations. Larvae of *T. confusum* was more susceptible to the treatments than *T. castaneum* larvae. Nano-DE was more effective than natural-DE. The larval mortality percentage of *T. confusum* recorded 45, 39, 29% and 70, 65, 49% in case of DE and Nano-DE at 1.0, 0.5 and 0.25 % concentrations, respectively in comparison to 2% mortality in untreated control . After 7, 21and 45 days, mortality numbers of *S. oryzae* were significantly increased to 41.4±4.4, 47.8±5.8 and 50.6±3.6 individuals after application with 3% of nano Al2O3 as compared to 1.0 ± 2.8, 2.0 ± 5.1 and3.0 ± 3.4in the control, respectively [14]. Both nanoparticles (silica and silver) were highly effective on adults and larvae of *Callosobruchus maculatus* with 100% and 83% mortality, respectively [19]. Nanoparticles are more reactive than their bulk counterpart because of their increased surface to volume ratio [20].

The influence of (DE) combined with three fungal pathogens: *Beauveria bassiana, Metarhizium anisopliae* and *Isaria fumosorosea* against three moth species, *Plodia interpunctella*, *Ephestia cautella* and *E. kuehniella* was evaluated [9]. Both (Ca-DE) and (Na-DE) achieved the highest mortality percentages. The sharp edges of the diatom remains attributed the insecticidal efficacy of DE. As the insects crawl through treated grain and dusted bins, the DEs contact the insects and the sharp edges bore the insect's exoskeleton. The powdery DEs then absorb the body fluids causing death from desiccation [13].

Ovipostional deterrent effect of tested natural DE and nano-DE against the target insects

The fecundity of tested insects was highly affected with both DE and nano-DE. The egg production was highly suppressed by nano-DE .The mean number of the eggs laid /female of *T. castaneum* significantly decreased to 198.6 ±2.4 and 58.6 ± 9.4 eggs/ female when treated with DE and nano DE , respectively as compared to 291.1 ± 1.3eggs/ female in the control . While , in case of *T. confusum* ,the number of eggs/female significantly decreased to 144.6±9.4 and 48.6 ± 9.1 eggs/ female when treated with DE and nano- DE as compared to 278.6 ± 3.4eggs/ female in the control (Table 2). These results are in agreement with the research by [21]. They studied the egg-laying behavior of *C.maculatus* on mung beans when treated with diatomaceous earth Fossil (Shield & Silico – Sec). Also, the population of *S. oryzae* (L.), *T. castaneum* (Herbst) and *R. dominica* (Fabricius) were reduced by 98 to 100% in comparison to controls when tested with treated wheat (0.5 and 0.75 g of diatomaceous earth Protect-It® per kg of wheat).This effect is due to the repellent properties of diatomaceous earth, and probably has very good dispersal capacity in the grain mass [22]. The application of Ca-DE was highly suppressed the egg production (3.88± 0.35), followed by Al-DE (6.75 ± 0.25) eggs/female of *C. maculatus* in comparison with untreated control (50.2 ± 0.49) [8]. The egg production of *P. interpunctella* was strongly suppressed by Ca-DE/*B.bassiana* treatment (54.6±5.8 eggs/female), in comparison to untreated control (288.3±3.4 eggs/female) [9].

Effect of different treatments against tested insects under store conditions

The mean number of deposited eggs per female (the fertility) and % of adult emergence(F1) of each tested insects were greatly affected by natural DE and nano-DE in comparison to untreated with highly significant differences during stored conditions (Tables 3-4). Nano-DE strongly suppressed the number of deposited eggs of ***T. confusum*** more than ***T. castaneum*** (3.8±1.5 , 17.8±7.5 ,26.6±3.5 eggs/female and (13.8±1.5 , 37.8±7.5 , 46.6±3.5 eggs/female) after 20,90 and 120 storage interval days ,respectively . Also, the % adult emergence (F1) of tested insects was strongly reduced by DE and Nano-DE treatments. Under laboratory and store conditions, silica gel Cab-O-Sil-750 and silica gel Cab-O-Sil-500 were tested against *S. oryzae* [15a]. The number of eggs laid/ female were significantly decreased to 6±1.0 and 11±0.51 as compared to 99.1±1.43 and 97.2±1.82 in the control after 100 and 120 days. Also, adult emergence was reduced to91 and 90%, respectively after 120 days [15a]. Insects of stored products showed a wide extent of susceptibility to DE [23, 24]. The insecticidal efficacy of SilicoSec® (diatomaceous earth) against *T. castaneum*, adults and larvae was estimated. The adult fecundity in treated wheat was suppressed when compared with untreated wheat with significant difference [25].

Efficacy of Nano-DE on infestation percentages of the target insect pests under stored conditions was presented in (Fig 1). *T. confusum* was more sensitive than *T. castaneum* to Nano-DE *.* The infestation percentages of the tested insects under stored conditions were greatly suppressed by nano-DE in comparison to untreated control during storage intervals. DE had less contact to *T. castaneum* cuticle than other storage beetles .So; the application rate for it can be used for controlling infestations of other beetles [26]. Silica Nanoparticle does not affect the looseness and bulk density of grain mass like DE even with the highest dose used [13]. DEs possess insecticidal properties due to their sharp edges of the diatom remains. (DEs) contact directly with insect bodies resulting in absorbing the oily or waxy outer cuticle layer and then the insect loses water and dies [3].

Different modes of action of nano-DE as (reducing oviposition, adult emergence (F1) and infestation percentages of tested insects) were displayed due to their persistent effect.

Table 1: The insecticidal efficacy of the DE and nano-DE on larval mortality% of the target insects

Treatment	Concentrations	Tested insects % Larval mortality	
		T. castaneum	*T. confusum*
Natural- DE	**1.0**	40.0	45.0
	0.5	32.0	39.0
	0.25	22.0	29.0
Nano-DE	**1.0**	67.0	70.0
	0.5	53.0	65.0
	0.25	44.0	49.0
Control	**0.0**	1.0	2.0

Table 2. Ovipostional deterrent effect of tested DE and nano-DE against the target insects

Treatment	Mean number of eggs/female ± SE	
	T. castaneum	*T. confusum*
Natural-DE	198.6 ± 2.4	144.6 ± 9.4
Nano- DE	58.6 ± 9.4	48.6 ± 9.1
Control	291.1 ± 1.3	278.6 ± 3.4
F test	31.21	
Lsd5%	19.10	

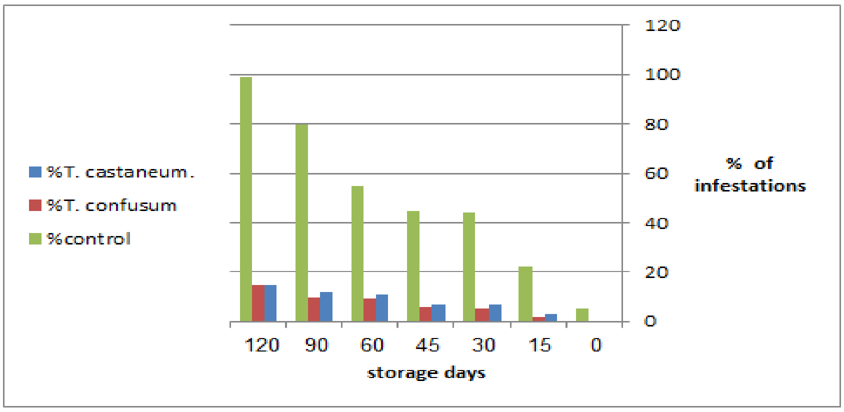
Table 3: Effect of different treatments on number of laid eggs/female and % of adult emergence (F1) of *T. confusum* during storage periods

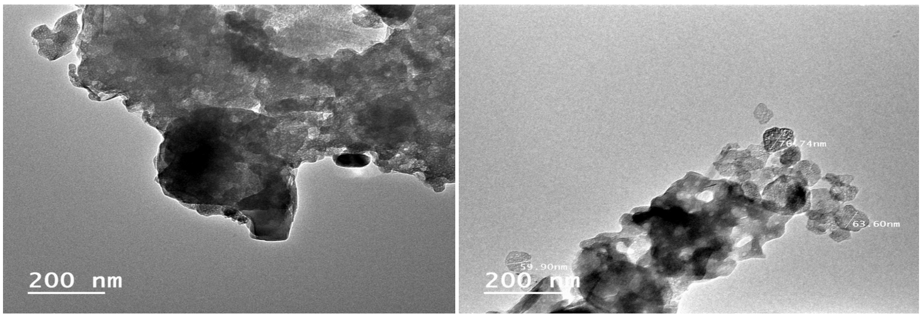
Storage Interval Days	Control		DE		Nano-DE	
	No. of Eggs/♀±S.E.	%adult Emergence (F1)	No. of Eggs/♀±S.E.	%adult Emergence (F1)	No. of Eggs/♀±S.E.	%adult Emergence (F1)
20	11.8±5.5	89	8.8±2.5	15	6.8±8.8	1
90	94.5±5.8	97	31.8±2.5	28	20.8±7.5	13
120	99.8±1.7	100	39.8±3.9	36	20.1±1.9	14
F value	20.1		21.1		10.1	
LSD 5%	12		17		9	

Table 4: Effect of different treatments on number of laid eggs/female and % of adult emergence (F1) of *T. castaneum* during storage periods

Storage Interval Days	Control		DE		Nano-DE	
	No. of Eggs/♀±S.E.	%adult Emergence (F1)	No. of Eggs/♀±S.E.	%adult Emergence (F1)	No. of Eggs/♀±S.E.	%adult Emergence (F1)
20	23.7±6.5	90	10.8±8.5	17	3.8±1.5	1
90	93.1±2.6	98	37.8±7.7	38	17.8±7.5	10
120	99.9±8.9	100	41.8±1.9	49	26.6±3.5	22
F value	10.9		17.3		12.1	
LSD 5%	11		14		10	

Fig 1. Efficacy of Nano-DE on Infestation percentages of the target insect pests under stored conditions





(a) (b)

Fig 2. Scanning electron microscopy (SEM)of Nano-DE particles

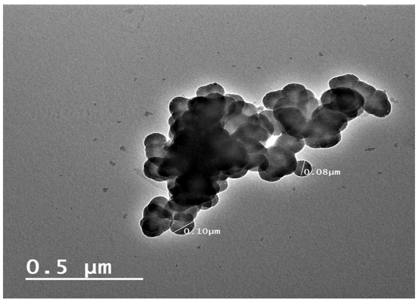


Fig 3. Scanning electron microscopy(SEM) of natural DE particles

CONCLUSIONS

The results showed that DE-nanoparticles can be used as a valuable tool in pest management programs of *T. confusum* and *T. castaneum* . The DE-nanoparticles can be removed by conventional milling process unlike sprayable formulations of conventional pesticides, leaving residues on the stored grain..The DE-Nanoparticles have an excellent potency as grain protecting agent if applied with adequate safety measures. Our results could lead to open up newer pass of using nanomaterial-based technology for the control of stored grain insects.

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CITATION OF THIS ARTICLE

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