

# LABORATORY EVALUATION OF EFFICACY OF THREE DIATOMACEOUS EARTH FORMULATIONS AGAINST *TRIBOLIUM CASTANEUM* HERBST (COLEOPTERA: TENEBRIONIDAE) IN STORED WHEAT

**B. G. J. Kabir**

Department of Crop Protection, Faculty of Agriculture,  
University of Maiduguri

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## Abstract

Laboratory experiments were conducted to evaluate three DE formulations - Protect-It, DiaFil 610 and Celite 209 at dose rates 500, 750 and 1000 mg/kg of wheat, against the red flour beetle *Tribolium castaneum* Herbst under ambient laboratory conditions (26 - 32°C and 48 - 65% r.h.). Mortality of exposed adults increased with increase in dose and exposure period; however consistency was notable only after 7 days of exposure. With all DE formulations, mortality was low on grains treated at 500 and 750 mg/kg after 3 and 7 days of exposure. Protect-It was highly effective at 1000 mg/kg causing 100% adult mortality after 3 days of exposure. Similarly, all the three DE formulations caused complete adult mortality on grains treated at 750 and 1000 mg/kg after 14 days of exposure. In addition, at these dose rates complete progeny suppression was recorded except on grain treated with DiaFil 610 at 750 mg/kg. The findings of this study have demonstrated that the efficacy of the three DE formulations to control *T. castaneum* and as potential alternative to synthetic insecticides.

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**Keywords:** DE, formulation, *T. castaneum*, efficacy, control

## Introduction

The red flour beetle *Tribolium castaneum* is one of the most common secondary part of stored grain and also an important grain pest, because when present in the grain contributes to contamination and depreciation of the commercial value of the flours of the cereals (Morsaro *et al.*, 2006).

The use of synthetic pesticides is under increasing scrutiny because of problems with environmental contamination, atmospheric ozone-depletion (i.e., methyl bromide), potential carcinogens, concern over residues in

commodities, exposure to applicators, resistance in pests, and general consumer aversion to the use of chemicals. Therefore, there is a growing need for nonchemical pest control methods (Fields and Korunic, 2000)

Diatomaceous earths (DEs) are very promising alternatives to traditional residual grain protectants (Athanassiou and Korunic, 2007). They are of natural origin, have negligible toxicity to mammals and can be applied with similar technology to that needed for residual pesticides (Subramanyam and Roesli, 2000). DE has a unique, non-chemical mode of action against, killing insects by adhering to and abrading the insect cuticle, thereby adsorbing lipids in the epicuticle and causing death due to water loss and desiccation (Korunic, 1998). Several DE formulations are now commercially available and many studies document that they are very effective against a wide range of stored-product insect species (Stathers *et al.*, 2004). However, several factors affect their efficacy including the DE formulation itself (Korunic, 1998) and the commodity protected (Athanassiou *et al.*, 2003; Kavallieratos *et al.*, 2005) Although the insecticidal properties of all DEs are affected by the same parameters (Korunic, 1997, 1998), the insecticidal value against a given species varies among commercially available DE formulations (Fields and Korunic, 2000; Vayias *et al.*, 2006; Athanassiou *et al.*, 2007).

These issues suggest that DEs must be assessed separately against each stored-product insect species and stored commodities; and therefore results obtained under one set of conditions could not be transferrable to another. Moreover, there is dearth of information on efficacies of DEs against local strains of stored-product insect species in Nigeria. In the present work, the insecticidal effect of three DE formulations was evaluated against the red flour beetle, *Tribolium castaneum* in treated wheat. The effects on parental mortality and progeny production were assessed.

## Materials And Methods

*Tribolium castaneum* individuals were reared on wheat flour and bakers' yeast mixture (19: 1 w/w). Adults, 1-3-weeks-old were used in the tests.

The three DE formulations used in the present test were Protect-It, DiaFil 610 and Celite 209. Protect-It (Hedley Technologies Inc., Canada) is an enhanced DE that contains 83.7% SiO<sub>2</sub> with 10% silica aerogel. The particle size distribution is between 5 and 6 µm (Korunic & Fields 1995). DiaFil 610 (Celite Corporation, USA) is a white fresh water DE containing 89 % amorphous SiO<sub>2</sub> and crystalline silica >0.1 %. The median particle size is 10 µm, (Korunic and Fields, 2006). Celite 209 (Celite Corporation, USA) is marine DE containing 87 % SiO<sub>2</sub>, 65.0% of particles <12 µm (Fields and Korunic, 2000; Saez and Mora, 2007). The three DE

formulations were obtained from Diatom Research and Consulting Inc., Canada.

The grain used was wheat (var. Cetia) selected and released by the Lake Chad Research Institute, Maiduguri, Nigeria. The grain came from the Foundation Seed stock of the Institute. Prior to setting up of experiment the grains were cleaned and disinfested by exposing to 60°C for three hours in an air-circulated oven. Thereafter, allowed to equilibrate with laboratory condition for 10 days.

The treatments consisted of three DE formulations and three DE dose rates and an untreated control, each replicated three times. Lots of 150 g of wheat were treated with four dose rates of 0 (control), 500, 750 and 1000 mg/kg of each DE formulation, giving a total of ten treatment combinations. The lots were placed in 500 ml capacity bottles and the appropriate amount of DE was added each lot. The bottles were shaken manually for 5 minutes to achieve an even distribution of the DE on the grains. Subsequently, three samples of 50 g of treated or control wheat were taken from each lot, and placed in 250 ml capacity glass jars. In each jar, 30 *T. castaneum* adults were introduced, then the jars were covered with perforated plastic lids fitted with filter papers to allow gaseous exchange. The jars were kept under ambient laboratory conditions at 26 - 32°C and 48 - 65% r.h. Adult mortality was assessed after 3, 7, 14 and 21 days of exposure. After 21 days count the parent adults were removed and discarded. The number of F1 progeny produced was counted after 40 days. The number of progeny included all live and dead adults found in treated and untreated wheat.

Where necessary mortality data were corrected using Abbott's formula (Abbott, 1925). Before analysis data on mortality and number of progeny were arcsine and square root [ $\sqrt{(x+0.5)}$ ] transformed, respectively, to normalize treatment variances. The transformed data were then subjected to analysis of variance (ANOVA) using the GLM procedure of Statistix 8.0 to determine differences among treatment means. Treatment means were separated using Tukey Kramer HSD test at the  $\alpha = 0.05$  level.

## Results

Three days of exposure of *T. castaneum* adults to three DE formulations at dose rate of 500 and 750 mg/kg, resulted in low mortality which did not exceed 12%. There were no significant differences ( $P > 0.5$ ) between the two dose rates or among DE formulations. However, on wheat treated at 1000 mg/kg mortality was significantly higher on wheat treated with Protect-It, which was about 2 and 5 times higher than with Celite 209 and DiaFil 610, respectively, used at the same rate (Table 1). Prolonged exposure to DE resulted in increased adult mortality at all dose rates. Thus, after 7 days of exposure, adult mortality increased to 100% when Protect-It

and Celite 209 were used at 1000 mg/kg. On wheat treated with Celite 209 at 750mg/kg, adult mortality was significantly higher than on Protect-It and DiaFil 610 which were comparable. Similarly, after 14 days of exposure, mortality was further increased and all adults were dead in wheat sample treated at 750 or 1000 mg/kg of all the three DE formulations. Even the lowest dose rate (500 mg/kg) of Celite 209 caused complete adult mortality, though this was not significantly different ( $P=0.3513$ ) from Protect-It and DiaFil 610. At the 21 days of exposure, complete adult mortality was recorded in all wheat treated at all dose rates of three DE formulations, with the exception of sample treated with DiaFil 610 at 500 mg/kg, which was even then not significantly different ( $P=0.4219$ ) from the other two DE formulations.

Progeny production by *T. castaneum* was greatly suppressed by DE treatment, given that on all treated grains, with the exception of sample treated with DiaFil 610 at 500 mg/kg, the mean number of F1 progeny was <1. In fact no progeny developed on wheat treated with Protect-It at all dose rates, Celite 209 at  $\geq 750$ mg/kg and DiaFil 610 at 1000mg/kg (Table 2). Generally, progeny development was poor, with the mean number of F1 progeny in the untreated control being  $16.7 \pm 3.2$ . However, in comparison with the untreated control, all treatment resulted in > 80% progeny suppression.

Table 1. Mean mortality (%  $\pm$  SE) of *T. castaneum* adults after 3, 7, 14 and 21 days of exposure to wheat treated with three diatomaceous earth (DE) formulations at three dose rates

DE Formulation	DE Dose rate (mg/kg of wheat)			F	P
	500	750	1000		
3 d					
Protect-It	0.0 $\pm$ 0.0bA	2.2 $\pm$ 1.1bA	88.9 $\pm$ 6.2aA	113	< 0.0001
DiaFil 610	8.9 $\pm$ 4.4aA	4.4 $\pm$ 1.1aA	17.8 $\pm$ 8.0aB	1.41	0.3141
Celite 209	1.1 $\pm$ 1.1cA	11.1 $\pm$ 4.0bA	44.5 $\pm$ 4.0aB	32.2	< 0.0001
F	2.68	3.18	25.1		
P	0.4177	0.1142	0.0012		
7 d					
Protect-It	4.4 $\pm$ 1.1cB	35.6 $\pm$ 9.5bB	100 $\pm$ 0.0aA	120	< 0.0001
DiaFil 610	25.6 $\pm$ 4.8bAB	24.4 $\pm$ 4.4bB	90.0 $\pm$ 10.0aA	17.2	0.0033
Celite 209	32.6 $\pm$ 10.9bAB	94.4 $\pm$ 1.3aA	100 $\pm$ 0.0aA	53.1	0.0002
F	7.58	40.2	1.0	-	-
P	0.0228	0.0003	0.4219	-	-
14 d					

Protect-It	86.7 ± 7.0 aA	100 ± 0.0 aA	100 ± 0.0 aA	1.87	0.2341
DiaFil 610	97.8 ± 1.1 aA	100 ± 0.0 aA	100 ± 0.0 aA	4.0	0.0787
Celite 209	100 ± 0.0 aA	100 ± 0.0 aA	100 ± 0.0 aA	-	-
F	1.25	-	-	-	-
P	0.3513	-	-	-	-
21 d					
Protect-It	100 ± 0.0 aA	100 ± 0.0 aA	100 ± 0.0 aA	1.0	0.4219
DiaFil 610	96.7 ± 3.3aA	100 ± 0.0 aA	100 ± 0.0 aA	-	-
Celite 209	100 ± 0.0 aA	100 ± 0.0 aA	100 ± 0.0 aA	-	-
F	1.0	-	-	-	-
P	0.4219	-	-	-	-

Within each exposure period, means for dose rates (rows, lower case letter) and for DE formulation (columns, capital letters) followed by the same letter are not significantly different ( $P > 0.05$ ; Tukey Kramer HSD Test).

Table 2: Progeny production (mean number of adults ± SE) of *T. castaneum* on wheat treated with each of three DE formulations at three dose rates. Mean number of progeny in the untreated control = 16.7±3.2.

DE Formulation	Dose (mg/kg grain)	No. of progeny (Mean ± SE)	Progeny suppression (%)
Protect-It	500	0.0±0.0	100
	750	0.0±0.0	100
	1000	0.0±0.0	100
	F	1.0	-
	P	0.4219	-
DiaFil 610	500	3.3±1.5	80.2
	750	0.7±0.7	95.8
	1000	0.0±0.0	100
	F	4.37	-
	P	0.621	-
Celite 209	500	0.3±0.3	98.2
	750	0.0±0.0	100
	1000	0.0±0.0	100
	F	-	-
	P	-	-

## Discussion

The result of the present study has shown that commercial DE formulations, Protect-It, DiaFil 610 and Celite 209 could be used with success against *T. castaneum* in stored wheat. Our results agree with

obtained by other authors (Athanassiou *et al.*, 2003; 2007; Kostyukovsky *et al.*, 2006; Vayias *et al.*, 2006; Shayeateh and Ziaee, 2007; Wakil *et al.*, 2008; Shams *et al.* 2011.) who observed that DE efficacy is highly influenced by dose of DE and time of exposure as well as type of DE formulation. In the present study after the 3 and 5 days of exposure adult mortality in grains treated at 500 or 750 mg/kg was lower than 36% except in samples treated with Celite 209 at 750mg/kg. This can be explained by the mode of action of DE, which cause dead of affected insect by desiccations (Korunic, 1998). At these rates the exposed insect might not have picked-up sufficient DE particles that cause excessive water loss which leads to mortality. Further, after the 3 days exposure there was a wide variation in mortality between dose rate and DE formulations. In the light of our results insect mortality as a result of exposure to DE stabilizes at least 3 days after treatment even at prescribed dose rates. This may perhaps be a drawbacks associated with use of DE to control stored-product insects. According to Subramanian and Roesli (2000) the “speed of kill” is perhaps more important than 100% mortality, because delayed mortality may allow insects to disperse from the treated grain and colonize untreated parts of the grain mass. This factor may be crucial for the use of DE to control *T. castaneum* since this species is more mobile and has a relatively short development period, which allows surviving adults to rapidly reproduce progeny and continue to cause damage. Another possible explanation of the variation is that, since mixed sex and differently aged adults were bioassayed, the variation might have been due to differences in their physiology.

Progeny development was poor even in the untreated control. This is probably because *T. castaneum* is a secondary pest and thrives better on broken grains than sound grains (Dobie *et al.*, 1991). Despite the poor progeny development, DE treatment has profound effect on the progeny production, given that effective progeny suppression (>95%) was noted at dose rates  $\geq 750$  mg/kg. The effect on progeny could be attributed to the life history of *T. castaneum* whose all life stages are found outside the grain, thus continuously exposed to DE particles. The larvae of *T. castaneum* are reported to be very susceptible to DEs (Kostyukovsky *et al.*, 2010) whereas the adults are tolerant (Fields and Korunic, 2000). Despite the high tolerance of adults of *Tribolium* spp to DEs (Vayias and Athanassiou, 2004) the susceptibility of the larvae may slowly control *Tribolium* spp population in treated commodities.

According to the results of the present study, Protect-It appears to be the most effective, followed by Celite 209 and then by DiaFil 610, at least in the short term exposure. In line with our findings, Korunic and Fields (2006) reported that Protect-It was more effective than DiaFil 610 when both were tested against *Sitophilus oryzae* Motschulsky. In another study, Fields *et al.*

(2003) tested the enhanced DE Protect-It and natural DE formulation Perma Guard, similar in physical and chemical characteristic to DiaFil 610, and found Protect-It significantly more effective against *S. oryzae* than Perma Guard.

Although all DEs have the same mode of action (Korunic, 1998), the different additives that commercially available DEs contain appear to be the main cause of efficacy difference (Athanassiou *et al.*, 2007). The presence of 10% Silica aerogel in Protect-It explains its relatively higher efficacy than Celite 209 and DiaFil 610.

In conclusion, the findings of this study indicate that, Protect-It, DiaFil 610 and Celite 209 could provide excellent control of *T. castaneum* on wheat when used at 750 - 1000 mg/kg. Within this dose range, abrasion and absorption of cuticular wax occurs faster, causing death in a short time compared with those at lower dose rates.

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### **Disclaimer**

This paper reports the results of laboratory research only. Mention of a proprietary or a trade name does not constitute a recommendation or endorsement.

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