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Efficacy and Persistence of Raw Diatomaceous Earth Against *Tribolium castaneum* Herbst (Coleoptera: Tenebrionidae) on Stored Maize, Sorghum and Wheat

B.G.J. Kabir, M. Lawan and F.M. Gambo

Department of Crop Protection, Faculty of Agriculture,
University of Maiduguri, P.M.B. 1069, Maiduguri, Borno State, Nigeria

Abstract: Laboratory experiments were carried out to evaluate the efficacy of raw diatomaceous earth (DE) against *T. castaneum* on maize: *Zea mays* (L.), sorghum: *Sorghum bicolor* (L.) (Moench) and wheat: *Triticum aestivum* (L). The tests were conducted under ambient laboratory conditions (26-34°C and 27-51% R.H.). Thirty adult insects were exposed to 50 g grain sample treated with the DE at five dose rates: 125, 250, 500, 1000 and 1500 ppm. Mortality of exposed adults was assessed after 7 d and 14 d exposure, while number of progeny from exposed adults was counted after 40 and 80 d. On all grain types used adult mortality increased with dose and exposure period. In addition, grain type significantly influenced DE effectiveness. On any grain type used none of the dose rates caused complete mortality after 7 d exposure. However, after 14 d exposure 100% mortality was observed on wheat and maize treated at 500 and 1000 ppm, respectively; whereas on sorghum treated at 1500 ppm mortality was 72.8±2.1%. Progeny production was significantly suppressed in DE treated grains in comparison with untreated controls. No progeny developed on maize treated at 1000 ppm or wheat at 1500 ppm over the exposure period, but progeny development was not totally avoided on sorghum. The result indicates that it is possible to control *T. castaneum* using the raw DE tested in this study.

Key words: Diatomaceous earth · *T. castaneum* · Grain type · Dose rate · Exposure period

INTRODUCTION

The red flour beetle, *Tribolium castaneum* (Herbst) is a very common pest of a wide range of stored products in tropical and sub-tropical regions. It is frequently the first pest to arrive on stored food, such as wheat flour, peanuts and milled rice and its number rises quickly once a population has become established [1]. In relation to grains it is the most common secondary pest of stored grain and an important one because when present in the grain it contributes to contamination and depreciation of the commercial value of cereals flour [2]. The major problem by this species is contamination rather than actual food weight losses, which seem to be rather small [3]. Besides contamination with the bodies and insect frass, the food substrate will be exposed to quinones that are released from the thoracic and abdominal defence glands [4].

The control of this pest is achieved mainly with chemical insecticides. However, widespread resistance to some organophosphates (e.g. Malathion) and pyrethroid (e.g. resmethrin and bioresmethrin) has been reported in this species [5, 6, 7]. Apart from the problem of resistance, consumers demand for pesticide residue-free food and health concerns have lead researchers to evaluate alternative control methods for stored-product pest management [8]. Currently trend use of reduced-risk or low-toxicity insecticides is being promoted as a replacement for conventional chemical grain protectants [9]. The use of diatomaceous earth (DE) is one of the most promising alternatives to traditional residual insecticides against several pest species, infesting stored grain including *T. castaneum* [10, 11] Diatomaceous earth (DE) is natural product mined from deposits of fossilized diatoms [12]. It is of natural origin, leave no harmful residues on the product and have low mammalian toxicity [13]. DE absorbs lipids and abrades the epicuticular wax layer of insects causing loss of body water and death within hours or days [14]. DE use for grain storage pest management has been undertaken and registered for use as grain protectants in many countries [13]. Though DEs were found to be very effective against stored-product

Corresponding Author: B.G.J. Kabir, Department of Crop Protection, Faculty of Agriculture, University of Maiduguri, P.M.B. 1069, Maiduguri, Borno State, Nigeria. Tel: +234 802 6798714, E-mail: bgkabir@yahoo.com.

insects, several factors affect their efficacy. These include target species [15], life stage [16, 17], strain [18, 19], the DE formulation itself [20] and grain commodity protected [15, 21, 22]. Most of the literature concern commercial DE formulations (e.g. Dryacide®, Protect-It®, SilicoSec®, Insecto® etc.). The information available suggests that each DE must be assessed separately against a stored-product insect species and grain commodity. Moreover, there is a dearth of documented information of studies relating to raw DEs.

In the present work, we examined the efficacy of raw DE against *T. castaneum*. The objectives were to: evaluate the effects of dose rate and exposure periods on adult mortality and progeny production; and assess the influence of grain type on efficacy of raw DE.

MATERIALS AND METHODS

Test Insects: The test insects used in our study, *T. castaneum* came from a laboratory culture on bread wheat flour under ambient conditions (26-34°C, 27-51% R.H.). For the different experiments the test insects were reared on wheat flour and bakers yeast (20:1 w/w). The adults used in this experiment were 1-3 weeks old.

Grains: The grains used were maize (Local cultivar), sorghum (*cv.* Ajama) and wheat (variety unknown) all obtained from commercial sources in Maiduguri. The grains were cleaned and disinfected by exposing the grains to 60°C for 3hrs in an air circulation elective oven (Chirana). Before the beginning of the experiments, the grains were left at ambient conditions for 7 days to equilibrate with the relative humidity level.

Diatomaceous Earth: The DE was obtained from the Department of Geology, University of Maiduguri, Nigeria. The DE was supplied in a form of crude soft chalky rock. The rock was milled finely in the laboratory using a pestle and mortar and passed through a fine sieve to obtain a powdery consistency. The fine powder was analyzed for pH and tapped density in accordance with methods described by Korunic (1998), while mineral composition was analyzed in the Laboratories of the Department of Geology, University of Maiduguri, by X-ray Fluorescence method on Minimate (Pananlytical Company, U.K.). The raw DE has the following properties: tapped density – 312.5 g/l, pH-9.2; mineral composition: SiO_2-28.7%, Al_2O_3-12.6%, CaO-26.5%, Na_2O-11.6%, K_2O-9.3%, FeO-0.9%, ZnO-0.33%, CuO-0.18%, MnO-0.55%.

Grain Treatment: The DE dose rates tested were 125, 250, 500, 1000 and 1500 ppm. For each grain type and dose rate combination lots of 250 were placed in the capacity jars and the appropriate amount of DE was added to the jars. The jars were then shaken manually for 5 minutes to achieve equal distribution of the dust in the entire grain mass. For each grain type there was an additional untreated 250 g lot which served as the control.

Bioassay: Exposure studies were carried out under ambient laboratory conditions (26-34°C, 25-51% r.h.). Five samples of 50 g of each treatment combination and the untreated control were taken from each lot and placed in 250 ml capacity jars. Thirty mixed-sex adult *T. castaneum* were introduced into each jar. The jars were capped with perforated plastic lids and kept on laboratory shelf. Dead adults were counted after 7 d and 14 d. After the 14 d count all (dead and alive) adults were removed. The jars returned to the shelf and held at the same conditions for 36 d more. After this the emerged adults were counted and the dead removed. The same procedure was repeated after additional 40 d later.

Data Analysis: The data were analyzed using the GLM procedure of statistical software (Statistix 8), with adult mortality as response variable and dose rate, exposure period and grain type as main effects. Where it was necessary, the mortality counts were corrected by using Abbott's formula [23]. Data on mortality were arcsine transformed to normalize variance. Given the importance of dose rate, grain type and exposure period in this study, for each grain type the mortality data were submitted to one-way analysis of variance (ANOVA) for dose by exposure period and then to ANOVA appropriate for factorial design to measure interactions between the main effects. Similar procedures were followed for data on progeny. In this case the data were square root v(x + 0.5) transformed and one-way analysis of variance (ANOVA) was performed within each dose rate to determine differences among grain types, the number of progeny and percentage of dead progenies were the response variables, while the main effects were dose rate and grain type. Differences between means were compared using Turkey-Kramer HSD test at $P<0.05$.

RESULTS

For mortality of *T. castaneum* adults, all the main effects (dose rate, exposure period and grain type) were significant. Similarly, as well as the associated interactions were significant (Table 1). Generally, increase in dose rate

Table 1: ANOVA parameters for main and interaction effects on mortality of *T. castaneum* subjected to three grain types treated at different dose rates of DE for two exposure periods

Source	df	F	P
Dose rate	5	636.20	<0.0001
Grain type	2	847.15	<0.0001
Exposure period	1	919.11	<0.0001
Dose rate x Grain type	10	39.34	<0.0001
Dose rate x exposure period	5	35.12	<0.0001
Grain type x exposure period	2	13.96	<0.0001
Dose rate x grain type x exposure period	10	27.25	<0.0001
Error	144	-	-
Total	179	-	-

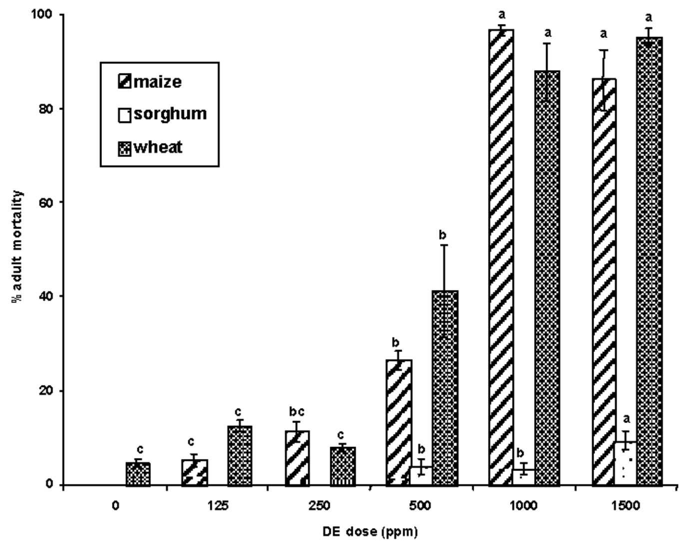

Fig. 1: Mortality (%) (mean ± SE) of *T. castaneum* adults on three grain types treated with different doses of raw DE after 7 d exposure (means within the same grain type followed by the same letter are not significantly different; Tukey-Kramer HSD test, at $P < 0.05$)

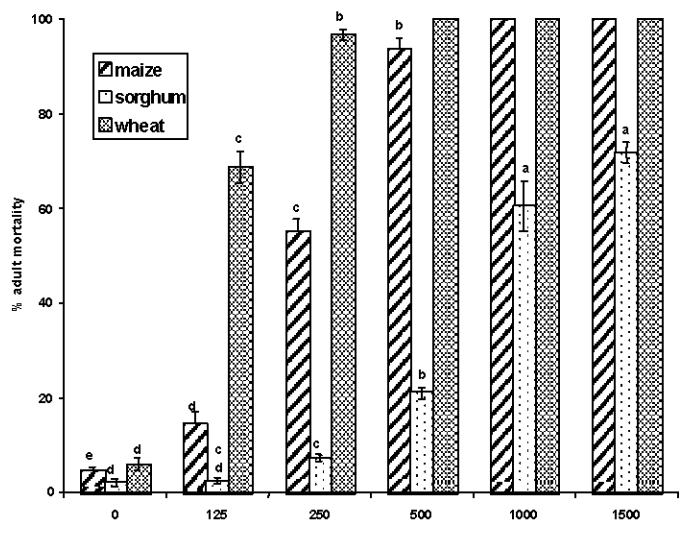

Fig. 2: Mean mortality (%± SE) of *T. castaneum* adults on three grain types treated with different doses of raw DE after 14 d exposure (means within the same grain type followed by the same letter are not significantly different; no letter appear where 100% mortality was recorded: Tukey-Kramer HSD test, at $P < 0.05$)

significantly increased adult mortality. After 7 d exposure to treated grain adult mortality was very low on sorghum and did not exceed 12% even at the highest dose rate. In contrast, more insects died on maize and wheat. Higher mortality was observed at 1000 ppm on maize and wheat, where 86.1 to 96.7% of exposed adult died. However, the difference between dose rates were not significant (P>0.05) on both grain types (Fig. 1).

Increase of exposure period to DE treated grains increased adult mortality. Thus 14 d of continues exposure resulted in increase in mortality consistent with dose rate (Fig. 2). However, the pattern for grain type remained similar to that of 7 d exposure. DE treatment was most effective on wheat with 100% mortality achieved at =500 ppm and even the lowest dose rate (125 ppm) resulted in >68% adult mortality. This was followed by maize with complete adult mortality observed at 1000 ppm. In contrast, on sorghum treated at 1000 and 1500 ppm adult mortality ranged from 60.6 to 71.8%, respectively, even then the difference was not significant (P>0.05).

Analysis of variance for number of progeny produced by *T. castaneum* indicated significant main effects of dose rate and grain type as well as interaction effects between the two (P<0.05). For percentage of dead progenies at 40 d both the main and interaction effects were significant, but for 80d effect of dose was not significant (P = 0.440), though interaction effect of dose rate and grain type was significant (P < 0.001).

DE treatment significantly influenced progeny production in *T. castaneum*. Increase in dose rate resulted in greater progeny suppression. In the untreated controls significantly (P<0.05) more progenies developed on sorghum and wheat than on maize (Table 2) when examined after 40 d. On DE treated grains, at any dose rate where they developed, significantly more progenies developed on sorghum than on wheat or maize, but the latter two commodities did not significant differ from each other. In addition, on maize and wheat no progeny developed when treated at =1000 ppm. The percentage of dead progenies did not follow an ordered pattern, as many replicates did not yield progeny.

Observation after additional 40 d incubation showed increase in the number of progenies in most treatment where earlier present increase, though the pattern remained similar (Table 3). In the untreated control the differences were similar to the preceding observation. At all dose rates <1000 ppm significantly (P<0.05) more progeny developed on sorghum and less on maize. On maize and wheat treated at =1000 ppm no progeny developed, but on sorghum even at 1500 ppm, complete progeny suppression was not achieved. Changes in percentages of dead progenies by dose rate remained more or less similar to the 40 d observation for the very reason that many replicate did not yield progeny.

Table 2: Progeny production (mean number of adults ± SE) and percentage (% ± SE) of dead individuals on each grain type treated with DE 26 days after removal of *T. castaneum* adults

DE application rate	Grain type	No. of progeny	% dead progeny
0.0 ppm ($F_{2,15}$ = 33.3, P<0.0001)	Maize	8.2±1.1b*	17.0±1.30
	Sorghum	28.6±1.7a	0.0±0.00
	Wheat	22.6±2.7a	1.5±0.90
125 ppm ($F_{2,15}$ = 79.5, P<0.0001)	Maize	3.0±0.8b	50.7±18.0
	Sorghum	28.4±0.8a	1.3±0.80
	Wheat	4.2±0.9b	12.3±5.20
250 ppm ($F_{2,15}$ = 57.8, P<0.0001)	Maize	2.0±0.3b	76.7±10.0
	Sorghum	19.0±2.3a	11.8±10.0
	Wheat	1.6±0.5b	46.7±16.2
500 ppm ($F_{2,15}$ = 47.5, P<0.0001)	Maize	0.4±0.3b	40.0±2.50
	Sorghum	14.4±2.5a	7.7±3.50
	Wheat	1.0±0.6b	26.7±19.4
1000 ppm ($F_{2,15}$ = 46.4, P =<0.0001)	Maize	0.0±0.0b	-
	Sorghum	10.2±0.4a	10.1±3.50
	Wheat	0.2±0.2b	20.0±20.0
1500 ppm ($F_{2,15}$ = 66.6, P <0.0001)	Maize	0.0±0.0b	-
	Sorghum	4.6±0.9a	32.3±5.10
	Wheat	0.0±0.0a	-

*Means within the same dose rate followed by the same letter are not significantly different: Tukey-Kramer HSD test, at P < 0.05)

Table 3: Progeny production (mean number of adults ± SE), percentage of dead individuals (% ± SE) on each grain type treated with DE 66 days after removal of *T. castaneum* adults

DE application rate	Grain type	No. of progeny	% dead progeny
0.0 ppm ($F_{2,15}$ = 41.7, P<0.0001	Maize	18.2±1.9b*	22.6±4.5
	Sorghum	44.6±1.5a	1.3±0.9
	Wheat	40.0±3.6a	12.4±2.2
125 ($F_{2,15}$ = 39.9, P<0.0001)	Maize	3.4±1.8c	5.0±3.9
	Sorghum	47.2±1.6a	3.8±0.4
	Wheat	21.6±5.2b	15.3±3.5
250 ppm ($F_{2,15}$ = 42.8, P<0.0001)	Maize	0.8±0.4c	50.0±22.4
	Sorghum	30.8±3.2a	3.3±1.7
	Wheat	7.4±2.2b	27.4±8.4
500 ppm ($F_{2,15}$ = 349, P<0.0001)	Maize	0.2±0.0b	100±0.0
	Sorghum	23.4±1.0a	12.7±1.5
	Wheat	0.6±0.4b	40.0±24.5
1000 ppm ($F_{2,15}$ = 110, P<0.0001)	Maize	0.0±0.0b	-
	Sorghum	12.0±2.1a	53.1±8.0
	Wheat	0.0±0.0b	-
1500 ppm ($F_{2,15}$ = 164, =<0.0001)	Maize	0.0±0.0b	-
	Sorghum	9.6±1.1a	66.5±6.8
	Wheat	0.0±0.0b	-

* Means within the same dose rate followed by the same letter are not significantly different: Tukey-Kramer HSD test, at P < 0.05)

DISCUSSION

Based on the results obtained, by using the raw DE higher mortality and progeny suppression of *T. castaneum* can be achieved. According to available literature, the efficacy of commercially available DE formulation has been sufficiently investigated and generally, the results reported are in agreement with the finding of this study. Fields and Korunic [24] by using different DEs noted that dose rate of 1000 ppm were required for satisfactory level of mortality of *T. castaneum*. Lorini and Beckel [11] recorded 100% mortality of *T. castaneum* following 20 d exposure to wheat treated with Keepdry® at 500, 1000 ppm and Insecto at 1000 ppm. Dowdy [25] showed that mortality of *T. castaneum* increase with longer exposure of insects to DE.

In the present study significant interaction effects were noted between all the main effects on mortality, confirming earlier reports that efficacy is influenced by interaction between dose rates, grain type and exposure period. Similarly significant interaction between dosage and exposure period in relation to mortality has been reported by Arthur [26] and dose and exposure period, dose and commodity and exposure and commodity by Vayias and Stephou [21].

The result of this study showed low mortality level at lower doses. Many workers reported similar observation. Low mortality rate of *T. confusum* in low dosages of 125 and 250 ppm were also observed by Athanassiou *et al.* [27] using DE at 250 ppm. They found that mortality of *T. confusum* was lower than 20% after 7 d exposure. Similarly Arnaud *et al.* [19] reported that some population of *T. castaneum* sustained low mortality (<10%) even after 21 d exposure to DE. The fact that longer exposure periods are required at low dose rate to achieve satisfactory level of mortality could be explained by the mode of action of DE. Death of exposed insects occurs because of desiccation provoked by abrasion and removal of epicuticular wax [20]. Therefore, at lower doses the process occurs slowly delaying death by dehydration for longer period when compared with higher doses.

Considerable variations in efficacy were observed among the grain types used in this study. In the light of our findings, the same dose rate does not provide the same level of protection when applied on different grains. Mortality of *T. castaneum* on sorghum was generally lower compared to maize and wheat. Even the highest dose rate applied to sorghum resulted in <74% mortality following 14 d exposure and did not completely stopped progeny production. This probably implies that sorghum grains retain less DE particles on the surface, thus higher dose rates may be needed to achieve the same effect as in maize or wheat. The efficiency of DEs against stored-product insects depend on several factors including grain commodity protected [15, 27, 28, 29]. Considerable variations in efficacy of DEs among different grain commodities were noted in previous studies. Athanassiou *et al.* [15] stated that DE efficacy in barley or rice was higher than in maize. Vayias *et al.* [30] found that DE was much more effective and stable when applied on wheat rather than on sorghum. Also Athanassiou and Kavallieratos [31] reported that retention of DE on wheat, barley or rice was much higher than on maize. Much earlier LaHue [32] found that less DE dust were retained on maize than on wheat and sorghum. In partial contrast we found high mortality of *T. castaneum* on wheat and maize than on sorghum. Varietal factor might have been responsible for this difference. For instance, when Dryacide® was tested against *R. dominica*, noticeable variations in efficacy among different classes of wheat were found [33].

Athanassiou and Korunic [10] emphasized that progeny production in a treated substrate is perhaps more important than parental mortality, because a grain protestant should protect the grain for a long storage period. In our work complete progeny suppression was obtained on maize and wheat treated at =1000 ppm. The emergence of few progenies on sorghum at these dose rates could be ascribed to the survival of some adults at the end of the exposure period. Moreover some species of stored–product insect exposed to DE lay eggs before they are killed [34]. The results of this study and those available in literature suggest that dose rates higher than those giving 100% mortality are required for complete progeny suppression.

In conclusion, the results obtained indicated that the raw DE tested here was effective against *T. castaneum* infesting stored grains. Application of 1000 ppm resulted in complete adult mortality and progeny suppression in maize or wheat. For sorghum, however doses higher than 1500 pmm would be required to achieve the same level of efficacy. However, further investigations are needed to confirm this inference. The factor that determine DE-grain interaction has not been studied in detail [15]. Investigations in this direction are equally useful in order to understand the factors involved in DE efficacy on grain commodities.

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