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Bioactivity of raw diatomaceous earth against *Rhyzopertha dominica* Fab. (Coleoptera: Bostrichidae): Effects of grain type, dose rate and exposure period

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Abstract: *Laboratory experiments were carried out to evaluate the efficacy of raw diatomaceous earth (DE) against R. dominica 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 bioassayed on 50 g grain sample treated with the DE at five dose rates: 125, 250, 500, 1000 and 1500 ppm. Adult mortality, progeny production and percentage of insect-damaged kernels (IDK) were assessed. On all grain tested mortality increased with higher dose rate and longer exposure period. After 14 days exposure to the highest dose rate, adult mortality was 98.0±0.8, 93.2±1.8 and 99.3±0.75% on maize, sorghum and wheat, respectively. Progeny production was considerably suppressed on treated grains in comparison with the untreated respective controls but significantly influenced by grain type. Similarly, the percentage of IDK decreased with increase in dose rate. At 1500 ppm IDK in maize, sorghum and wheat were 1.2±0.5, 2.8±0.5 and 2.2±0.8 % as compared to 54.1±8.6, 45.1±1.8 and 84.1±1.75% in the untreated control, respectively. To provide effective control of R. dominica using the raw DE, dose rates higher than 1500 ppm are required.*
Keywords*: raw DE, R. dominica, adult mortality, progeny production, grain damage*

I. Introduction

The lesser grain borer, *R. dominica* is a major pest of stored grains worldwide [1]. The females lay eggs externally on the grain, and the larva bores its way into the kernel and develops there until reaching the adult stage. Upon reaching the adult stage, the insect bores out of the kernel, creating a large exit hole [2]. This exit hole is often indicative of an insect-damaged kernel (IDK) which is often a factor in the grading and marketing of grain [3]. The life history makes *R. dominica* a primary pest and one that is difficult to manage because most of the life cycle is spent inside the grain kernel [4]. The control of stored-product insects relies heavily on the use of synthetic insecticides with residual activity and fumigants [5,6]. Several problems have emerged with the use of synthetic chemicals such as development of resistance by many species of stored-product insects including *R. dominica* [7], worker exposure, residues in human and animal foods, and environmental contamination [5,8].

Search for alternative control methods of stored-product insects has received renewed attention with emphasis to reduce the application of insecticides and minimize associated hazards. This led to the development of inert dusts formulations, which include diatomaceous earth [9]. DE is a soft rock that is the fossilized remains of unicellular algae called diatoms. DE is an inert dust that had been extensively tested for control of stored grain insect pests [10]. DE particles absorb wax from insect cuticle, causing death due to desiccation [9]. DE is largely composed of amorphous silicon dioxide which is non-toxic to mammals [11], does not break down rapidly and does not affect end use quality [12]. Because of these properties, DEs have been described as promising alternative to conventional insecticides [5]. Recently sufficient information on the efficacies of commercial DE formulations have been documented. Many DE formulations are now commercially available and are generally effective against stored-product insects [13].

However, there is scarcity of published information on studies concerning raw DEs. Moreover DEs differ considerably in their efficacies against stored-product insects. More recent studies [5,14,15,16,17,18,19] reveal that type of grain commodity also affects efficacy of DEs. The present study was, therefore, undertaken to evaluate the efficacy of raw DE applied at different dose rates on maize, sorghum and wheat grains to control *R. dominica*.

II. Materials And Methods

2.1 Test Insects

The test insect, *R. dominica* used in this study was obtained from a laboratory culture maintained on wheat. The adult insects used in this experiment were also cultured on wheat and were 1-3 weeks old.

2.2 Grains

The grains used were maize (Local cultivar), sorghum (*cv*. Ajama) and wheat (variety unknown) all obtained from commercial sources in Monday Market, Maiduguri north eastern Nigeria. The grains were cleaned and disinfested by exposing thin layers of grain to 60^0C for 3 h in an air circulation electric oven (Chirana). Before the beginning of the experiments, the grains were left at ambient conditions as stated above for 7 d to equilibrate with the relative humidity level.

2.3 Diatomaceous Earth

The DE was obtained from the Department of Geology, University of Maiduguri, Nigeria. The soft chalky rock was milled finely and passed through a fine sieve to obtain a powdery consistency. The fine powder was then analysed for pH and tapped density according to Korunic [20] while mineral composition was analyzed 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%.

2.4 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 g of grain were place in 1L capacity jars, and the appropriate amount of DE was added. 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 250 g untreated lot, which served as the control.

2.5 Bioassay

Exposure studies were carried out under ambient laboratory conditions (26–34^0C, 25–51% r.h.). Five samples of 50 g grain of each treatment combination and the untreated control were taken from each lot and placed in 250 ml capacity jars. Thirty mixed-sex adult *R. dominica* were introduced into each jar. The jars were capped with perforated plastic lids and kept on a shelf. Adult mortality was assessed after 7 d and 14 d. After the 14 d counts all dead and live adults were removed. The jars were held under the same conditions for 40 d more. After this, the emerged adults were counted and the dead removed. The same procedure was repeated after additional 40 d later. After the second progeny count, IDK was assessed by sub-sampling 5 g kernels from each replicate. Grain kernels with emergence holes were counted and expressed as percentage of the number of kernels in a sub-sample.

2.6 Data analysis

Where it was necessary, the mortality counts were corrected by using Abbott's [21] formula. Data on mortality were arcsine transformed to normalise variance. 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. Given the importance of dose rate, grain type and exposure period in this study, for each grain type the mortality date 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 $\sqrt{(x + 0.5)}$ and then analysed with number of progeny and percentage of dead progenies as the response variables, while the main effects were dose rate and grain type. One-way ANOVA was performed within each dose rate to determine differences among grain types. In all cases differences between treatment means were compared using Tukey–Kramer (HSD) test at (P<0.05)

III. Results And Discussion

The main effects of dose rate, exposure period and grain type on adult mortality of *R. dominica* were all significant (P<0.05). Similarly, all associated interactions were also significant except for interaction effects between grain type x exposure period (P=0.167) and dose rate x exposure period x grain type (P=0.206).

On all grain types significant differences (P<0.05) in mortality levels between dose rates were noted after 7 d exposure, with mortality levels in controls significantly lower than in treated grains at lowest dose rate (Fig. 1). In general, increase in dosage resulted in increased adult mortality. Regardless of grain type, adult mortality increased after longer exposure period. Thus, 14 d exposure resulted in increased adult mortality on all grain types. In this case, mortality was considerably higher even at lowest dose rates, notably on maize and wheat. In contrast, sorghum treated at ≤500 ppm showed that adult mortality did not exceed 21.8% and differences were significant. At the highest dose rate, mortality was > 93.2%, although complete mortality was not achieved on any grain type (Fig. 1).

The observed adult mortality after 14 d exposure at 1500 ppm to this raw DE product was not a complete kill but an acceptable level of control. This rate was more than the labeled rates for commercial DE products. For instance, the labeled rates for Protect-It, Insecto and Dryacide are 400, 500 and 1000 ppm, respectively [22]. It is well established that DEs differ in efficacy. Fields and Korunic [23] investigated the efficacy of six commercial DEs from different geographical locations against five stored-products insect species including *R. dominica* and found up to 70% differences in mortality between DE sources depending on experimental conditions. Hence the results of this study suggest that relatively higher dose of this product is required to kill *R. dominica* within 14 d.

The most important finding of this study is that the raw DE product used affects *R. dominica* in ways similar to commercial DE formulations. Our results are in agreement with the findings of La Hue [24], Wakil et al. [25] and Athanassiou et al. [26] who also reported that adult mortality increased with increase in dose rate and exposure period, and efficacy varied between grain types. Variations in efficacy of DEs among different grain types has been noted in a series of recent studies, involving tests with different DE products on different grain types [14,15,27,28]. The significant interaction effect ($P<0.001$) between dose rate and grain type on mortality of *R. dominica* noted in this study, confirms the aforementioned statements that DE efficacy is influenced by grain type.

The number of progeny produced by *R. dominica* and their subsequent survival was significantly affected by DE dose rate and grain type. All the main effects and associated interactions concerned were significant ($P<0.001$). Noticeable decline in progeny number was observed with increase in dose rate. Also higher proportion of emerged adults died with increase in dose rate. Fourty days after removal of exposed parents, at rates ≤500 ppm and untreated control progeny production was significantly ($P<0.05$) higher on wheat and lowest on maize (Table 1). The lowest dose rate resulted in more than two-thirds progeny reduction on all grain types compared to their respective untreated controls. At the highest dose rate, fewer progenies (<4/jar) were produced and more than half died. Similar trend was observed after additional 40 d. However, none of the treatments completely killed emerging adults except for maize treated at highest dose rate.

Generally, the number of emerged progeny on maize was considerably lower than on wheat or sorghum and more progenies died on maize. This was expected as type of grain affects biological parameters of insects such as development, oviposition rate or progeny production [29].

In this study, none of the dose rates gave 100% adult mortality even after 14 d continuous exposure, which probably explains the emergence of few progenies in the treated grains. In a similar test, using Protect-It against *S. oryzae*, Arthur and Throne [6] reported that progenies emerged in treatment where 100% mortality of exposed adults was recorded. This could be attributed to the mode of action of DEs. It appears that stored-product insect can lay eggs following initial exposure to DEs before toxicity and subsequently lethality manifest. Considering the biology of *R. dominica* some first instar larva might tolerate short-term exposure to DE before burrowing into grain kernels where they are protected from further exposure, thus completing development within the grain kernel.

Percentage of IDK reflected the effects of raw DE on number of progeny. All the main and interaction effects were significant ($P<0.001$). Similarly, significant differences in levels of grain damage were noted between dose rates on all grains (Table 2). Damage was relatively lower on maize and sorghum, while wheat sustained heavy damage with >50% kernels damaged at ≤500 ppm. Finally, none of the dose rates completely stopped grain damage, but IDK did not exceed 3.5% on any grain treated at 1500 ppm. The emergence of some progenies in grains treated at 1500 ppm explains the presence of damaged kernels in the treatments.

While commercial DE formulations provided complete control of *R. dominica* at dose rate range of 500 to 1000 ppm [22, 25]. Similar effect was not achieved with this raw DE at 1500 ppm. According to Fields and Korunic [30], effective DE should have >80% SiO_2, a pH below 8.5, and a tapped density < 300 g/L. The raw DE used in this study has in particular has very low (28.7%) SiO_2 content, the component responsible for insecticidal effect. On the other hand, most commercial DE products contain other substances such as silica aerogel or baits, which either improve physical properties or enhance efficacy [10,11] . The aforementioned could be the reasons for reduced efficacy of this raw DE at high dose rates. Nevertheless, the efficacy observed can be considered satisfactory given that >90% mortality level and considerable prevention of progeny production and grain damage were noted.

IV. Conclusion

The results of this study indicate that the raw DE was effective against *R. dominica* and can provide substantial level of control of *R. dominica* despite low SiO_2 content. Efficacy varied with dose, exposure period and grain type. To achieve 100% adult mortality, progeny suppression or prevention of grain damage, dose rates higher than 1500 ppm should be used. Further investigation involving higher dose rates that can provide complete control of *R. dominica* are recommended.

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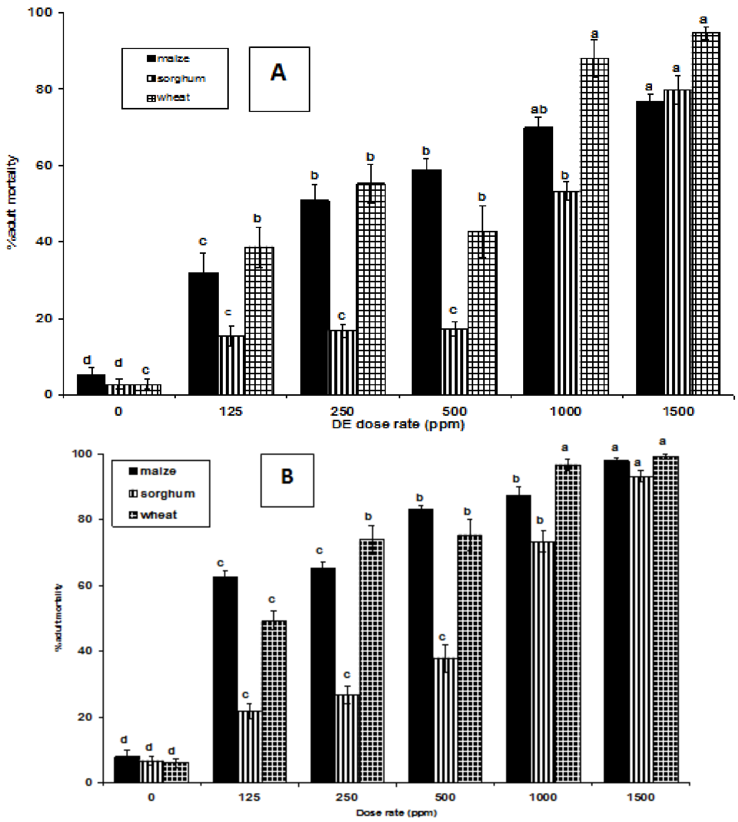

Figure 1. Mean ± SE mortality (%) of *R dominica* adults on three grain types treated with different doses of raw DE after 7 d (A) and 14 d (B) exposure (means within the same grain type and exposure period followed by the same letter are not significantly different; Tukey-Kramer HSD test, at $P < 0.05$)

Table 1. Progeny production (mean number of adults ± SE) and percentage (% ± SE) of dead progenies on eachgrain type treated with DE incubated for 40 and 80 days after removal of parent *R. dominica* adults. Means within the same dose rate and exposure period followed by the same letter are not significantly different: Tukey-Kramer HSD test, at $P < 0.05$)

DE application rate (ppm)	Grain type	Incubation period			
		40 d		80 d	
		Mean no. progeny	%dead progeny	Mean no. progeny	%dead progeny
0.0 ppm	Maize	14.4±1.7c*	11.0±2.0	47.8±4.8c	12.3±1.2
	Sorghum	106.6±5.1b	3.2±0.6	247.4±13.5b	1.7±0.3
	Wheat	237.6±15.8a	1.3±0.4	602.0±69.9a	7.9±0.9
125 ppm	Maize	8.4±1.4c	15.0±4.3	17.4±3.1c	42.7±5.5
	Sorghum	78.8±3.7b	3.3±0.5	218.1±1.8b	4.2±0.3
	Wheat	126.2±19.9a	2.6±0.4	339.0±37.3a	12.9±1.7
250 ppm	Maize	5.0±0.3c	27.6±3.9	3.6±1.1c	52.6±13.8
	Sorghum	45.6±5.3b	7.7±1.3	177.6±24.9b	8.9±1.3
	Wheat	93.4±8.9a	4.3±0.9	346.8±26.4a	9.8±0.3
500 ppm	Maize	2.6±0.7c	61.9±16.6	7.0±0.6c	68.7±8.1
	Sorghum	50.4±0.7b	4.7±0.6	127.4±5.9b	13.5±0.5
	Wheat	80.0±9.3a	7.6±1.3	332.6±26.4a	16.1±0.9
1000 ppm	Maize	2.2±0.4b	73.3±11.3	1.6±0.8b	55.0±22.9
	Sorghum	17.0±4.0a	47.2±9.8	41.85.2a	31.3±4.2
	Wheat	10.2±2.4a	44.9±7.9	50.6±11.7a	60.4±7.6
1500 ppm	Maize	1.6±0.5a	80.0±12.7	1.6±0.4a	100±0.0
	Sorghum	3.4±0.8a	74.8±7.1	4.8±1.1a	62.0±7.6

Wheat	2.9±0.5a	69.4±7.4	7.4±4.8a	55.6±22.8

Table 2. Percentage of insect-damaged kernel (IDK) (mean ± SE) from exposing 30 parental *R. dominica* for 14d on three grain types and 5 DE application rates. Grains were held for 80 days after parent adults were removed. Means within the same column followed by the same letter are not significantly different: Tukey-Kramer HSD test, at P <0.05

DE application rate (ppm)	%IDK (mean±SE)/ grain type		
	Maize	Sorghum	Wheat
0	54.1±8.6a*	45.1±1.8a	84.1±1.7a
125	10.0±2.8b	57.3±7.4a	66.5±5.6bc
250	8.3±2.0bc	30.8±2.3b	74.5±1.4ab
500	4.5±0.9bcd	22.5±1.3b	57.0±2.9c
1000	1.8±0.5cd	8.7±0.7c	6.7±0.7d
1500	1.2±0.5d	2.8±0.5d	2.2±0.8e

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