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Insecticidal Effect of Bularafa Raw Diatomaceous Earth (BRDE) Against *Rhyzopertha dominica* (F.) on Stored Maize (Zea mays L.) and Sorghum (Sorghum bicolor [L.] Moench) Grains

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ABSTRACT

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The toxicity of Bularafa raw diatomaceous earth (BRDE) to Rhyzopertha dominica adults on stored maize and sorghum grains was conducted under ambient laboratory conditions (31-34°C and 24 - 30% R.H.). The BRDE was tested at doses: 0 (untreated control), 250, 500, 1,000 1,500, and 2000 mg/kg and Protect-It[®] at 1000 mg/kg of grain which served as a check. Thirty (30) mixed-sex adult insects were exposed to 50 g of freshly treated grains, and mortality was observed after 7 and 14 days. The results showed that a higher number of exposed Rhyzopertha dominica adults died on maize than on sorghum, and mortality increased with an increase in dose and exposure period. After 7 days of exposure to treated maize and sorghum grains, the LD₉₅ values were 4981 and 5890 mg/kg of BRDE, respectively. The corresponding values after 14 days were 1561 and 3206 mg/kg, respectively.

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INTRODUCTION

Cereals belong to the monocot families and are cultivated widely to obtain the edible components of their fruit seeds. Cereal grains are grown in greater quantities and provide more food energy worldwide than any other type of crops. They are therefore called staple crops (Idem and Showemimu, 2004). Cereal grains provide essential nutrients and energy

in the everyday human diet through direct human consumption and also via meat production since they comprise a major livestock feed. According to the Food and Organization, Agriculture total crop production during 2016 reached 2577.85 million tons, whereas the production of coarse grains (cereal grains other than wheat and rice used primarily for animal feed or brewing) reached 1330.02 million tons (FAO-AMIS, 2017). Among the cereal crops, maize, wheat, and rice were the most cultivated crops representing about 90% of all cereal crops produced globally (FAOSTAT, 2021). Cereals have high level of utilization in Nigeria and are mainly processed traditionally due to their abundant availability (Ukwuru et al., 2018).

Maize (Zea mays L.), is an important food, cash, and industrial crop (Adebayo and Omoloye, 2016). It is the most extensively cultivated cereal crop in Nigeria after guinea corn and millet, and provides families with the much needed nutrients such as carbohydrates, proteins, fats, vitamin B and minerals (Tongjura et al., 2010). Nigeria accounts for 96 % of the total maize production in the Sub-Saharan Africa (SSA) where it is the most widely-grown staple cereal food occupying more than 33 million ha each year (FAOSTAT, 2015). Nigeria produces about 7.7 million tonnes of maize grains annually, representing 0.9% of the world production (Amudalat, 2015).

Sorghum (Sorghum bicolor [L.] Moench) is the 5th most important world cereal crop after maize (Zea mays L.), wheat (Triticum aestivum L.), rice (Oryza sativa L.), and barley (Hordeum vulgare L.) (FAO 2019). It is a staple food crop in the drier parts of Africa, China, and India (Ajeigbe et al., 2018; Mrema et al., 2020). Sorghum is the largest staple cereal crop accounting for 50% of the total output and occupying about 45% of the total land area devoted to cereal crops production in Nigeria (FAO 2019). Nigeria's bulk of sorghum production is derived from the

Northern Guinea and Sudan/Sahel ecologies of Northern Nigeria (Yahaya *et al.*, 2021)

Rhyzopertha dominica (F.) is a major primary and cosmopolitan pest of stored grains worldwide, it is an internal feeder of various stored raw grains and well adapted to dry conditions (Emekci et al., 2004). It reduces the essential amino acid contents of wheat, maize and sorghum (Jood et al., 1995). Females lay eggs on the surface of grain kernels, and upon hatching, the larva enters the kernel (Neethirajan et al., 2007; Ozkaya et al., 2009) and remains inside until maturity (Chanbang et al., 2007). Significant physical damage and weight loss result from internal and external feeding by larvae and mature adults, respectively (Kaoud et al., 2013).

Stored grains are susceptible to infestation by insect pests (Beriş *et al.*, 2011) especially in developing countries in the tropics due to favorable climatic conditions (Bekele *et al.*, 1997). These pests are responsible for decline in quantity, quality and germination potential (Olakojo and Akinlosutu, 2004). Controlling insects, especially during post-harvesting stages that can involve long-term storage (in silos or bunkers), to prevent infestation and provide this high quality standard of grain is a very challenging problem (Losic and Korunic 2018).

Synthetic pesticides have been considered the most effective means to control insect pests of stored products. Indiscriminate use of these chemicals have given increase to many serious problems, including resistance of pest species, toxic residues, and environmental and human health concerns (Sanna *et al.*, 2004; Tapondjou *et al.*, 2005; Koul *et al.*, 2008). The public awareness and concern for food and environmental safety led to an increased search for eco-friendly methods to protect stored grains. Diatomaceous earth is one of the materials that can be easily used due to its effective properties. Diatomaceous earths have

proved to be capable of controlling various groups of insect pests, and can replace the common chemical insecticides (Islam *et al.*, 2010).

Diatomaceous earth is an inert dust; it contains fossilized diatoms. single-celled algae. Basically, it is amorphous silicon dioxide with an internal structure that is very effective in the absorption of insects' protective cuticle wax layer, leading to insects' death through desiccation and, to much lesser extent, by (Korunic, 2013). However, abrasion insecticidal efficiency depends significantly physical, chemical the upon morphological characteristics of the diatomite species in DE (Korunić et al. 2016, Rojht et al. 2010) and particle size (Korunić 2013). It also depends upon the resistance of the host insects (Aoues et al., 2017). Diatomaceous earths have several advantages given that they do not affect the end use quality of grains, are effective for long durations, low mammalian toxicity, they are also applied using same technology as conventional synthetic insecticides and can control synthetic insecticide- resistant strains of insects (Shah and Khan, 2014; Baldassari and Martini, 2014). Unlike the conventional contact insecticides DEs function through their physical properties and are therefore generally slower in action (Arthur, 1999). The effects of DE on a wide range of stored product beetles including Rhyzopertha dominica have been reported by previous workers (Kavallieratos et al., 2007; Kostyukovsky et al., 2010).

Several studies have demonstrated the insecticidal potential of local Raw DEs from different geographic locations including Africa (Machekano *et al.*, 2017; Mvumi *et al.*, 2006), Argentina (Dal Bello *et al.*, 2018), Egypt (Abd El-Aziz and Abd El-Ghany, 2018), South-Eastern Europe (Vayias *et al.*,

2009), Serbia (Andrić et al., 2012; Kljajić et al., 2010), Turkey (Akçali et al., 2018; Gultekin et al., 2018; Mortazavi and Ferizil, 2018) Greece and Romania (Athanassiou et al., 2016) and Croatia (Liška et al., 2018) for controlling stored-product insects. In Nigeria, most farmers store grains in small quantities using different storage structures such as pots, bags, silos, metal and plastic containers, while commercial grain dealers resort to use of storage bags (Kabir et al., 2012). Application of Raw diatomaceous Earth can easily be adopted for such storage practices. In Northern Nigeria, large deposits of Raw diatomaceous earth have been identified in Borno, Gombe, Niger and Yobe states and studies have shown that it has insecticidal activities and useful in production of dermatological products, human hygiene products as well as in food and beverages industry (Nwaubani et al., 2014: Otitodun et al., 2015 and NEITI, 2019). It can be used to control Sitophilus zeamais (Motschulsky), Rhvzopcrtha dominica (F.), SitophiIus oryzae (L.), Callosobruchus subinnotatus (Pic.), Callosobruchus maculatus (F.) and Tribolium castaneum (Herbst) effectively on stored grains and its efficacy is comparable to many commercial formulations (Kabir et al., 2011, 2012, 2013; (Nwaubani et al., 20 14; Abdulrahman et al., 2017; Audu et al., 2017; Abdulrahman and Kabir, 2018) and Medugu et al., 2020).

The insecticidal potential of the vast deposits of Raw Diatomaceous earth in Northern Nigeria is not fully utilized for the control of an important storage insect pest such as R. dominica. In this study, we examined the insecticidal effect of RDE from Bularafa on R. dominica. The objectives were to (1). Evaluate the influence of dose rates and exposure periods on toxicity; and (2). Assess the effect of grain types on BRDE efficacy.

METHODOLOGY

Study Site and Experimental Material

The experiment was conducted in the Research Laboratory Department of Biological Sciences, University of Maiduguri, Nigeria. The BRDE in the form of soft chalky rock was obtained from the Bularafa community in Yobe State, Northeastern Nigeria and identified in the laboratory of the Department of Geology, University of Maiduguri. It was milled and its mineral composition analyzed X-ray was by Fluorescence method on Minimate (Pananlytical Company, U.K.). Its pH and tapped density were analyzed by methods described by Korunic (1997). The BRDE has a tapped density of 312.5 g/l, pH-9.2 and a silicon dioxide (SiO₂) content of 28.7%. The commercial formulation of DE (Protect-It®) was obtained from samples provided by Diatom Research and Consulting Inc., Toronto, Canada. It is an enhanced DE that contains approximately 83.7% amorphous SiO₂ with 10% silica aerogel, with a median particle size between 5 and 6 µm. Insecticidefree maize grains (var. Yarchadi) and sorghum grains (var. Chakalare) were obtained from Borno State Agricultural Development Programme (BOSADP) Maiduguri, Borno State. Before setting up the experiment all grains were cleaned and disinfected by exposure to 60°C for 3 h in an air circulation electric oven and were left at ambient conditions for 7 days to equilibrate with the relative humidity level (Kabir, 2013).

Sources of Insects and Culturing Procedure

Rhyzopertha dominica adults were obtained from stock cultures maintained in the Entomology Laboratory, Department of Crop Protection, University of Maiduguri Nigeria. They were used to establish insect cultures on wheat grains and maintained under ambient conditions in the laboratory. Culturing

required placing 200 mixed-sex adult insects on 500 g of wheat grains in a jar. All insects were removed after 15 days and grains were maintained and observed for the emergence of new adults which were used for the study.

Grain Treatment

The BRDE dose rates tested were 250, 500, 1,000, 1,500, 2000 mg/kg and 1000 mg/kg Protect-It® as a check. For each grain type (maize and sorghum) and dose rate combination, 200 g lots of grains were placed in 1L capacity jars and the appropriate amount of dust 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 200 g untreated lot which served as the control.

Bioassay

The lots above were then divided into four samples of 50 g for each treatment combination and the untreated control and placed in 250 ml capacity glass bottles. Thirty mixed-sex *R. dominica* adults (1–2 weeks old) were introduced into each bottle, they were then covered with muslin cloth fitted with a rubber band to allow gaseous exchange. The bottles were then kept at laboratory conditions of 31-34° C and 24 - 30% R.H. Adult mortality was recorded on 7 and 14 days of exposure (Kabir *et al.* 2012).

Adult Mortality of *Rhyzopertha dominica*:

Adult mortality in both treated and untreated (control) bottles were assessed at 7 and 14 days after infestation with weevils (an insect is assumed dead when there is no movement on probing with a pin). All adult insects were removed from each bottle, and both dead and living insects were counted and recorded. After the 7-day count, live insects were returned to their respective bottles to wait for the 14-day count.

Experimental Design and Data Analyses

The toxicity test was a factorial experiment in a Complete Randomized Design replicated four times. The main plot factor was the grain type, exposure period was the sub-plot factor and DE dose rates sub-sub plot factor. Adult mortality data were corrected for mortality in the control by Abbott's formula (Abbott, 1925) and then transformed to arcsine transformation before the analysis. Data were

RESULTS

Toxicity of BRDE to R. dominica Adults

The main effects of grain type, exposure period and BRDE doses on *R. dominica* mortality were all significant (P. Significantly higher adult mortality was recorded on maize grain, than on sorghum grain. Similarly, more beetles died at 14 days

subjected to Analysis of Variance (ANOVA) using the General Linear Model (GLM) procedure of statistical software (Statistics 8.0). Differences between treatment means were separated using Tukey-Kramer Honestly Significant Difference (HSD) test at a 5% level of probability. The lethal doses for 50% (LD₅₀) and 95% (LD₉₅) of the population were calculated by probit analysis using StatsDirect® statistical software.

than at 7 days of exposure to treated grains. Mortality of exposed beetles increased with an increase in BRDE dose, and the differences were significant (P among all doses tested. Protect-It® applied at 1000 mg/kg resulted in more beetle mortality than the BRDE applied at 2000 mg/kg (Table 1).

Table 1: Effects of grain type, exposure period and BRDE doses on adult *R. dominica* mortality.

Treatment	Mean adult mortality (%)	
Grain type (A)		
Maize	70.1(56.9)a	
Sorghum	53.9(47.2)b	
SE±	0.9	
Exposure period (B)		
7 days	55.6(48.2)b	
14 days	68.3(55.7)a	
SE±	0.8	
DE doses (mg/kg)(C)		
0	1.5(7.0)g	
250	27.9(31.9)f	
500	48.3(44.0)e	
1000	62.3(52.1)d	
1500	73.9(59.3)c	
2000	90.6(72.2)b	
Check	95.4(77.6)a	
SE±	1.7	
Interaction		
AXB	ns	
AXC	*	
BXC	*	
AXBXC	*	

Means within a treatment in a column followed by the same letter are not significantly different, (Tukey Kramer, HSD Test, $P \le 0.05$). Values in parenthesis are arcsine transformed values.

^{*-} significant; ns- not significant Check (Protect-It® at 1000 mg/kg)

Table 2 shows the interaction effect of grain type and BRDE doses on adult *R*. *dominica* mortality. Adult mortality in the untreated control was very low (< 2%) on both grain types. The mean percentage of adult *R*. *dominica* mortality caused by 1000 mg/kg Protect-It® and the highest dose of BRDE

(2000 mg/kg) on both grain types was not significantly different. The RBDE applied at 500-1500 mg/kg caused significantly higher adult mortality in maize grain than in sorghum grain. However, the efficacy of the commercial DE (check) against *R. dominica* was not influenced by grain type.

Table 2: Interaction effect of grain type and BRDE doses on adult R. dominica mortality

BRDE dose (mg/kg)	Adult mortalit	y (%)
	Grain	type
	Maize	Sorghum
0	1.7(7.5)i	1.3(6.6)i
250	40.4(39.4)f	15.4(23.1)h
500	68.8(56.0)e	27.9(31.9)g
1000	78.3(62.2)d	46.3(42.9)f
1500	85.4(67.5)cd	62.5(52.2)e
2000	90.4(72.0)bc	90.8(72.3)a-c
check	97.5(80.9)ab	93.3(75.0)a-c
SE±	2.5	,

Means followed by the same letter(s) are not significantly different, (Tukey Kramer, HSD Test, *P*≤0.05). Values in parenthesis are arcsine transformed values. Check (Protect-It® at 1000 mg/kg)

The interaction effect of exposure period and BRDE doses on adult R. *dominica* mortality is presented in Table 3. There was a general increase in mortality with increase in exposure period. Thus, significantly higher beetle mortality was observed during the 14 day

exposure on all BRDE treated grains. Except for the untreated control and 1000 mg/kg Protect-It[®], in addition to the increasing adult mortality with increase in all BRDE doses, significantly higher proportions of beetles died after 14d than 7 day exposure.

Table 3: Interaction effect of exposure period and BRDE doses on adult R. dominica mortality

	Adult mortalit	y (%)
	Exposure p	period
BRDE dose (mg/kg)	7d	14d
0	0.4(3.6)i	2.5(9.1)i
250	18.8(25.7)h	37.1(37.5)g
500	39.2(38.8)g	57.5(49.3)f
1000	52.9(46.7)f	71.7(57.9)de
1500	62.1(52.0)ef	85.8(67.9)bc
2000	84.6(66.9)cd	96.7(79.5)a
Check	93.3(75.0)ab	97.5(80.9)a
$SE\pm$	2.4	

Means followed by the same letter(s) are not significantly different, (Tukey Kramer, HSD Test, $P \le 0.05$). Values in parenthesis are arcsine transformed values. Check (Protect-It® at 1000 mg/kg)

Table 4 shows the effect of grain type, exposure period and BRDE doses on the mortality of *R. dominica* adults. Adult mortality in the untreated grains was similar and did not exceed 2% irrespective of grain type and exposure period. Similarly, mortality levels on treated grains caused by Protect-It® and the highest dose (2000 mg/kg) of BRDE were comparable irrespective of the exposure period. Beetles were more susceptible to BRDE treatment on maize grain than sorghum grain and their susceptibility increased with the exposure period. For instance, after the 14-

day exposure, adult *R. dominica* mortality on sorghum grain treated with 1500 mg/kg BRDE was similar to the mortality recorded on maize grain treated with 500 mg/kg BRDE. The same was noted between sorghum grains treated with 1000 mg/kg of BRDE and maize grains treated at 250 mg/kg. Furthermore, the mortality of beetle exposed to sorghum grain treated with 250 mg/kg of BRDE for 14 days was comparable to that observed when beetles were exposed to sorghum grain treated with 500 mg/kg of BRDE for 7 days.

Table 4: Interaction effect of grain type, exposure period and BRDE doses on adult *R. dominica* mortality.

		Adult mortality (%) Grain Type		
		Maize	Sorghum	
Exposure period (days)	BRDE dose (mg/kg)			
	0	0.8(5.1)o	0.0(0.0)o	
	250	26.7(31.1)k-m	10.8(19.1)mn	
	500	57.5(49.3)h-j	20.8(27.1)lm	
	1000	69.2(56.3)g-i	36.7(37.2)j-l	
7d	1500	78.3(62.2)f.h	45.8(42.6)j	
	2000	81.7(64.7)d-g	87.5(69.3)c-f	
	check	95.0(77.1)a-c	91.8(73.4)c-f	
	0	2.5(9.1)no	2.5(9.1)no	
	250	54.2(47.4)ij	20.0(26.6)lm	
	500	80.0(63.4)e-g	35.0(36.3)j-l	
14d	1000	87.5(69.3)c-g	55.8(48.3)ij	
	1500	92.5(74.1)b-f	79.2(62.9)e-g	
	2000	99.0(84.3)ab	94.2(76.1)b-e	
	check	100.0(90.0)a	95.0(77.1)a-c	
	$\mathrm{SE}\pm$	3.4		

Means followed by the same letter(s) are not significantly different, (Tukey Kramer, HSD Test, $P \le 0.05$). Values in parenthesis are arcsine transformed values. Check (Protect-It[®] at 1000 g/kg)

Table 5 shows the LD_{50} and LD_{95} values for BRDE against *R. dominica* adults. The BRDE was found to be more toxic to *R. dominica* on maize grain than sorghum grain, with lethal dose value decreasing with increasing

exposure period. After 7 days exposure to maize grain, the LD_{50} and LD_{95} values for R. dominica were 521 and 4981 mg/kg, respectively. Increasing exposure to 14 days reduced these values by more than 50% (215)

and 1,561 mg/kg, respectively). However, on sorghum grain, the corresponding values after 7 days of exposure were higher than those noted on maize grains, thus, 1,147 and 5,890 mg/kg of BRDE may be required to kill 50%

and 95%, respectively of adult R.dominica. After 14 days of exposure, the LD_{50} and LD_{95} values were approximately 691 and 3,206 mg/kg, respectively.

Table 5: The LD₅₀ and LD₉₅ and their 95% confidence limits of BRDE for adult R. dominica on maize and sorghum grains

Grain type	Exposure Period (Days)	LD ₅₀ (95% CL)mg/kg	LD ₉₅ (95% CL)mg/kg	Slope	Chi square (X²)	p-value
Maize	7	521 * (417-622)	4,981 (3399-8999)	1.68	7.47	0.88
	14	215 (146-278)	1,561 (1210 - 2286)	1.91	6.29	0.93
Sorghum	7	1,147 (923 -1442)	5,890 (3332-12008)	2.31	29.34	0.01
	14	691 (605 - 781)	3,206 (2542 - 4400)	2.47	14.16	0.36

^{*}Values in parenthesis are 95% lower and upper fiducial limits

DISCUSSIONS

The direct comparison of results from this study and those reported by others is difficult despite using DE from the same source. Differences in DE efficacy were observed even between laboratories, for example, the findings of Audu and Ibrahim (2021) revealed that raw diatomaceous earth applied at a dose rate of 1.5g/kg bambaranuts killed 76.6% of seed beetles just 24 hours after exposure. The findings of their study are in line with Kabir et al. (2010; 2011 and 2013) who noted that 500 - 1500 ppm of raw diatomaceous earth caused 72.3 % and 100 mortality of R. % dominica and T. castaneum at 7 and 14 days after exposure periods, and protected maize, sorghum and wheat grains against the beetles. This could, according to Sakenin Chelav et al. (2013), be a result of differences in experimental conditions, the population of insects, proficiency of formulation or types and varieties of commodities. Fields and Korunić (2002) noted up to 70% differences in

mortality between different DE sources depending on experimental conditions.

Mortality

In the present study, the BRDE proved to be less toxic to R. dominica than the commercial DE formulation (Protect-It®), given that after 14 days of exposure to treated commodities, even the highest dose level of 2000 mg/kg did not result in complete adult mortality. For example, the lethal dosage required to kill 95% of R. dominica adults (LD₉₅) after 14 days of exposure to treated maize and sorghum grains were 1,561mg/kg and 3,206 mg/kg, respectively. This relatively low efficacy of the BRDE could be attributed to its high Al₂O₃ and NaCO₃ content, as a result of the presence of these impurities, Stathers et al. (2004) and Mvumi et al. (2006) concluded that DE of African origin could be effective against stored-product insects only when used at doses as high as 2500 mg/kg. However, Subramanyam and Roesli (2000)recommended that DE doses should range from 60 to 5000 ppm depending on the source

of DE, type of formulation and environmental condition. Furthermore, the Bostrychids are reported to be more tolerant to DEs (Subramanyam *et al.*, 1994; Korunić 1998 and Stathers *et al.*, 2004), this could likely be due to their behavioural characteristics of boring and tunnelling, and are protected from direct exposure to DEs because they develop internally. Furthermore, Vardeman *et al.* (2007) established that DE treatments reduced the locomotion ability of *R. dominica* adults as such are less likely to pick up a substantial amount of the DE dust.

Dose Rates and Exposure periods

The results of this study indicate that DE dose rates and exposure time to the treated grains influenced the efficacy of tested BRDE, and lethal dose values (LD₅₀ and LD₉₅) decreased with increasing exposure periods. This trend of toxicity to R. dominica was similar to that of commercial DE formulations. Mortality of exposed adult beetles increased with an increase in BRDE dose and exposure period, which was in agreement with the findings of Vayias et al. (2006); Wakil et al. (2006); Athanassiou et al. (2007); Shayesteh and (2007);Ceruti et al. (2008); Kostyukovsky et al. (2010); Shams et al. (2011); Medugu et al. (2020); Audu and Ibrahim (2021). According to Athanassiou et al., (2006), this could be a result of the increase in the number of active ingredients picked by the insects. The exposure time is crucial for the effectiveness of DEs because insects' movement increases the contact of the cuticle with the dust particles. According to Subramanyam and Roesli (2000), the death of insects by diatomaceous earth is attributed to dehydration provoked by the abrasiveness of the small particles of inert dust and by the adsorption of oils in the body of the insect, which breaks the layer of wax on the epicuticle, causing loss of water and death.

Therefore, in higher dosages the adsorption of wax and abrasiveness caused by the DE occurs quickly, causing death in a shorter time when compared with the lower dosages.

Grain Type

The efficacy of DE is determined by the type of storage product to the dust is applied, the BRDE used in this study was more effective on maize than on sorghum grains because it did not provide the same level of protection even when the same dose rate was applied to the two grains. For example, the RBDE at 500-1500 mg/kg applied caused significantly higher adult mortality in maize grain than in sorghum grain. Previous studies have reported the influence of grain type on DE efficacy (Keszthelyi and Pál-Fám, 2012; Chiriloaie et al., 2014 Athanassiou et al., 2016). They attributed the differential performance to differences in physical and chemical characteristics of the grains which affect the adherence of DE dusts to the kernels and effectiveness against insect infestation. However, in the present study, performance of the check (Protect-It®) was not influenced by grain type, suggesting that other factors might be involved. Perhaps particle size might have played a role in adherence as the BRDE was not fractioned to a particular particle size.

CONCLUSION

BRDE used in this study is easily accessible to resource-poor farmers in large deposits, however, efficacy varied with dose, exposure period and grain type. To effectively protect stored maize and sorghum grains dominica infestations, against *Rhyzopertha* dose rates higher than 2000 mg/kg and a longer exposure period may be required. It is, therefore, necessary that BRDE is combined with other suitable biorational insecticides to adequately enhance its effectiveness in protecting these grains.

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