# EFFICACY OF DIFFERENT DIATOMACEOUS EARTH FORMULATIONS IN THREE WHEAT VARIETIES AGAINST THE LESSER GRAIN BORER RHYZOPERTHA DOMINICA (F.) (COLEOPTERA: BOSTRICHIDAE)

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## Abstract

Laboratory tests were conducted to evaluate the efficacy of three diatomaceous earth (DE) formulations; Celite 209, DiaFil 610 and SilicoSec in three wheat varieties, Atilla-Gan-Atilla, Cetia and Seri M82, against *Rhyzopertha dominica* (F.). The DE formulations were applied at four dose rates; 250, 500, 750 and 1000 mg/kg. Bioassays were carried out at 26-34°C and at 32-45 % relative humidity (RH). Efficacy was evaluated by recording adult mortality after 7 and 14 days, and progeny production at 56 and 112 days after treatment (DAT). Adult mortality levels significantly differed among varieties, exposure periods, DE formulations and their dose rates, ranging from 27.7 to 100 %. With all DE formulations and wheat varieties, complete adult mortality was achieved at 1000 mg/kg and 14 days of exposure. SilicoSec was more effective, proving higher mortality within shorter exposure period than other formulations. Progeny production was significantly higher in untreated Cetia than in the two other varieties and always greater in untreated than treated grains. Complete progeny suppression was recorded at >750 mg/kg. Generally, the performance of all DE formulations was greater in Atilla-Gan-Atilla and Seri M82 compared to Cetia.

KEY WORDS: diatomaceous earth, formulation, wheat variety, Rhyzopertha dominica

## Introduction

Wheat, *Triticum* spp., is the world's most widely grown cereal crop and it is a major food staple for most of the world (Iftikhar *et al.*, 2004). Nigeria requires 3.7 million metric tons of wheat annually and spends about 635 billion Naira (USD 3.91 billion) annually to import the commodity. Yet, current local production stands at a meager 50,000-60,000 metric tones (Magaji *et al.*, 2012). Wheat is currently produced commercially in Nigeria under irrigation conditions, mainly in the Sudan/Sahelian zones where night temperatures during

most of the growing period (November – March) range from 15 to 20°C. Wheat growing (and storage) is the key driver of sustained economic development and food security in the wheat growing Savannah belts of Nigeria (Falaki *et al.*, 2009; Nwaubani *et al.*, 2014). Therefore, harvested wheat must be protected from the agents of deterioration during storage to maintain a high quality for processing and consumption.

Insects are the major cause of deterioration of stored wheat. The lesser grain borer, *Rhyzopertha dominica*, is a major cosmopolitan pest of stored wheat. It is also considered to be one of the most destructive and widespread species in stored cereal grains (Athanassiou *et al.*, 2008). Females lay eggs on the outside of the kernel and the first instars bore inside, where they continue development until adulthood. Upon reaching the adult stage, the mature insect bores out of the grain kernel and creates a large exit hole (Potter, 1935). This internal development makes management of the insect difficult (Chanbang *et al.*, 2007). This pest has also developed a considerable level of resistance to some synthetic insecticides (Lorini & Galley, 1995). While use of synthetic insecticides had been relied upon for the control this pest, their adverse effects on the environment, development of resistant strains and residues in food has motivated the search for safer alternative methods.

Diatomaceous earths (DE) are fossilized skeletons of diatoms comprising amorphous or shapeless silicon dioxide (silica) and small amounts of other mineral elements. In contrast to synthetic chemicals, DE adsorbs the epicuticular lipid layers, inducing mortality mainly as a result of excessive water loss through the cuticle of the insects (Korunic, 1998). There are several commercial DE formulations that are effective against an array of insect pests. However, insecticidal efficacy differs significantly in relation to the physical, chemical and morphological characteristics of species of diatoms (Korunic, 1998; Rojht *et al.*, 2010). Other factors affecting the effectiveness of the treatment are insect species, strain, stage of development and morphological characteristics (Korunic, 1998; Rigaux *et al.*, 2001), grain type and even variety within a grain type (Athanassiou, *et al.* 2003; Kavallieratos *et al.*, 2005; Kavallieratos *et al.*, 2010). Distomaceous earth could be very effective in the management of stored product insects in the wheat producing savannah zones of Nigeria, which are characterized by high temperature and low relative humidity during the hot season (March – May) when daytime temperatures in the open commonly reach 50°C and relative humidity as low as 10-20 % (Lale, 1998). These conditions increase DE efficacy (Fields & Korunic, 2000).

Because of the numerous factors influencing the insecticidal activity of DEs, it appears necessary to test the different commercial products to evaluate their efficacy against different insect species infesting stored grain under a given set of conditions. Despite the plentiful literature data on DEs, there are no publications on DE studies involving *R. dominica* and local wheat varieties in Nigerian. Moreover, there are no DE studies conducted under real or simulated conditions of arid environments. In this work, we evaluated the insecticidal activity of three commercially available DE formulations against *R. dominica* in three wheat varieties under ambient conditions characterized by high temperature and low relative humidity. The survival of adult insects and their ability to reproduce in the treated wheat were measured.

## Materials and Methods

#### Insect species

The test insect, *Rhyzopertha dominica*, was from a field population obtained from stored paddy rice in a rice mill store in Muna village, outskirts of Maiduguri, Nigeria, in 2016. The samples were reared on wheat (*var.* Norman B) in the laboratory under fluctuating ambient conditions (26-34°C and at 32-45 % RH). One hundred adults along with 250 g of disinfested wheat grains were placed into 1L culture jars. After 10 days,

the adults were removed. The emerging unsexed adults from the first generation reared on wheat, aged 1 to 2 weeks-old, were used for the bioassays.

#### Wheat varieties

The wheat varieties used in this study were Atilla-Gan-Atilla, Cetia and Seri M 82, and were obtained from the selecting Institute, Lake Chad Research Institute (LCRI), Maiduguri. Brief characteristics of these varieties are presented in Table I. Foundation seeds of the varieties were used in the tests. One-kg lots of each variety were cleaned by shaking the wheat through a sieve (mesh 2 mm) to remove inert materials and extraneous particles. Before use in experiments, the wheat was stored for at least 72 h at 6 to -18°C to kill any residual insect infestation, and then transferred to ambient conditions to equilibrate for two weeks. Moisture content was determined using Grain Moisture Tester PM 410 (Kett, USA). The initial moisture contents of the grains ranged from 12.2 to 12.6 %. These were adjusted to a uniform moisture content of 14 % by the addition of the appropriate amount of distilled water, tumbled for 5 min and kept for 7 days in airtight glass jars (Zakladnoy, 1985).

Oracia Ohanna tarriatia		Wheat variety	
Grain Characteristic —	Atilla-Gan- Atilla	Cetia	Seri M82
Protein (%)	13.2	15.1	14.1
Carbohydrate (%)	76.3	70.3	72.2
Fat (%)	1.5	1.9	1.7
Gluten (%)	12.7	12.9	12.5
Weight of 100 kernels (g)	2.9±0.1	3.4±0.2	3.0±0.1

Table I. Plant and grain characteristics of the tested wheat varieties. Source: LCRI (2012).

#### DE Formulations

The DE formulations used were Celite 209, DiaFil 610 and SilicoSec. These were obtained from Diatom Research and Consulting Inc., Canada. Celite 209 (Celite Corporation, USA) is a marine DE containing 87 % SiO2, 65.0 % of particles <12  $\mu$ m (Fields and Korunic, 2000); 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  $\mu$ m, (Korunic and Fields, 2006). SilicoSec® (Biofa GmbH Munsingen, Germany) is a DE formulation of fresh water origin, and contains approximately 92 % SiO<sub>2</sub>, 3 % Al<sub>2</sub>O<sub>3</sub>, 1 % Fe<sub>2</sub>O<sub>3</sub>, and 1 % Na<sub>2</sub>O. The average particle size is between 8 and 12 mm (Shaayesteh and Ziaee, 2007).

#### Bioassay procedure

The experiments were carried out at 26-34 °C and at 32 % and 45 % relative humidity (RH). Four concentrations of each DE formulation were used (250, 500, 750 and 1000 mg/kg). Each glass jar contained 150 g of wheat grain and an individual dose of DE. An additional jar of untreated wheat served as control. All jars were shaken manually for approximately 5 min to achieve distribution of the DE in the entire grain mass (Kavallieratos *et al.*, 2007a). Jars were left for 10 min to allow dust to settle before dividing into test vials. From each treatment, 3 samples of 50 g each were taken from each jar and each sample was placed in a vial. Then, 30 *R. dominica* adults were introduced into each vial and the vials were closed with perforated lids

lined with Whatman filter paper on the inside to prevent the insects from escaping. After adult introduction, vials were placed on a laboratory shelf. Mortality of the exposed adults was measured after 7, and 14 days of exposure. After the 14 days count, dead and live individuals were counted and removed, and then the test vial containing wheat was returned to the shelf. The number of live progeny was counted 56 days after treatment (DAT), returned to the jars and counted again after 112 DAT. Progeny suppression was calculated using the formula:  $\{(1 - no. of progeny in a treatment)/no. of progeny in control\} x100 % (Nwaubani$ *et al.*, 2014).

#### Experimental design and data analysis

The treatments consisted of three wheat varieties and three DE formulations tested at (250, 500, 750 and 1000 mg/kg), replicated three times and laid in a factorial design. Adult mortality data were corrected for adult mortality in the control using Abbott's (1925) formula. Data on adult mortality and progeny were arcsine and square root transformed, respectively, and then subjected to the analysis of variance (ANOVA) procedure of the Statistix 8.0 (Statistix Inc.). Tukey-Kramer HSD tests at P<0.05 was used to determine statistical differences among treatments. Non-transformed data are presented in Tables II-V.

## Results

After seven days of exposure to treated wheat, mortality levels of *R. dominica* adults ranged from 27.8 % on the variety Atilla-Gan-Atilla treated with 250 mg/kg of Celite 209 to 100.0 % on Atilla-Gan-Atilla and Seri M82 treated at 1000 mg/kg of SilicoSec (Table II).

Table II. Mean mortality ( $\%\pm$ SE) of *R. dominica* adults after 7 days of exposure to three wheat varieties treated with different DE formulations and doses. For each DE formulation, means within a column followed by the same lowercase letter are not significantly different, and each dose means within a row followed by the same uppercase letter is not significantly different; there no letter exist, differences are not significant: Tukey-Kramer (HSD) test at *P*< 0.05.

DE formulation		Wheat variety				
	Dose rate (mg/kg)	Atilla-Gan-Atilla	Cetia	Seri M82		
Celite 209	250	32.2±6.2c	27.8±7.3c	45.6±2.9c		
	500	43.3±3.8cB	69.9±5.0bA	51.1±8.0bcAB		
	750	79.9±2.0b	76.5±1.9ab	73.3±7.7b		
	1000	93.3±1.9a	91.9±2.2a	95.6=1.1a		
DiaFil 610	250	35.7±4.8c	27.7±4.0c	36.7±1.2c		
	500	67.8±4.0b	51.1±4.9b	62.2±6.2b		
	750	80.0±1.9ab	84.4±2.9a	75.6±2.9b		
	1000	88.9±1.1a	87.8±1.1a	88.9±1.1a		
SilicoSec	250	57.8±4.9c	42.2±4.0c	53.3±5.1b		
	500	84.5±2.2b	78.9±4.4b	72.2±2.2b		
	750	92.2±1.1b	85.6±2.9ab	95.6±2.9a		
	1000	100±0.0a	96.7±1.9a	100±0.0a		

Significant differences in mortality levels were noted among dose rates of all DE formulations. Mortality level increased with DE dose rate. Hence, >70 % of exposed adults died at dose rates of ≥750 mg/kg, irrespective of DE formulation and wheat variety. Differences in mortality levels among varieties were only significant when Celite 209 was applied at 500 mg/kg.

More *R. dominica* adults died with increased exposure period. Thus, after 14 days of exposure, >70 % of adults died when any DE formulation was applied at 500 mg/kg or higher (Table III). Within this exposure period, complete mortality was observed at the highest dose rate of all the DE formulations, except on Cetia treated with Celite 209. Generally, the most effective formulation was SilicoSec causing 100 % mortality on all the varieties treated at  $\geq$ 500 mg/kg, except on Cetia. Even then, the difference was not significant. This was followed by DiaFil 610, which caused complete mortality at the highest dose rate. Also, within this exposure period, significant differences in mortality levels among varieties were noted only with Celite 209 applied at 500 and 750 mg/kg (Table III).

Diatomaceous earth treatment significantly affected progeny production in *R. dominica*. Progeny production of this species at 56 DAT was significantly higher in untreated Cetia compared to the other varieties. However, this trend was not consistent in the treated grains. Also, significantly less F<sub>1</sub> individuals emerged from the treated varieties in comparison with their respective control grains (Table IV).

Table III. Mean mortality (%±SE) of *R. dominica* adults after 14 days of exposure to three wheat varieties treated with different DE formulations and dose. For each formulation, means within a column followed by the same lowercase letter are not significantly different, and each dose mean within a row followed by the same uppercase letter is not significantly different; where no letter exist, differences are not significant: Tukey-Kramer (HSD) test at *P*<0.05.

DE formulation		Wheat variety				
	Dose rate (mg/kg) -	Atilla-Gan-Atilla	Cetia	Seri M82		
	250	53.3±5.1c	38.9±8.0c	60.2±9.6c		
Celite 209	500	94.6±1.1bA	72.2±4.0bB	84.3±6.0bcAB		
Cente 209	750	96.7±1.9ab 87.8±3.0ab		98.8±1.2ab		
	1000	100±0.0a	97.8±1.1a	100±0.0a		
	250	63.3±5.1c	60.0±5.0d	62.7±3.2c		
DiaFil 610	500	88.9±1.1b 78.9±4.8c		88.0±5.3b		
DIAFII 610	750	98.9±1.1a 94.4±1.1b		97.6±1.2ab		
	1000	100±0.0a	100±0.0a	100±0.0a		
SilicoSec	250	76.7±1.9b	84.4±2.9b	81.8±2.0b		
	500	100±0.0a	98.9±1.1a	100±0.0c		
	750	100±0.0a 100±0.0a		100±0.0c		
	1000	100±0.0a	100±0.0a	100±0.0c		

Table IV. Mean $\pm$ SE number of live *R. dominica adults* on three wheat varieties treated with three DE formulations dust formulation at four doses 56 DAT. For each variety, means within a column followed by the same letter are significantly different: Tukey-Kramer (HSD) test at *P*< 0.05.

DE formulation	Dose rate (mg/kg)	Wheat variety						
		Atilla-Gan-Atilla		Cetia		Seri M82		
		No. of progeny	Progeny suppression (%)	No. of progeny	Progeny suppression (%)	No. of progeny	Progeny suppression (%)	
Control	0	62.3±4.5a	-	106.7±8.5a	-	69.3±7.1a	-	
	250	18.0±2.5b	71.1	29.7±2.0b	90.9	8.3±1.2b	88.0	
0 111 000	500	1.0±0.0cd	98.4	0.7±0.3e	99.3	1.3±1.9d	98.1	
Celite 209	750	0.0+0.0d	100	0.3±0.3e	99.7	0.0+0.0d	100	
	1000	0.0+0.0d	100	0.0+0.0e	100	0.0+0.0d	100	
DiaFil 610	250	18.0±1.2b	71.1	11.7±1.5c	89.0	5.7±2.2bc	91.8	
	500	2.3±0.9cd	86.7	2.3±0.9de	97.8	0.3±0.3d	99.5	
	750	0.0+0.08d	100	0.0+0.0e	100	0.0+0.0d	100	
	1000	0.0+0.0d	100	0.0+0.0e	100	0.0+0.0d	100	
SilicoSec	250	3.5±1.5c	94.7	5.7±1.8cd	94.7	1.7±0.7cd	97.6	
	500	0.0+0.0d	100	1.3±0.9de	98.8	0.0+0.0d	100	
	750	0.0+0.0d	100	0.0+0.0e	100	0.0+0.0d	100	
	1000	0.0+0.0d	100	0.0+0.0e	100	0.0+0.0d	100	

Table V. Mean $\pm$ SE number of live *R. dominica* adults on three wheat varieties treated with three DE formulations dust formulation at four doses 112 DAT. For each variety, means within a column followed by the same letter are significantly different: Tukey-Kramer (HSD) test at *P*< 0.05.

DE formulation	Dose rate (mg/kg)	Wheat variety						
		Atilla-Gan-Atilla		Cetia		Seri M82		
		No. of progeny	Progeny suppression (%)	No. of progeny	Progeny suppression (%)	No. of progeny	Progeny suppression (%)	
Control	0	163.3±23.1a	-	246.0±19.2a	-	125.3±18.3a	-	
Celite 209	250	10.0±1.5b	93.9	12.3±2.9b	95.0	6.7±2.0b	94.7	
	500	0.0±0.0c	100	1.0±0.6c	99.5	1.0±0.6bc	99.2	
	750	0.0±0.0c	100	0.0±0.0c	100	0.0±0.0c	100	
	1000	0.0±0.0c	100	0.0±0.0c	100	0.0±0.0c	100	
DiaFil 610	250	3.3±1.5bc	98.0	6.0±2.2b	97.5	2.0±1.0bc	98.4	
	500	0.0±0.0c	100	0.3±0.3c	99.9	0.3±0.3c	99.7	
	750	0.0±0.0c	100	0.0±0.0c	100	0.0±0.0c	100	
	1000	0.0±0.0c	100	0.0±0.0c	100	0.0±0.0c	100	
SilicoSec	250	0.0±0.0c	100	3.7±0.7bc	98.5	0.7±0.3c	99.4	
	500	0.0±0.0c	100	0.3±0.3c	99.9	0.0±0.0c	100	
	750	0.0±0.0c	100	0.0±0.0c	100	0.0±0.0c	100	
	1000	0.0±0.0c	100	0.0±0.0c	100	0.0±0.0c	100	

## Discussion

The results obtained from this study showed that the DE products could be used to control *R. dominica* in stored wheat under arid conditions. In the current study, adult mortality increased with increasing dose rate and time of exposure from 7 to 14 days. These results are consistent with previous reports (Athanassiou *et al.*, 2005; Ziaee *et al.*, 2007; Vayias *et al.*, 2009) indicating that the efficacy of DEs requires an extended exposure interval and elevated dose rate.

DE has a physical mode of action, since the DE particles grasp the insect cuticles while insects are moving on treated grain. Further exposure means active contact with DE particles, which damages the cuticular wax layer and insects die through desiccation (Korunic, 1998). Arthur (2004) reported that higher application rates or longer exposure intervals were necessary for control of *R. dominica* because adults in DE treated grains bored into kernels, stayed inside and avoided contact with the dust. Desmarchelier & Dines (1987) reported that at least 1.0 g/kg rate of Dryacide in wheat at 28 days of exposure was necessary for the complete control of *R. dominica* adults and their progeny, while 0.5 g/kg of Dryacide was sufficient for containment of the adult population only.

Our results showed significant effects of DE formulation against *R. dominica* although the differences were evident at lower concentrations. Previous studies indicated that the efficacy of DEs would be different against the same insect species and different results were obtained, showing the influence of origin of DEs on their insecticidal activity. For instance, Kavallieratos *et al.* (2007) noted that the DE formulation PyriSec was more effective than SilicoSec and Insecto, rendering 100 % mortality to *R. dominica* adults after 14 days of exposure on wheat. Baldassari *et al.* (2014) showed that the DE formulation Protector caused higher mortality of *R. dominica* adults than SilicoSec, after two days of exposure when both were applied at 1000 mg/kg; thereafter differences were not significant. This differential performance could be ascribed to DE origin. According to Snetsinger (1988) DEs of marine origin are less efficacious as an insecticide than freshwater ones. Korunic (1998) indicated that DEs differ in species of diatoms (shape), origin (marine or freshwater), particle size distribution, SiO<sub>2</sub> content and so on. Hence, these properties influence their insecticidal activities. In the present study, SilicoSec containing 92 % SiO<sub>2</sub> of fresh-water origin had the highest efficacy, followed by DiaFil 610, also of fresh-water origin, and Celite 209 with marine origin was less efficacious. These properties might have been responsible for the differences observed.

DEs are generally more slow-acting than traditional grain protectants (Golob, 1997; Korunic 1998), which may allow adults to oviposit before dying and continue to cause grain damage (Subramanyam and Roesli, 2000). This probably explains why progeny production, even in cases of very high parental mortality, was not prevented in some of the treatments. Similar observations were made by Athanassiou *et al.* (2003) and Vardeman *et al.* (2006), and they concluded that, even if 100 % parental mortality occurs, progeny production is unlikely to be prevented. Interestingly the number of progeny declined in treated grains, in contrast to the more than two-folds increase in the untreated control at 112 DAT. Generally, the progeny suppression rate was >93 %. These results suggest that the presence of DE may slowly eliminate *R. dominica* populations in treated wheat. Moreover, as inert materials, DEs have a considerable level of persistence, which makes them suitable for long-term grain protection.

Our results show the significant effect of wheat variety on the mortality of *R. dominica*, indicating that variation among varieties of a particular grain type can influence the results of DE studies. The fact that DE efficacy varied among the tested varieties could be attributed to differences in the physical or chemical characteristics of the grain. Our results show that *R. dominica* was harder to control on Cetia, the variety on which a higher number of progeny was recorded compared to the other varieties tested. The variety Cetia had heavier kernels and high protein and gluten contents. These characteristics might have a negatively

impacted on DE efficacy. In contrast to our results, Kavallieratos *et al.* (2010), found that protein content was related to high DE efficacy levels in certain wheat varieties. Kernel size is also likely to play an important role in the efficacy of dust formulations, since better distribution of the DE particles is achieved on smaller kernels compared to larger ones (Athanassiou *et al.*, 2003). The interactions among physic-chemical characteristics of grain on DE efficacy is not well understood and requires further investigations.

In conclusion, this study confirmed the insecticidal effect of Celite 209, DiaFil 610 and SilicoSec against *R. dominica* under an arid storage environment. This pest could be controlled by using these DE formulations on stored wheat under the dry conditions of northern Nigeria. The recommended rate of 1000 mg/kg is acceptable to the environment and peoples' health and it has no influence on the quality of treated grains (Korunic *et al.*, 1996). Significant variations in DE efficacy may occur even among varieties of a specific grain type. However, the mechanisms involved in the differential performance of DE formulations among varieties are not established. Further investigations on varietal differences need to be carried out with a view to incorporating varietal resistance as a component of a DE-based integrated pest management (IPM) strategy and deriving maximum benefits from DE applications.

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# ЕФИКАСНОСТ РАЗЛИЧИТИХ ФОРМУЛАЦИЈА ДИЈАТОМЕЈСКЕ ЗЕМЉЕ НА ТРИ СОРТЕ ПШЕНИЦЕ ПРОТИВ ЖИТНОГ ЖИШКА *RHYZOPERTHA DOMINICA* (F.) (COLEOPTERA: BOSTRICHIDAE)

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#### Извод

Спроведена су лабораторијска испитивања за процену ефикасности три формулације дијатомејске земље (DE); Celite 209, DiaFil 610 и SilicoSec на три сорте пшенице, Atilla-Gan-Atilla, Cetia и Seri M82, против *Rhyzopertha dominica* (F.). Формулације дијатомејске земље су примењене у четири дозе; 250, 500, 750 и 1000 mg/kg. Биотестови су изведени на 26-34 °C и при 32-45 % релативне влажности. Ефикасност је процењивана евидентирањем морталитета адултних јединки после 7 и 14 дана, а потомства након 56 и 112 дана након третмана. Ниво смртности адултних јединки значајно се разликовао међу сортама, периодима излагања, формулацијама дијатомејске земље и сортама пшенице, потпуни морталитет код адулта је постигнут на 1000 mg/kg и 14 дана излагања. SilicoSec је био ефикаснији, што је узроковало већу смртност у краћем периоду излагања него друге формулације. Продукција потомства је била значајно виша у нетретираној Cetia него у друге две сорте и увек већа у нетретираном него у третираном зрну. Потпуна супресија потомства је забележена применом дозе ≥750 mg/kg. Карактеристике свих формулација дијатомејске земље биле су веће у Atilla-Gan-Atilla и Seri M82 у односу на Cetia.

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