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Full Length Research Paper

Evaluation of four variant diatomaceous earths and a commercial DE Insecto® against *Callosobruchus maculatus* F. (Coleoptera:Chrysomelidae) on two varieties of stored cowpea in Nigeria

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Nigerian Stored Products Research Institute (NSPRI) has patented a Diatomaceous Earth, a non-toxic pesticide NSPRIDUST® Patent No. 000744 for storage of grains with Trade Marks Section of the Federal Ministry of Trade and Investment, Abuja, Nigeria. Efficacy of four Nigerian Diatomaceous Earths (DEs): Bularafa, Abakire, Share and Kwami as grain protectants of stored cowpea against cowpea bruchid were compared to a commercial DE Insecto®. The grains were admixed with two varieties of cowpea (Ife brown and IT 98-12 white) separately at 0.1% w/w (1000 ppm). All treatments were infested with 30 unsexed adults of *Callosobruchus maculatus* (48 h-old). Adult mortality, progeny production, IDK, repellency test and germination of seeds were assessed in NSPRI laboratories in 2016. The Insecto®, Bularafa, Abakire, Share and Kwami caused corrected mortalities of 90, 80, 76, 76 and 43% respectively against adult *C. maculatus* on Ife brown cowpea while 86, 80,76, 73% and 73% were recorded respectively on IT 98-12 after 72 h exposure. There was F_1 progeny suppression. Bularafa was as effective as Insecto®. Results showed that the number of emerged F_1 progeny reduced in proportion with increased DE dose rate, but could not prevent progeny production even where complete adult mortality was observed within 5 days. This study showed that progeny suppression is a more important criterion to be considered in efficacy of the DEs on cowpea than adult mortality as the adults are short-lived, do not feed or cause damage but only lay eggs. Repellency showed that test insects avoided treated grains of the two cowpea varieties. There was no significant effect on germination capacity observed in the study.

Key words: Nigerian diatomaceous earth, *Callosobruchus maculatus*, infestation, stored cowpea.

INTRODUCTION

Nigeria is the world largest producer of cowpea [*Vigna unguiculata* (L) Walp.] of which the bulk comes from the drier states of Northern Nigeria (Singh et al., 2002). Cowpea grain is nutritious and is a source of plant protein and minerals for both rural and urban consumers in Nigeria and other subtropical countries in Africa (Bamaiyi et al., 2006).

Callosobruchus maculatus (F.) is the most serious insect pest of cowpea both in the field and storage (Turaki, 2012). *C*. *maculatus* is a primary grain (pulse) beetle which is widely distributed throughout the world. In Nigeria alone, the dry weight loss due to *C. maculatus* exceeded 2,900 tonnes each year. In some cases damage in terms of holes produced by adult emergence from seed increased to 99% after 6 months of storage (Singh, 2005; Umeozor, 2005).

The losses incurred during cowpea storage by *C*. *maculatus* cannot be compensated; this therefore requires urgent and effective pest management strategies for year round availability of cowpea that is the major source of plant protein for the population for food quality and safety. In this work, we focused on novelty strategies that will transform both the smallholder farmers and grain aggregators to meet food security, reduce malnutrition resulting from protein deficiencies and sustain economic growth.

Various studies on the efficacy of inert dusts have been reported particularly those based upon activated silica which are finding increasing use as storage protectants in the grain industry (Obeng-Ofori, 2010). These materials can be classified into different groups depending on their composition and particle size. Non-silica dusts and those composed of coarse grain silicates, such as kaolin, sand and Attapulgite Based Clay Dust (ABCD), have been used traditionally as grain protectants by small-scale farmers in the developing world (Okonkwo and Okoye 2000). Materials including diatomaceous earths and silica aerogels have been used increasingly in commercial storage in the developed world, replacing conventional chemicals (Golob, 1997).

Another advantage of DEs over conventional insecticides is their low mammalian toxicity. In the USA, diatomaceous earths are 'Generally Recognized as Safe' by the US Food and Drug Administration and are registered for use as food additives (Subramanyam et al., 1994).

There has been a renewed interest in diatomaceous earth as a grain protectant because of concerns of insecticide residues in grain, worker exposure to insecticides and resistant insect populations for over three decades (Fields et al, 2002). Admixture of inert dusts with grains, especially DEs are gaining acceptability among grain storage practitioners in the developing world as protectants against stored products insect pests being effective alternatives to chemical insecticides and plant materials (Korunic, 1998; Arthur, 2000; Fields and Korunic, 2000; Subramanyam and Roesli, 2000; Athanassiou et al., 2003; 2007). Diatomaceous earth products are composed of microscopic fossils of diatoms. Insecticidal activity depends on the DE capacity to damage insects' cuticle and cause water loss from their bodies, so that they die of desiccation (Korunic, 1988). These products are attractive because they have very low mammalian toxicity, are inert, leave no toxic residues on grains, control the insecticide resistant pests and are long-lasting and are applied using the same technology for conventional grain protectants (Vayias et al., 2006; Athanassiou et al., 2007).

There are several commercially available DE formulations which have been successfully evaluated as grain protectants against a wide range of insect species: *Sitophilus oryza*e (L.), *Rhyzopertha dominica* (Fab.), *Tribolium castaneum* (Herbst.) (Subramanyam and Roesli, 2000; Stathers et al., 2004). The efficacy of DE products depends on several parameters, such as insect morphology, type of grains, DE physical parameters, temperature and relative humidity (Korunic, 1998). Insecto® DE of marine origin has been found to be effective against several stored grain insect species at 0.5-1.0g/kg (Golob, 1997).

Although, there are numerous studies on commercially formulated DEs to control stored product insects, only few studies have been evaluated against *C. maculatus* despite the importance of cowpea and the destructive nature of *C. maculatus*. Apart from documented literature on use of commercially formulated DEs to control *C. maculatus* of stored cowpea in Nigeria (Kabir and Gaya, 2013; Kabir and Wuglo, 2014), the authors did not find documented literature on the use of raw Nigeria-derived DEs, and commercially formulated DE Insecto® against *C. maculatus* on stored cowpea. Previous works by Nwaubani et al. (2014) and Otitodun et al. (2015) reported the effectiveness of a variant Nigeria DE against two species of stored wheat pests - *Sitophilus oryzae* and *Rhyzopertha dominica*.

Diatomaceous Earths are already registered in some countries to control stored products pests. Insecto® is registered in the United States for use on stored grains and empty grain-holding facilities to control insects.

NSPRIDUST® is registered in Nigeria with Trade Marks Section of Federal Ministry of Trade and

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Investment, Abuja in 2017 by Nigerian Stored Products Research Institute as an effective grain protectant for stored insect pests with Patent No. 000744.

Based on this, the present work describes further studies of additional variants of DEs Insecto®, Bularafa, Abakire, Share and Kwami from different geographical locations in Nigeria against the stored cowpea bruchid.

MATERIALS AND METHODS

All studies were conducted in the Entomology Laboratories of Nigerian Stored Products Research Institute, Headquarters, Ilorin, Kwara State, Nigeria at uncontrolled conditions of temperature fluctuation between 28.4 to 35.1°C and 34.9 to 67.4% RH.

Test insects

C. maculatus was used in the experiments. *C. maculatus* was obtained from the stock cultures maintained on cowpea seeds in the insectary of Entomology Department. New cultures were reared on the cowpea seeds (IT98-12 white and Ife brown); 9% moisture content in Kilner jars. The F_1 generation was put into another set of Kilner jars containing IT98-12 white or Ife brown which was used to culture subsequent generations. Adults emerging from the cowpea seeds, aged 1 to 48 h old were used in the experiments.

DE formulations

The DEs used were commercially formulated Insecto®, and raw Nigeria-derived DEs namely - Bularafa, Abakire, Share and Kwami. The raw DEs were dried in ventilated oven, pulverized into dust, sieved with 90 µm sieve (Endecott Laboratory Standard Sieves, London). Insecto® is a marine DE (Natural Insecto® Products, Inc. Costa Mesa, CA 92627, USA) with 10% food grade bait. It is a gray coloured powder containing 87% (w/w) amorphous silicon dioxide, with 2 to 4% m.c.; and a chemical composition of 3% Al_2O_3 and 1% Fe_2O_3, and less than 1% CaO, MgO, TiO_2 and P_2O_3 (Subramanyam et al., 1994; Arnaud et al., 2005). The physical characteristics of the formulation are as follows: mean particle diameter, 6.89 µm; medium particle size is 8.2 µm and particles range from 1.0-34.3 µm, retention 325 mesh, 0.5% oil adsorption capability, 175% by weight; pH, 6.0; bulk density, 0.128 g/cm^3; specific gravity, 0.23; and surface area of 10 to 20 m^2/g (Subramanyam et al., 1994). A sample of dry formulation of Insecto® was obtained from Natural Insecto® Products, Inc. Costa Mesa, CA 92627, USA.

The fresh water crude Bularafa DE ore was obtained from Bularafa community in Gulani LGA, Yobe state. The fresh water crude Abakire DE ore was obtained from Abakire community, Fika LGA, Yobe state. Bularafa is a fine whitish dust containing 80.98% amorphous silica, with 1.4% m. c. Bularafa is composed of 4.9% Al_2O_3 and 2.30% Fe_2O_3, and less than 1% CaO, Na_2O, K_2O, MgO, TiO_2 and P_2O_5, MnO, Cr_2O_3. The composition of elements is: Ba 130 ppm; Ni 45 ppm; Sr 66 ppm; Zr 107 ppm. Particle sizes ranged from 1.0 to 13.5 µm (Nwaubani et al., 2014; Otitodun et al., 2015). Abakire is a whitish dust containing 60.17% silicon dioxide with …% m.c.. Abakire is composed of 18.39% Al_2O_3 and 5.09% Fe_2O_3, and less than 1% CaO, Na_2O, K_2O, MgO, TiO_2 and P_2O_5, MnO, Cr_2O_3.

The composition of elements is: Ba 293 ppm; Ni 45 ppm; Sr 115 ppm; Zr 288 ppm. Minimum particle size, 1.8µm; mean particle size, 16.3 µm and particles range from 1.0 to 100 µm (Nwaubani et al., 2014; Otitodun et al., 2015).

Share is a fresh water crude DE ore obtained from Share community in Share LGA, Kwara State while Kwami is a fresh water crude DE ore obtained from Gombe State. Share and Kwami are whitish dusts. Geochemical, physical parameters and pH analyses of Share and Kwami DEs have not been determined. Share and Kwami DEs were dried to 4% m.c. for use during the study.

Cowpea

The cowpea varieties IT98-12 white and Ife Brown were obtained from Institute of Agricultural Research and Training (IAR&T), Ibadan, Oyo State. The seeds were already disinfested by fumigation with phosphine for 72 h before purchase; ventilated for 7 days in a plastic basin covered with muslin cloth to allow entire volatilization of the phosphine gas from the seeds; then cleaned by sieving, picked before packed in polythene bag and kept in the domestic deep freezer at -18°C for 7 days. After 7 days, the seeds were brought out and kept on the laboratory table for equilibrium for 2 weeks.

DE and cowpea grain Bioassay

A protocol developed for standardized testing of diatomaceous earth (Fields et al., 2002) was followed.

Each DE–Insecto®, Bularafa, Abakire, Share and Kwami was tested at three concentrations of 500, 1000 and 1500 ppm (ppm: parts per million, mg of DE per g of cowpea; equivalent of 0.05%, 0.1% and 0.15% w/w). DE was added to each jar containing 300 g of Ife brown or IT98-12 white cowpea (9% m.c). The cowpea seeds and each DE were shaken in jars by hand for 2 min. After mixing, the treated cowpea was divided into three 100 g samples, one for each replicate. Each treated and untreated jar was infested with 30 unsexed adults of *C. maculatus* (1 to 48 h old) and covered with muslin cloth held tightly with a rubber band. The necks of the jars were coated with non-sticky Polytetrafuloroethylene (PTFE) Fluon emulsion to prevent insects climbing to the top. Insecto® was used as positive control, while untreated grains served as negative control. Adult mortality was assessed after 3 and 5 days. After 3 days the contents of each jar were poured onto stainless aluminum tray gently avoiding loss or damage to eggs. The number of live and dead adults were counted and recorded. After 5 days the seeds were sieved, all adults removed and the number of dead and live counted, recorded and discarded. The seeds were returned to their respective jar for offspring production and kept under the same conditions. After 35 days post-treatment, seeds in each jar were sieved and the total number of F_1 adults counted. To determine the seed damage, in each jar, 100 seeds were randomly taken and examined for exit hole. The number of seeds with exit holes were termed damaged seeds were expressed as percentage of seeds in the sample.

Germination of seeds

Each DE dust was added to 100 g of IT98-12 white or Ife brown cowpea separately at (0.05%, 0.1%, 0.15% w/w). Untreated control was set up. No insect was added to seeds in different treatments and the untreated control. The jars were stored for 180 d in the laboratory. This was to determine the effect of the five DE dusts on germination capacity. One hundred seeds from each treatment and untreated jar were selected at random, divided into five batches of 20 seeds and placed in Petri dishes containing moistened cotton wool. Initial germination was determined at the start of the experiment. The percentage of germination was calculated after 7

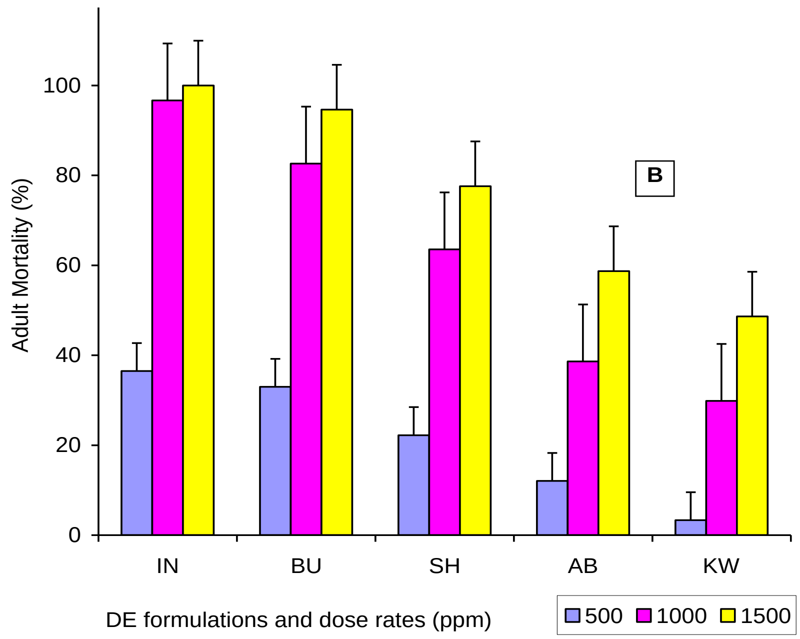


Figure 1. Mortality of adult *C. maculatus* in IT98-12 White cowpea after 5 days of exposure to different dose rates of five DEs. IN, Insecto®; BU, Bularafa; SH, Share; AB, Abakire; KW, Kwami.

days.

Repellency test

This test was conducted for Insecto®, Bularafa, Share, Abakire and Kwami DE dusts, using the two-way choice method (Nwaubani and Fasoranti, 2008) to assess the likelihood of bruchids avoiding contact with DE-treated grain in natural storage condition. Samples of 100 g of each variety of cowpea treated separately with Insecto®, Bularafa, Share, Abakire and Kwami dusts at 0.1% w/w and untreated 100 g samples were placed 10 cm apart in a long plastic chamber (30 x 12 x 10 cm) covered all round with black tape to avoid photo effect of sunlight on the distribution of the bruchid. Thirty 1 to 48h old adults of *C. maculatus*, starved for 48 h, were placed between the treated and untreated cowpea through a centrally-located opening on the lid. Insects found within 1 cm of treated or untreated cowpea were counted after 1, 3 and 5 days. Dead insects were replaced with live ones during each count. There were four replications for each cowpea variety set up.

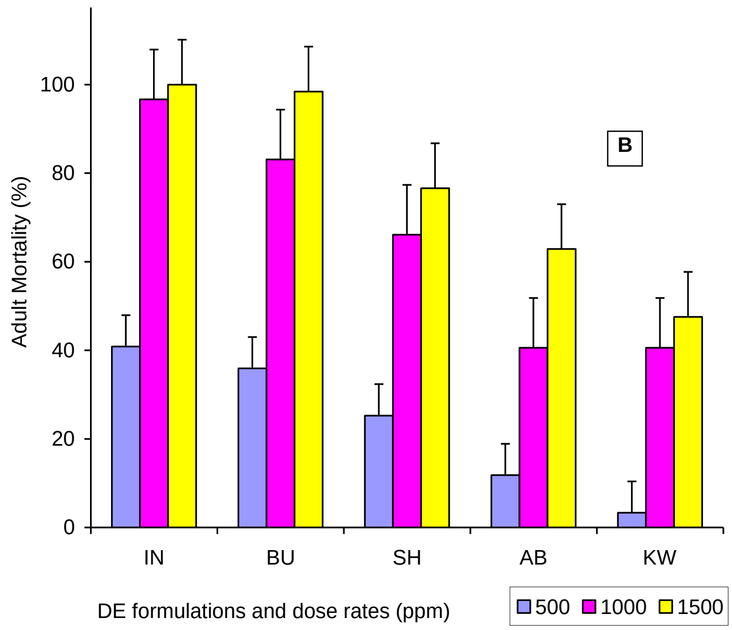
Statistical analysis

Data on adult mortality were first corrected for mortality in controls using the Abbott's formula (Abbott, 1925). To equalize variances, corrected mortality data was transformed using the square root of the arcsin. Data on number of progeny was square-root transformed. All percentage data were arcsin-transformed. The transformed data were subjected to analysis of variance (ANOVA) using Stata Statistics Program Version 12. The lethal dose for 50% of the population (LD_{50}) was estimated using probit analysis (SPSS Version 20). Differences between treatment means were compared by Tukey-Kramer HSD test at $p < 0.05$. For repellency test, Student's t-test was used to determine deviation from the expectation of equal distribution of bruchids in treated and untreated cowpea seeds.

RESULTS

Mortality of *C. maculatus*

The insecticidal efficacy of the DE dusts tested against *C. maculatus* on two varieties of cowpea, IT98-12 white and Ife Brown is presented in Figures 1 and 2. Mortality was observed to increase with increase in the DE concentration and exposure interval. The highest corrected mortality of *C. maculatus* adults after 3 days of exposure to DE treated cowpea was recorded to increase with increase in DE dose rates. Significant differences were noted among dose rates (500 and 1000/1500 ppm) within each DE dust treatment and between treatments (Insecto®/Bularafa and the other three DEs). *C. maculatus* had lowest 27.4% mortality and the highest 85.6% mortality recorded on Kwami and Insecto® applied at 1000 ppm respectively for IT98-12 white cowpea; and lowest 34.3% and highest 85.1% recorded on Kwami and Insecto® applied at 1000 ppm respectively for Ife brown cowpea. The effectiveness of the five DE treatments in decreasing order was Insecto®, Bularafa, Share, Abakire and Kwami. *C. maculatus* mortality increased as exposure interval increased from 3 to 5 days. The highest corrected mortality of 100% was by Insecto® at 1500



Figures 2. Mortality of adult *C. maculatus* in Ife brown cowpea after 5 days of exposure to different dose rates of five Des IN (Insecto®); BU (Bularafa); SH (Share); AB (Abakire) KW (Kwami).

Table 1. Mean ± SE for main effects and interactions for percentage corrected mortality of *Callosobruchus maculatus* after 5 d of exposure on IT98-12 white and Ife Brown cowpea treated with five Diatomaceous earth dusts at three dose rates.

DE Dose rate (ppm)	DEs				
	Insecto®	Bularafa	Share	Abakire	Kwami
500	36.49±4.0^{bA}	33.0 ±3.3^{bA}	22.22±0.0^{cB}	12.04±1.9^{cC}	3.3±2.2^{cD}
1000	96.7±2.2^{aA}	82.6±1.1^{aB}	63.5±1.9^{bC}	38.6±1.1^{bD}	9.8±0.0^{bE}
1500	100±0.0^{aA}	96.7±1.9^{aA}	77.6±1.1^{aB}	58.7±1.9^{aC}	8.6±2.2^{aD}
500	40.9 ±2.9^{bA}	35.9±4.4^{bA}	25.3±1.1^{bB}	11.8±2.2^{cC}	6.0±2.00^{bD}
1000	96.7±2.2^{aA}	83.1±1.1^{aB}	66.1±1.1^{aC}	40.6±1.1^{bD}	40.6±1.1^{aD}
1500	100±0.0^{aA}	98.4±1.9^{aA}	76.6±2.9^{aB}	62.9±2.2^{aC}	47.5±2.2^{aD}

Means within a column accompanied by same lower case letters and within a row- upper case letter are not significantly different: Tukey-Kramer HSD test; P=0.05.

ppm at 27.3±2°C.

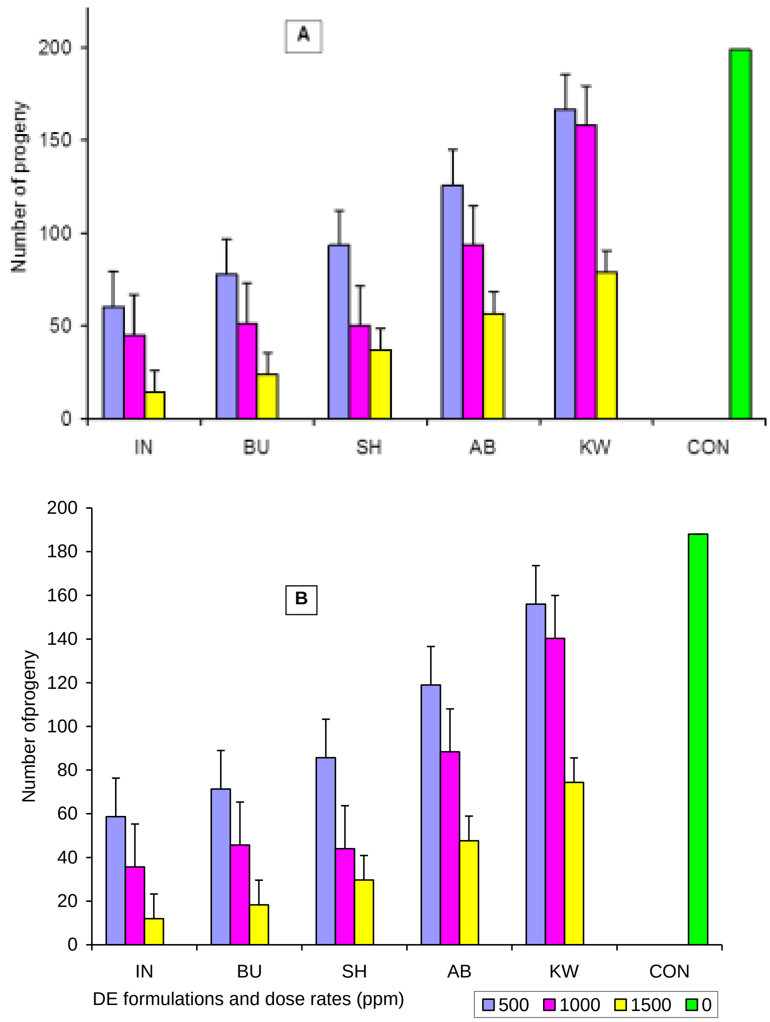
The highest mortality of *C. maculatus* adults after 5 days of exposure to DE treated cowpea was recorded to increase with increase in DE dose rate (Figures 1 and 2). Significant differences were noted among dose rates within each DE formulation. *C. maculatus* had lowest 29.8% mortality and the highest 96.7 mortality recorded on Kwami and Insecto® applied at 1000 ppm respectively for IT98-12 white cowpea; and lowest 40.6% and highest 96.7% recorded on Kwami and Insecto® applied at 1000 ppm respectively for Ife brown cowpea.

The main effects and interactions for percentage corrected mortality of *C. maculatus* after 5 days of exposure on IT98-12 white cowpea and Ife brown cowpea treated with five Diatomaceous earth dusts at three dose rates are shown in Table 1.

Tukey test conducted to compare the efficacy of the five DE formulations, showed there were no significant difference (P>0.05 (P>0.05) between Insecto® and Bularafa in any dose rate tested, whereas Share, Abakire and Kwami were significantly different (P<0.05) from Insecto® and Bularafa in any dose rate tested.

F_1 Progeny production

The main effects of DE formulations and dose as well as interactions were significant (p≤ 0.05) for number of F_1



Figures 3. Number of progeny of *Callosobruchus maculatus* in (A) IT98-12 White cowpea and (B) Ife Brown cowpea treated with IN (Insecto®); BU (Bularafa); SH (Share); AB (Abakire) KW (Kwami), CON (control).

progeny and reduction in progeny. The mean number of progeny in untreated control (199±2.5 and 188±7.2) for IT98-12 white and Ife brown cowpea respectively was significantly higher (P<0.05) than the numbers that were produced on treated seeds after 35 days post-treatment (Figure 3a and b). Progeny production was reduced by increasing DE dose rate. On treated cowpea seeds the lowest number of progeny and the highest progeny suppression were 13.2 and 12; and 92.8 and 93.7% for IT98-12 white and Ife brown cowpea respectively (Figure 4a and b).

Percentage seed damage

Percentage of cowpea seeds damaged by *C. maculatus* was significantly affected by DE dusts and dose rate and interactions within the treatments and between the treatments (Table 2). The untreated control recorded significantly higher (P<0.05) seed damage of 66.3±0.0% than in the other four treatments. There were no significant difference (P>0.05) in seed damage between Insecto® and Bularafa in any dose rate tested. Seed damage decreased with increased dose rate. Seed damage of <5% and <3% were recorded for both IT98-12 white and Ife brown treated with Bularafa and Insecto® at 1000 and 1500 ppm respectively.

Germination capacity of treated seeds

The DE dusts had no effect on the germination of cowpea seeds treated and stored for 180 d. Treated seeds with different doses for the five DE dusts had between

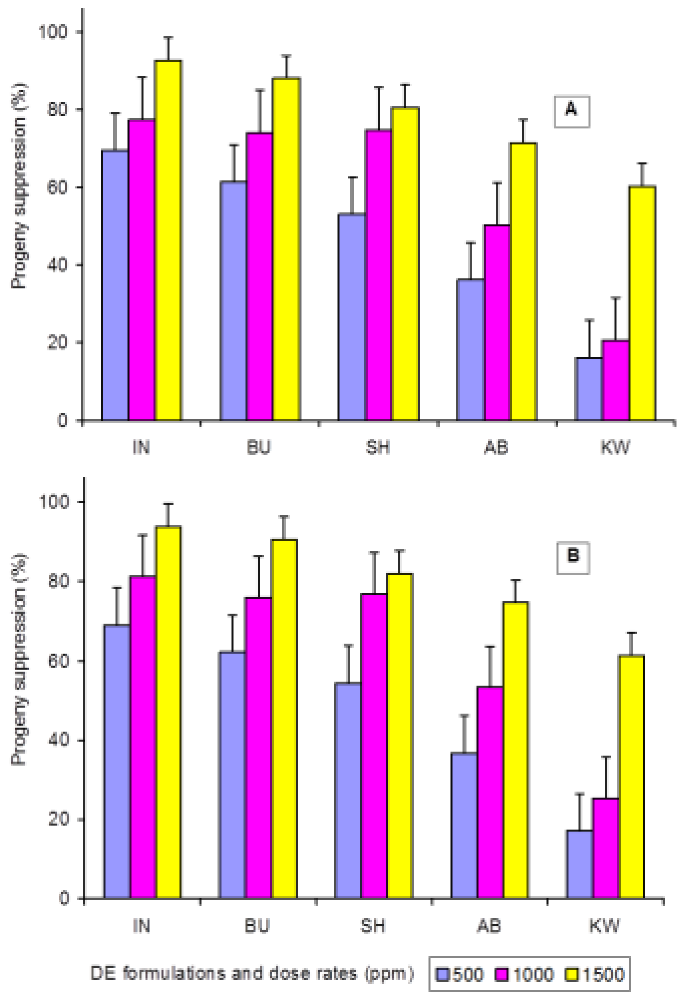


Figure 4. (A and B). Reduction in number of progeny of *Callosobruchus maculatus* in Ife Brown cowpea treated with IN is Insecto; BU is Bularafa; SH is Share; AB is Abakire and KW is Kwami

Table 2. Percentage seed damage of IT98-12 white and Ife brown cowpea varieties treated with five Diatomaceous Earth dusts at three doses after 35 day post-treatment. Mean percentage weight seed damage in untreated control were 66.3±0.0% and 66.0 ±0.0% respectively

DE Dose rate (ppm)	DEs				
	Insecto®	**Bularafa**	**Share**	**Abakire**	**Kwami**
	Mean ± SE for IT98-12 White cowpea				
500	11.0±0.6^{aC}	11.7±0.9^{aC}	15.7±0.9^{aB}	15.3±0.9^{aB}	23.3±0.9^{aA}
1000	4.0±0.6^{bC}	4.7±0.7^{bC}	12.0±1.2^{bB}	11.7±0.9^{bB}	17.7±0.9^{bA}
1500	2.3±0.3^{bD}	2.7±0.3^{bD}	6.0±0.6^{bC}	8.7±0.3^{bB}	13.0±0.6^{cA}
	Mean ± SE for IT98-12 White cowpea				
500	10.3±0.3^{aC}	10.7±0.3^{aC}	15.0±0.6^{aB}	14.3±1.2^{aB}	22.3±0.3^{aA}
1000	3.7±0.3^{bC}	4.7±0.7^{bC}	11.0±0.6^{bB}	10.7±0.3^{bB}	17.0±0.6^{bA}
1500	2.0±0.0^{cD}	2.3±0.3^{cD}	5.7±0.3^{cC}	8.0±0.6^{bB}	12.7±0.7^{cA}

Means within a column accompanied by same lower case letters and within a row- upper case letter are not significantly different: Tukey-Kramer HSD test; P=0.05.

Table 3. Mean ± SE Percentage seed germination of IT98-12 white and Ife brown cowpea treated with five Diatomaceous earth dusts at three dose rates after 180 days.

DE Dose rate (ppm)	DEs				
	Insecto®	Bularafa	Share	Abakire	Kwami
		Mean ± SE for IT98-12 White cowpea			
500	100.0±0.0^{aA}	100±0.0^{aA}	100.0±0.0^{aA}	100.0±0.0^{aA}	99.0±1.0^{aA}
1000	100.0±0.0^{aA}	100.0±0.0^{aA}	100.0±0.0^{aA}	100.0±0.0^{aA}	97.0±2.0^{aA}
1500	99.0±1.0^{aA}	99.0±1.0^{aA}	98.0±1.2^{aA}	96.0±2.4^{aA}	94.0±2.4^{aA}
		Mean ± SE for Ife brown cowpea			
500	100.0±0.0^{aA}	100±0.0^{aA}	100.0±0.0^{aA}	100.0±0.0^{aA}	98.0±2.0^{aA}
1000	100.0±0.0^{aA}	99.0±0.0^{aA}	98.0±2.0^{aA}	99.0±1.0^{aA}	98.0±2.0^{aA}
1500	99.0±1.0^{aA}	98.0±2.0^{aA}	99.0±1.0^{aA}	99.0±1.0^{aA}	97.0±2.0^{aA}

Means within a column accompanied by same lower case letters and within a row- upper case letter are not significantly different: Tukey-Kramer HSD test; P=0.05.

Table 4. Repellency Test - Number of *C. maculatus* adults found within 1 cm of cowpea seed for five DE-treated and untreated seeds after 3 d in IT98-12 white and Ife brown.

DEs	Number of adults within 1.0 cm of seed			
	Untreated seed	Treated seed	T-value	P-value
IT98-12 White				
Insecto®	24.5	5.5	24.3	0.002
Bularafa	22.0	8.0	6.6	0.001
Share	21.0	9.0	12.2	0.000
Abakire	21.7	8.3	6.1	0.005
Kwami	20.0	10.0	5.5	0.002
Ife brown				
Insecto®	25.7	4.3	10.3	0.002
Bularafa	22.0	8.0	18.4	0.004
Share	20.7	9.3	7.8	0.001
Abakire	21.5	8.5	7.9	0.002
Kwami	20.3	9.7	5.7	0.005

Values are means of four replicates.

94.0±2.4 and 100±0.0% germination; while the untreated control seed had mean germination of 100±0.0%. There was no significant difference (p>0.05) between the untreated control and treated seeds or among seeds treated at any dose rates of the five DE dusts (Table 3).

Repellency

The result on avoidance test is summarized in Table 4. The test data showed that adults of *C*. *maculatus* avoided contact with treated cowpea seeds.

DISCUSSION

In this study, raw Des derived from freshwater diatoms was used and compared to Insecto® which is derived from saltwater diatoms. The Insecto®, Bularafa and Abakire have silica content of 87, 80.98 and 60.17% respectively. Share and Kwami silica content had not been analysed. Insecto® is recommended at 1000 ppm. But we evaluated the DEs using 500, 1000 and 1500 ppm because different insects have different susceptibility to DE depending on different factors, such as DE type and concentration, grain moisture content, temperature, relative humidity of the environment, insect species, insect density and type of grain commodity (Korunic, 1997; Rigaux et al., 2001; Fields et al., 2003; Korunic and Fields, 2006). Diatomaceous earths differ in species of diatoms (shape), origin (marine or freshwater), particle size distribution, SiO_2 content. These propertiesof DEs influence their insecticidal activities (Korunic, 1997, 1998).

It was observed that increasing DE concentrations resulted in increased *C. maculatus* mortality (Shams et al., 2011) and that DE concentration affects mortality which was confirmed in this study. Bualrafa DE was significantly most effective of the four Nigeria-derived DE dusts against *C*. *maculatus*, both in terms of causing adult beetle mortality and in suppressing progeny production, while Insecto® was the most effective of the five DEs tested. We observed that Insecto® and Bularafa were at most efficient DE formulations. The high efficacy of Bularafa could be explained by the size of its particle, almost equal to Insecto®. The 10% of food-grade bait present in Insecto® may had influenced its efficacy against insects through internal desiccation due to feeding compared to Bularafa which is in raw state. Small percentage of added silica gel to Protect-It enhanced the efficacy of the DE (Korunic and Fields, 1995). Insecto® dose at 0.5 g and 1.0 g/kg of wheat or barley (500 and 1000 ppm has been found to achieve 94 to 100% mortality of seven insect species within 7-14 days (Subramahayam et al., 1994).

The results of the work shows that for adult mortality, the number of emerged F1 progeny reduced in proportion with increased DE dose rate, but could not prevent progeny production even where complete adult mortality was observed within 5 days. This is in agreement with the studies of Arnaud et al. (2005) who observed that mortality increased with concentration of DEs but live Tribolium castaneum were observed at highest 1000 ppm of Perma-Guard®, Insecto® and Dryacide®. This study shows that at dose rate of 1500 ppm, the five DE formulations did not prevent progeny emergence. Similar observations were recorded in previous studies with DEs against C. maculatus (Stathers et al., 2004; Kabir and Gaya, 2013; Kabir and Wuglo, 2014) and it seems this trend is common to all internal feeders as the developmental stages - larvae and pupae are inside the grain.

This study shows that longer exposure period interval at high dose rate had less progeny emergence in the treated seeds (Athanassiou et al., 2003; 2005; Wakil et al., 2010). Our results showed that DEs do not exhibit ovicidal effect; because 1500 ppm did not prevent oviposition before the death of the insects (Kabir and Wuglo, 2014). The effective progeny suppression (>90%) may be compensated for progeny production at the highest dose rate. This study showed that progeny suppression is a more important criterion to be considered in efficacy of the DEs on cowpea than adult mortality as the adults are short-lived, do not feed or cause damage but only lay eggs (Wakil et al., 2010; Kabir and Wuglo, 2014). The highest dose rate 1500 ppm used in our study was above the Insecto® dose of 1000 ppm for grain commodities. The dose rate was increased because the initial seed damage of cowpea was <2%; and high relative humidity when the study was conducted. In addition, Insecto® dosages up to 0.15% (w/w) gave complete mortality of *T*. *castaneum* adults (Subramanyam et al., 1994).

Studies have shown that DE efficacy decreases with increased relative humidity or grain moisture content (Arthur, 2000; Fields and Korunic, 2000). Wakil et al. (2010) reported 100% adult mortality of C. *maculatus* at 30°C and 50% rh and 91.7% at 30°C and 60% RH. It has been reported by studies that at high relative humidity levels, insects moderate water loss and the survival rate is increased after exposure in a DE-treated substrate (Fields and Korunic, 2000; Athanassiou et al., 2007).

The results indicated that the DEs could be used to control *C*. *maculatus* under the condition of optimum relative humidity (67%), based on the level of adult mortality, progeny suppression and prevention of seed damage achieved. There was no significant effect on germination capacity observed in the study which confirmed that there is no adverse effect on the quality of treated commodities (Korunic et al., 1996; Shayeateh and Ziaee, 2007; Kabir and Wuglo, 2014).

Repellency test data showed that adults of *C*. *maculatus* avoided contact with treated cowpea. Similar observation has been reported in studies with inert dusts against *Sitophilus zeamais* and *Rhyzopertha dominica* (Nwaubani and Farsoranti, 2008; Nwaubani et al., 2014). A possible negative implication of this is that stored grain beetle pests could reduce the effectiveness of DEs through this behavioral response (Rigaux et al., 2001).

Conclusion

Findings of this study indicate that DEs could be effective against *C*. *maculatus* in stored cowpea. The DEs were ranked in decreasing order of efficacy against *C*. *maculatus*: Insecto®=Bularafa>Share>Abakire>Kwami. As was previously reported for wheat, Bularafa seems to be an effective grain protectant in IPM program strategies for cowpea by both smallholder farmers and grain aggregators in Nigeria. The focus should be higher temperature and lower relative humidity combinations for storage of grain commodities in order to add value to the product and derive the benefit for application of DE-based strategy. As the DEs do not have adverse effect on germination capacity, Bularafa and the other three DEs could be used to protect cowpea seeds. In an effort to provide residue-free commodities for the consumers, Bularafa DE is a good alternative to synthetic insecticides. Additional studies should be conducted on oviposition and residual efficacy of the DEs against subsequent generations of the bruchid.

CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

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