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EFFICACY OF NIGERIA-DERIVED DIATOMACEOUS EARTH, BOTANICALS AND RIVERBED SAND AGAINST *Sitophilus oryzae* AND *Rhyzopertha dominica* ON WHEAT

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ABSTRACT

Powdered seeds of *Dennettia tripetala* (Baker f.) and *Piper guineense* Schumach, Bularafa diatomaceous earth (DE), Riverbed Sand, and combinations of the two botanicals with Bularafa DE and Riverbed Sand were evaluated as protectants of wheat seed against *Sitophilus oryzae* (L.) and *Rhyzopertha dominica* (F.). Insecto®, a commercialised DE, was tested as a standard check. The rates tested were 60 g kg^{-1} of wheat (for botanicals) and 1 g kg^{-1} of wheat (for other dusts). The botanicals singly and in combination with Bularafa DE and sand induced 100% mortality and total progeny suppression of both species in 7 days. Insecto and Bularafa DE treatments each induced ≥69% mortality and >78% progeny suppression against *R. dominica*, respectively. Sand was ineffective, inducing <10 and <13% mortality and progeny suppression, respectively. In *S. oryzae* assay, Insecto and Bularafa DEs singly caused ~ 100% mortality and >87% progeny suppression; while Riverbed Sand caused <9% mortality and <14% progeny suppression. Micrographs of dusted *S. oryzae* cuticle showed Bularafa and Insecto DEs to be abrasive. The relatively larger size of sand particles compared to DE particles probably contributed to its poor activity.

Key Words: Bularafa DE, *Dennettia tripetala*, *Piper guineense,* stored-product pest

RÉSUMÉ

Les grains poudrées de *Dennettia tripetala* (Baker f.) et *Piper guineense* Schumach, la terre à diatomées (DE) de Bularafa, les sables de rivière et des combinaisons de deux plantes avec Bularafa DE et sables de rivière ont été évalués comme agents protecteurs du blé contre *Sitophilus oryzae* (L.) et *Rhyzopertha dominica* (F.). Insecto®. Une terre à diatomées commercialisée a été utilisée comme référence. Les concentrations testées étaient 60 g kg^{-1} de blé (for les plantes) et 1 g kg^{-1} de blé (pour les autres). Les plantes seules, en combinaison avec Bularafa DE et sable ont engendré 100% de mortalité des pestes et la suppression totale des larves des deux espèces en 7 jours. Les traitements avec Insecto et Bularafa DE ont engendré chacun respectivement ≥ 69% de mortalité et >78% de suppression des larves de *R. dominica*. Le traitement de sable était inefficace, causant seulement <10% de mortalité et <13% de suppression de larves. Chez *S. oryzae*, Insecto and Bularafa DEs seuls ont causé approximativement 100% de mortalité et >87% de suppression de larves, tandis que les sables de rivière ont causé <9% de mortalité et <14% de suppression de larves. La microscopie électronique de la cuticule dégradée de *S. oryzae* montre que les terre à diatomées Bularafa Insecto DEs sont abrasifs. La taille relativement plus large des particules de sable par rapport aux particules de terres à diatomées est probablement responsable de la faible activité des sables.

Mots Clés: Bularafa DE, *Dennettia tripetala*, *Piper guineense,* stocké-produit antiparasitaire

INTRODUCTION

Wheat *(Triticum aestivum* L.) is attacked by various insect pests between harvest and storage. *Sitophilus oryzae* (L.) (Coleoptera: Curculionidae) and *Rhyzopertha dominica* (F.) (Coleoptera: Bostrichidae) are among the most important pests of wheat (Adedire, 2001). In Nigeria, insect-related postharvest losses in wheat of over 30% have been reported (Ivbijaro, 1989). For small-scale farmers in Africa, the main purpose of storage is to ensure household food supplies (reserves) and seed for planting (Adetunji, 2007).

Despite success in controlling insect pests using synthetic insecticides, their persistence in the environment, the toxic residues they leave in food, and development of resistance by insect pests require that more reduced-risk alternatives are sought (Ileke and Oni, 2011). Control measures with little or no negative impacts on the environment and human health are logical alternatives (Ileke, 2008). Compounds that occur naturally in plants (e.g. spices) and some environmentally benign substances like diatomaceous earth are good potential alternatives. *Piper guineense* Schumach and *Dennettia tripetala* (Baker f.) are spice plants that have been used to control stored-product insects (Okonkwo and Ewete, 2000; Abdullahi and Muhammad, 2004; Oparaeke and Bunmi, 2006), and an example of environmentally benign substance is Nigeria-derived Bularafa diatomaceous earth (Nwaubani *et al*., 2014).

The use of spices for postharvest pest control is inexpensive, locally available for most of sub-Saharan Africa, relatively safe, and poses little or no hazard to human health and the environment (Aslam *et al*., 2002; Mahdi and Rahman, 2008). *Piper* is a climbing plant with more than 700 species throughout the tropics and subtropics (Purseglove *et al*., 1991). It is known by different local names in Nigeria: Igbo (Uziza), Yoruba (Iyere) and is sometimes referred to as Guinea pepper or Ashanti pepper. It is a good alternative insecticide because it is also used for culinary and medicinal purposes, and has proven safe for humans *D. tripetala* is a well-known woody and spicy indigenous forest medicinal plant, widely distributed in West Africa; and concoctions prepared from its parts are used in traditional medicine for the treatment of skin infections, candidiasis, cough, fever, dysentery and stomach ache (Okigbo *et al*., 2005). In Nigeria, it is locally called 'Nkarika' by the Efiks of Calabar, 'Imako' by the Urhobo tribe of the Niger-Delta region, 'Igberi' by the Yorubas, and 'Umimi' by the Igbos. It is red when ripe and green in the unripe form, with a pungent spicy taste.

Diatomaceous earth (DE) is a natural product mined from deposits of fossilised skeletal remains of diatoms, which inhabited marine and fresh water bodies (Vayias and Athanassiou, 2004). Diatomaceous earths leave no harmful residues on treated grains and have low mammalian toxicity (Stathers *et al*., 2004). DE absorbs lipids and abrades the epicuticular wax layer of insects, causing loss of body water and death within hours or days (Subramanyam and Roesli, 2000).

Vast deposits of DE have been discovered in the Nigerian towns of Bularafa, Gujba, and Maluri in Yobe state (RMRDCN, 2009). Research needs to be conducted to find ways to enhance the effectiveness of Nigeria-derived DEs against stored-product pests (Nwaubani *et al*., 2014) and one of the possible ways is in a mixture with plant materials (Vayias *et al*., 2006). Therefore, the present study investigated if the efficacy of Bularafa DE or Riverbed Sand (RBS) against *S. oryzae* and *R. dominica* could be enhanced by combining with *D. tripetala* and *P. guineense*.

MATERIALS AND METHODS

Study insects. Adults of *S. oryzae* and *R. dominica* used in this study were originally collected in or near Payne Co., OK, U.S.A. The insects were taken from cultures that were kept in an incubator on 95% whole wheat and 5% Brewer's yeast (wt/wt), and maintained at 28 ±1°C and 65% relative humidity.

Botanical formulations. Mature seeds of *D. tripetala* and ripe fruits of *P. guineense* were obtained from Nnewi markets in Anambra State, Eastern Nigeria. Seeds of *D. tripetala* were removed with a scalpel from the soft pericarp, washed using tap water, and each of the botanical spices was thinly spread on the laboratory table under a shade to dry (Okonkwo and Okoye, 2001).

Seeds were then oven-dried at 30°C for 72 hours (Donald *et al*., 2008) and ground to dust by means of a laboratory electric blender. They were sieved using a U.S. Standard #200 sieve (0.075 mm or 75 µm openings) (Seedburo Equipment Company, Chicago, IL), and kept in air-tight Kilner jars for 24 hours. The two botanicals were applied at a dose rate of 60 g kg^{-1} of wheat (Okonkwo and Okoye, 1996).

DE formulations. A crude DE ore of fresh water origin was obtained from Bularafa community in Yobe State, Northern Nigeria. It was oven-dried at 40°C for six hours to 4.5% moisture content (Arnaud *et al*., 2005), ground to dust by means of a laboratory mortar and pestle. It was sieved using a U.S. Standard #200 sieve, and kept in airtight Kilner jars for 24 hours. One commercially available DE formulation, Insecto (Insecto Natural Products, Costa Mesa, California), a DE that contains 10% food grade additives (Subramanyam *et al*., 1994) was also assessed. Both types of DE were applied at a dose of 1 g kg^{-1} of wheat, the upper dose limit for Insecto application (500 - 1,000 ppm) (Subramanyam *et al*., 1994; Golob, 1997). Insecto was included as a positive control. It is registered by the U.S. Environmental Protection Agency for control of insect pests on stored grain and treatment of empty grain storage structures (Subramanyam *et al*., 1994).

Riverbed Sand formulation. Riverbed Sand was obtained from River Niger in Onitsha community of Anambra State, in Eastern Nigeria. It was air dried at room temperature (22–23°C) in the laboratory for 72 hours. It was ground to dust by means of a laboratory mortar and pestle, sieved using a U.S. Standard #200 sieve, and kept in airtight Kilner jars. The Riverbed Sand was applied at a dose rate of 1 g kg^{-1} of wheat.

Bioassays. Hard red winter wheat (*Triticum aestivum* L., variety Endurance) used was tempered to a moisture content of 11.5% by equilibrating for 18 days prior to the commencement of the experiment. Equilibration was conducted at controlled laboratory temperatures in a plastic box (42 cm x 29 cm x 24 cm) that contained saturated $NaNO_2$ solution beneath its false floor to maintain 63 ± 5% RH (Greenspan, 1977). Nine lots of 100 g of wheat were placed separately in 360-ml cylindrical glass jars, and admixed with Bularafa DE, Riverbed Sand, Insecto, *P. guineense*, *D. tripetala*, Bularafa DE + *P. guineense*, Bularafa DE + *D. tripetala*, Riverbed Sand + *P. guineense*, or Riverbed Sand + *D. tripetala* at dose rates described above. Another lot of 100 g of wheat that was not treated was placed in a 360-ml jar containing wheat for the negative control. The jars were manually shaken vigorously for approximately three minutes, to achieve proper and uniform coating of the grains with the applied dusts.

From each of the treated 100-g lots, nine 10-g samples were placed in cylindrical plastic vials (3.5 cm diameter, 6 cm high). Nine additional vials, each containing 10-g of untreated wheat served as vials for the control. Subsequently, 20 adults of mixed sexes and ages, of *S. oryzae,* were placed in each vial. Each vial was closed using a cap lid, which had a 1.5-cm diameter hole that was covered with a screen (U.S. #40 mesh) to allow aeration and prevent insects from escaping. The top third of the inner surface of each vial was coated with Fluon® (polytetrafluoroethylene; Northern Products, Woonsocket, RI) to prevent insects from climbing up and resting on the cap lids. Altogether, there were 90 vials for the *S. oryzae* test. The same procedure was repeated for *R. dominica*.

Vials for *S. oryzae* and *R. dominica* were randomly placed in a plastic box (42 cm x 29 cm x 24 cm) painted black to maintain dark conditions; and containing saturated $NaNO_2$ solution, below a perforated false floor to maintain 63 ± 5% RH. There were six boxes in total (three boxes for each species), each containing a total of 90 vials for each insect species, which were prepared on different dates, i.e., the experiment had three temporal replications. Boxes were incubated at 30 ± 1°C. These conditions (30 ± 1°C and 63 ± 5% RH) were chosen to simulate the hot dry ambient conditions in the grain producing Savannah belt of Northern Nigeria where the tested protectants could eventually be used for grain storage.

Temperature and relative humidity inside the boxes were monitored using HOBO data loggers (Onset Computer Corporation, Bourne, MA; model U12-011). For each box, three of the nine treated vials for each treatment and species and three of the nine untreated control vials were examined for insect mortality at 7 and 14 days post-treatment (here admixing any of the protectants with wheat is referred to as "treatment"). Insects that did not move spontaneously or respond to slight pressure applied using a blunt probe were considered dead and later discarded (Dyte and Forster, 1973).

Fourteen days after addition of insects to the treated and untreated wheat, contents (wheat) in the remaining vials were sieved to remove all insects (dead and alive), and then the vials were closed and returned to the incubators for an additional 54 days (68 days post-treatment) after which the number of adults in each vial was determined (Nwaubani, 2006). Reduction in progeny production relative to the control was calculated using the formula of Arthur and Throne (2003):

$$\text{RPP}(\%) = 1 - \frac{\text{Number of } F_1 \text{ in treatment}}{\text{Number of } F_1 \text{ in control}} \times \frac{100}{1}$$

Where: RPP = Reduction in progeny production

The experimental design for determining 7- and 14-day mortality and 68-day progeny production (F_1) was a completely randomised design.

Mineralogy, particle size, and pH analyses of Nigeria-derived DE and Riverbed Sand. Samples of Bularafa DE and Riverbed Sand were analysed at the Acme Analytical Laboratories Ltd, Vancouver, Canada. The determination of chemical composition of samples involved analysing 0.2-g samples using Inductively Coupled Plasma Emission Spectroscopy, following lithium metaborate/tetraborate fusion and dilute nitric digestion for determining total abundance of major oxides and several minor elements.

For determining particle sizes, scanning electron microscope images taken using a scanning electron microscope, were analysed using an image analysis programme called AnalySIS (Intertek, Allentown, PA, U.S.A.). Fifty particles were measured for each sample. The pH levels of Bularafa DE and Riverbed Sand were also determined. The solution for determination of pH of DE was prepared using a procedure described by Korunic (1997), which comprised of mixing 1.75 g of DE with 15.75 ml of double distilled water (DI) and stirring for 15 s. For Riverbed Sand, 20 g of ground sand was mixed with 40 ml of distilled water (Test Procedure 283, AMCOL Metalcasting, 2013), and the pH value measured using a pH meter (Five Easy, model FE20, Mettler Toledo, Pittsburg, PA).

Insect cuticle. Effects of *P. guineense*, Bularafa DE, Insecto, and Riverbed Sand on the insect cuticle were examined using a scanning electron microscope. *Sitophilus oryzae* and *R. dominica* species (7–10 insects) were obtained from different treatments for assessment. The specimens were mounted on an aluminium stub that had double sided carbon tape attached to it, with the help of a fine forceps. The samples were sputter coated with gold-palladium (Au-pd) (Cressington Carbon Coater; Balzers Union Med 010 Au/Pt coater) for 1 minute, and viewed under a scanning electron microscope (FEI Quanta 600 field emission gun ESEM with Evex EDS and HKL EBSD) in the microscopy laboratory, Oklahoma State University, Stillwater, OK, USA. Scanning electron micrographs were taken at a working distance of 10 mm and at 10 kV.

Data analysis. Data were analysed by species. Mortality data for 7- and 14-day exposure periods were corrected using Abbots formula (Abbot 1925). All statistical procedures were accomplished using Statistical Analysis System software (SAS Institute, 2013). PROC MIXED was used for a two-way analysis of variance (ANOVA) to determine the effects of treatments and exposure interval on adult insect mortality. To equalise variances, percentage data were transformed using the square root of the arcsine; untransformed data are reported to simplify interpretation. Where appropriate, the square root transformation was used to stabilise variances in relation to progeny production (number of progeny) data, but untransformed data are reported. The significance of mean differences was determined by Tukey's HSD (honest significant difference) test at $P<0.05$.

RESULTS

Mortality and progeny production suppression. *Piper guineense* and *D. tripetala* powders resulted in a corrected mortality of 100% for adult *R. dominica* within 7 days, when applied alone and when combined with Riverbed Sand or Bularafa DE. Riverbed Sand, Bularafa DE, and Insecto caused mortalities of 9.5, 69, and 71%, respectively, against *R. dominica* (Fig. 1A; Table 1). There was no significant increase in *R. dominica* mortality when exposure interval increased from 7 to 14 days (Fig. 1B; Table 1). All *P. guineense*- and *D. tripetala*-related treatments resulted in total progeny production suppression (Fig. 2; Table 1); whereas progeny production in the Riverbed Sand and control treatments was similar. Riverbed Sand, Bularafa DE, and Insecto treatments resulted in 12.7, 80.1, and 78.1% reduction in progeny production, respectively, compared with the control (Fig. 2B). The effectiveness of the treatments in suppressing *R. dominica* progeny production, in a decreasing order was botanicals alone, botanicals in combination with Bularafa DE, or with Riverbed Sand, Bularafa DE, Insecto, and Riverbed Sand (Fig. 2).

As for *S. oryzae* adults, *Piper guineense, D. tripetala*, Insecto, Bularafa DE, and combinations of botanicals with Bularafa DE or Riverbed Sand treatments were highly effective (Fig. 3A; Table 1). However, Riverbed Sand alone was ineffective and caused only 8% mortality (Fig. 3; Table 1). Adult mortality after 14 days was significantly greater than mortality after 7 days (Fig. 3B). All *P. guineense* and *D. tripetala* related treatments resulted in total progeny production suppression (Fig. 3; Table 1); whereas progeny production in the Riverbed Sand and control treatments was similar. Riverbed Sand, Bularafa DE, and Insecto treatments resulted in 13, 88 and 93% reduction in progeny production, respectively (Fig. 4B), compared to the control. The effectiveness of the treatments in suppressing *S. oryzae* progeny production in decreasing order were botanicals alone, botanicals in combination with Bularafa DE or Riverbed Sand, Insecto, Bularafa DE, and Riverbed Sand (Fig. 4).

Mineralogy, particle size, and pH analyses of Nigeria-derived DE and Riverbed Sand. The amount of silica (SiO_2) found in Bularafa DE and Riverbed Sand were 80.98 and 82.21%, respectively (Tables 2 and 3). Other compounds with the second and third highest concentrations in Bularafa DE were aluminium oxide (Al_2O_3; 4.94%) and iron oxide (Fe_2O_3; 2.3%), respectively (Table 2); and for Riverbed Sand, Al_2O_3 (7.25%) and Fe_2O_3 (2.23%), respectively (Table 3). In relation to elements found in Bularafa DE, barium (Ba; 130 ppm) and zirconium (Zr; 107 ppm) had the highest and second highest concentrations, respectively (Table 2). In Riverbed Sand, elements with the highest and second highest concentrations were zirconium (Zr; 2,380 ppm) and barium (Ba; 724 ppm), respectively (Table 3).

The mean, minimum, and maximum particle sizes for Bularafa DE were 4.3, 1.2, and 13.5 µm, respectively. These parameters for Riverbed Sand were 156.8, 75.0, and 405.4 µm, respectively. The

TABLE 1. ANOVA results for main effects and interactions for corrected mortality of the two beetle species, and for effects of protectant on number of progeny and percentage reduction in the number of progeny of the two beetle species

Parameter	Source	df	*R. dominica*		*S. oryzae*	
			F	P	F	P
Corrected mortality	Protectant	8,34	168.5	<0.001	289.3	<0.001
	Exposure interval	1,34	0.7	0.42	10.0	0.004
	Protectant x Exposure interval	8,34	0.3	0.97	1.5	0.20
Number of progeny	Protectant	9,18	326.6	<0.001	195.2	<0.001
Percentage reduction in number of progeny	Protectant	8,16	190.3	<0.001	105.6	<0.001

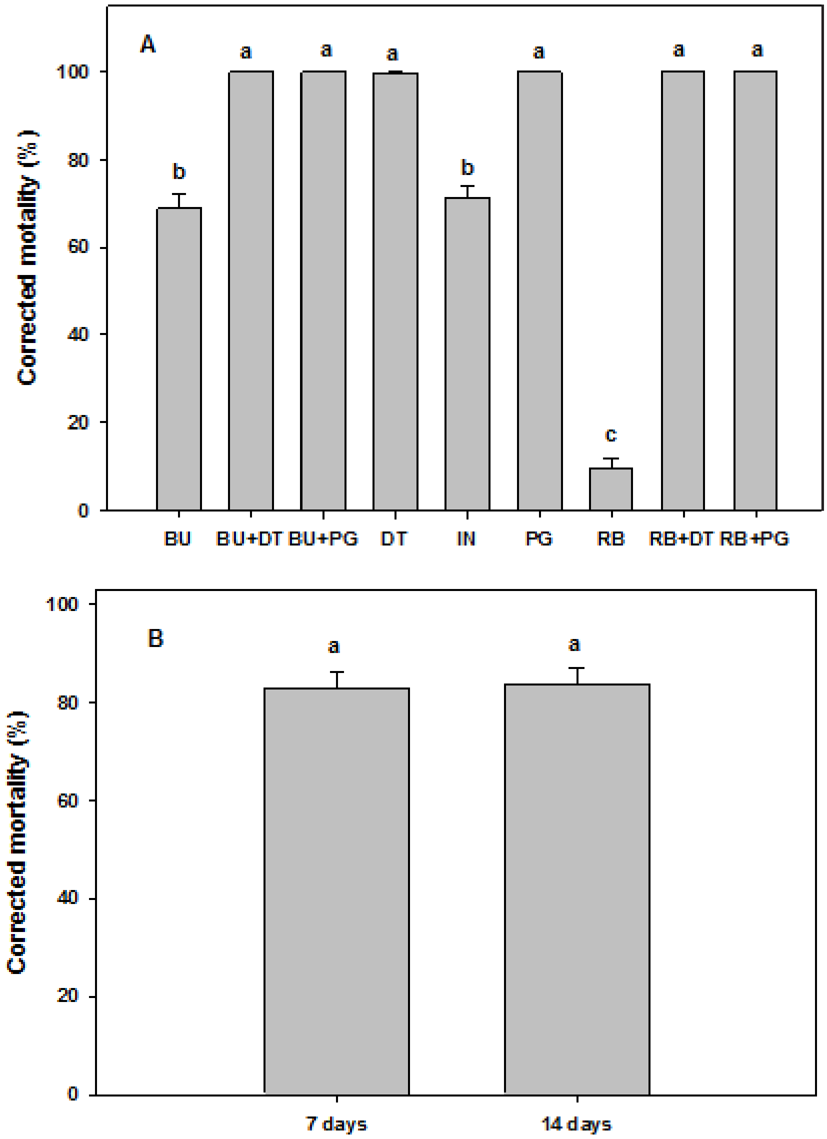

Figure 1. Corrected percentage mortality of *R. dominica* (mean ± SE) as a result of using different protectants (A) and due to the two exposure periods (B) in vials containing wheat treated using different protectants. BU is Bularafa diatomaceous earth; DT is *D. tripetala*; PG is *P. guineense*; CO is control; IN is Insecto®; and RB is Riverbed Sand. Means followed by different lowercase letters are significantly different ($P < 0.05$).

pH values for Bularafa DE and Riverbed Sand were 7.91 and 9.03, respectively.

Insect cuticle. Micrographs of *S. oryzae* cuticle showed that all treatments involving Bularafa DE, Insecto, or *P. guineense* caused markedly more abrasion of the cuticle compared to the control and Riverbed Sand treatments (Fig. 5). Surprisingly, Riverbed Sand seemed to cause very little abrasion (Fig. 5E), whereas *P. guineense* and related treatments caused marked abrasion of *S. oryzae* cuticle (Fig. 5C and F).

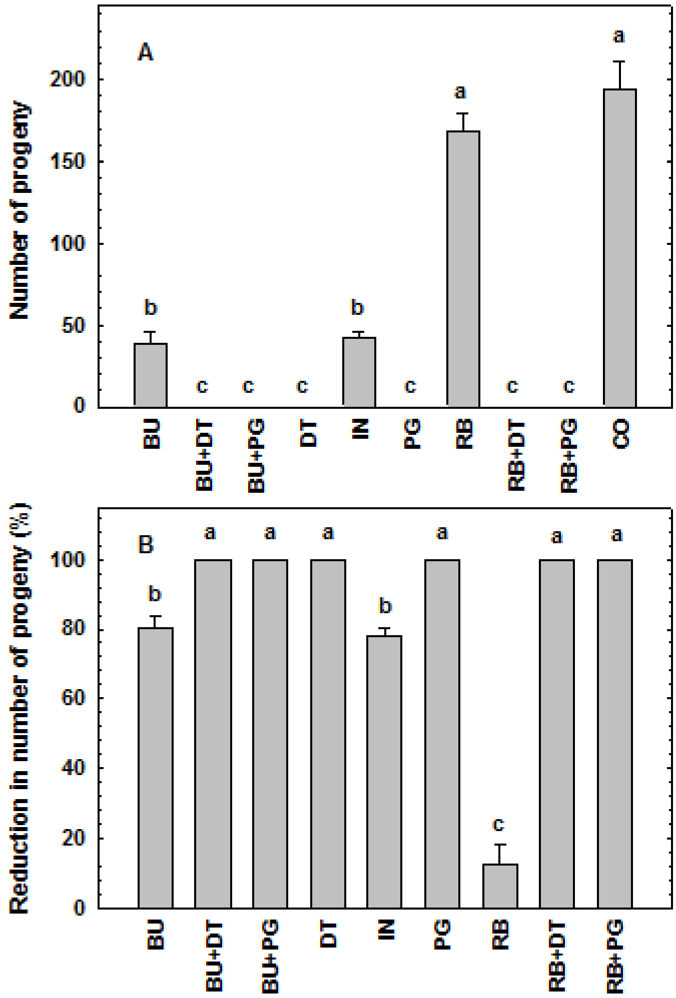

Figure 2. Number of progeny (mean ± SE) (A) and percent reduction in the number of progeny (B) of *R. dominica* found in vials containing wheat treated with different protectants. BU = Bularafa diatomaceous earth; DT = *D. tripetala*; PG = *P. guineense*; CO = control; IN = Insecto®; and RB = Riverbed Sand. Means followed by different lower case letters are significantly different ($P < 0.05$).

DISCUSSION

Based on our data, *D. tripetala*, and *P. guineense* were highly effective against both *R. dominica* and *S. oryzae* in terms of causing mortality in adults and suppressing progeny production (Table 1). These data corroborate with laboratory findings from studies by Okonkwo and Okoye (1996) and Udo (2005), that *P. guineense* and *D. tripetala*, applied at a concentration of 5–10% were highly effective against species in the genera *Sitophilus* and *Rhyzopertha*. Our data support the increasing evidence for the use of plant insecticidal compounds for stored-product insect pest management because they cause high adult mortality and inhibit pest reproduction (Regnault–Roger, 1997).

In the current study, mortalities caused by Bularafa DE against *R. dominica* and *S. oryzae,* and the reduction in progeny production caused by Bularafa DE for these two species show that it is as effective as Insecto (Figs. 1-4). Our findings corroborate the data obtained from a study conducted by Nwaubani *et al*. (2014) on

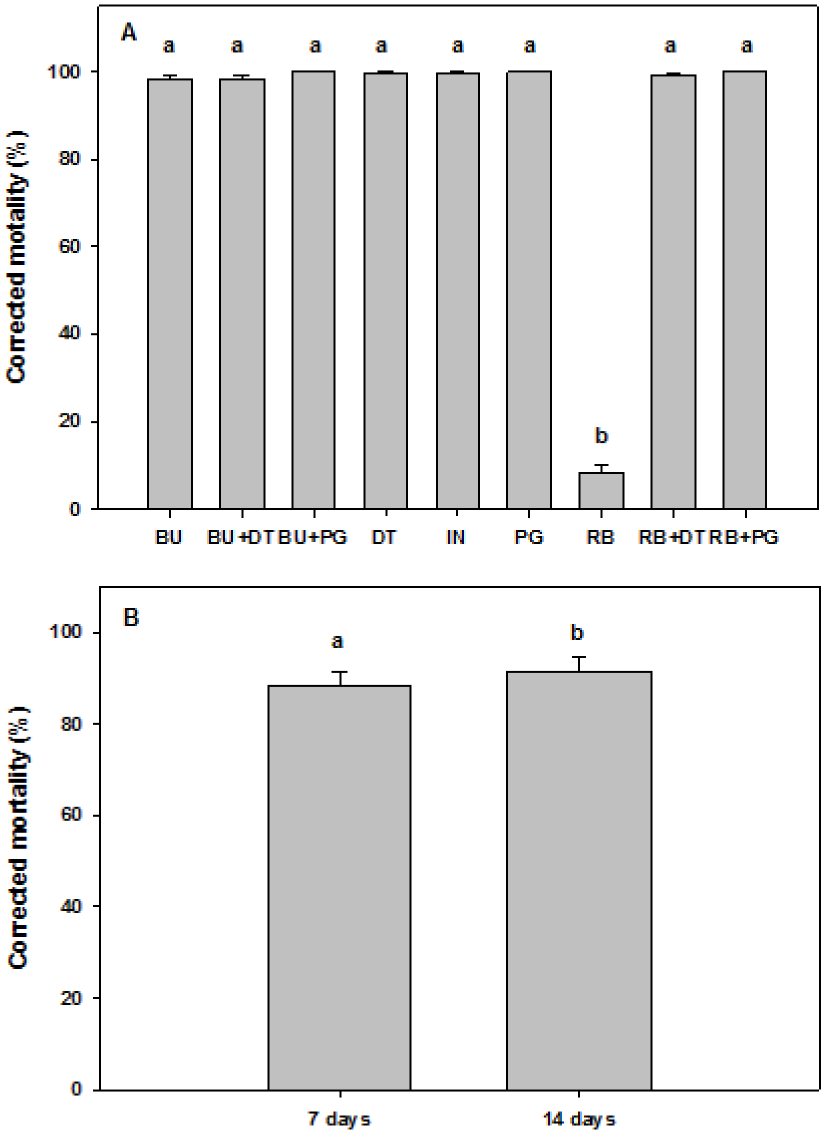

Figure 3. Corrected percentage mortality of *S. oryzae* (mean ± SE) as a result of using different protectants (A) and due to the two exposure periods (B) in vials containing wheat treated using different protectants. BU is Bularafa diatomaceous earth; DT is *D. tripetala*; PG is *P. guineense*; CO is control; IN is Insecto®; and RB is Riverbed Sand. Means followed by different lowercase letters are significantly different ($P < 0.05$).

the efficacy of Bularafa DE in which they found that this DE caused 61.2% mortality in *R. dominica* after 14 days in treated wheat. For *S. oryzae*, mortality after 7 and 14 days in treated wheat was 79.6 and 100%, respectively.

The current study shows that Bularafa DE can be enhanced with local botanicals to achieve 100% mortality of *R. dominica*. According to our data, *R. dominica* was more tolerant to the DEs than *S. oryzae* on wheat. However, the result could differ if the DEs are tested on commodities other than wheat, because efficacy of DEs against *R. dominica* has been found to vary among different commodities (Kavallieratos *et al.*, 2005). Although reasons for the relatively low susceptibility of bostrichids (*R. dominica*

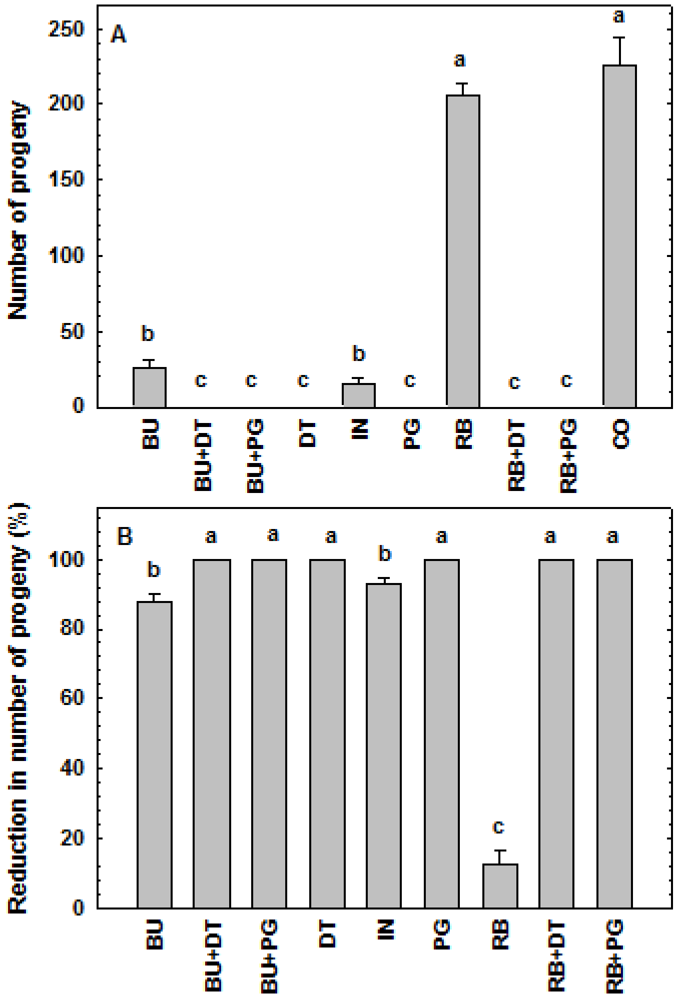

Figure 4. Number of progeny (mean ± SE) (A) and percent reduction in the number of progeny (B) of *S. oryzae* found in vials containing wheat treated with different protectants. BU = Bularafa diatomaceous earth; DT = *D. tripetala*; PG = *P. guineense*; CO = control; IN = Insecto®; and RB = Riverbed Sand. Means followed by different lowercase letters are significantly different ($P < 0.05$).

included) to DEs are not well understood, their low mobility which results in reduced contact between the cuticle and dust particles (Athanassiou *et al*., 2005), is a plausible explanation. It could also be due to taxonomic differences in the nature of the cuticles of the two test species which caused differential rates of water loss (Nawrot *et al*., 1994). Other reasons for the higher tolerance of *R. dominica* can be found in Nwaubani *et al*. (2014).

According to Nwaubani *et al.* (2014) and data from the current study, Bularafa DE is only moderately effective against *R. dominica,* because mortality of at least 80% is considered effective. This study showed that the effectiveness of Bularafa DE against *R. dominica* can be greatly enhanced by addition of *D. tripetala*, or *P. guineense*. However, the concentrations of the two botanicals needed to enhance the effectiveness of the DE are likely to be much lower than the concentration of 60 g kg^{-1} used in this study.

Now that we have shown the potential of *D. tripetala* and *P. guineense*, future studies can

TABLE 2. Concentrations of compounds and elements found in Bularafa – DE

Group	Molar dilutions	Composition (%)
Compounds		
SiO_2	0.01	80.98
Al_2O_3	0.01	4.94
Fe_2O_3	0.01	2.30
MgO	0.01	0.44
CaO	0.01	0.46
Na_2O	0.01	0.16
K_2O	0.01	0.36
TiO_2	0.01	0.41
P_2O_5	0.01	0.03
MnO	0.01	<0.01
Cr_2O_3	0.01	0.02
Elements		**(in ppm)**
Ba	5	130
Ni	20	45
Sr	2	66
Zr	5	107
Y	3	8
Nb	5	15
Sc	1	4

TABLE 3. Concentrations of compounds and elements found in Riverbed Sand

Group	Molar dilutions	Composition (%)
Compounds		
SiO_2	0.01	82.21
Al_2O_3	0.01	7.25
Fe_2O_3	0.04	2.23
MgO	0.01	0.26
CaO	0.01	0.84
Na_2O	0.01	0.95
K_2O	0.01	2.62
TiO_2	0.01	0.93
P_2O_5	0.01	0.06
MnO	0.01	0.04
Cr_2O_3	0.002	<0.01
Elements		**(in ppm)**
Ba	5	724
Ni	20	<20
Sr	2	166
Zr	5	2380
Y	3	32
Nb	5	12
Sc	1	4

focus on determining the lowest concentrations of botanicals that can be combined with DEs to achieve complete mortality (100% mortality) of stored-product insect pests.

We have also shown that Insecto and Bularafa DE are significantly more effective than Riverbed Sand, both in terms of causing beetle mortality and in suppressing progeny production. This could be because Bularafa DE has very similar chemical composition to Insecto (Subramanyam *et al*., 1994) and most likely because Bularafa DE has much uniform particles compared to Riverbed Sand. Our data reveal that the chemical composition of Bularafa DE (Table 2) was similar to that of Insecto as further elaborated by Subramanyam *et al*. (1994) and Golob (1997). This explains the similarities they displayed in their effectiveness against *S.oryzae* and *R.dominica* in causing mortality and reducing progeny formation. The mean particle sizes for Bularafa DE and Riverbed Sand were 4.3 and 156.8 µm, respectively. For this study, a laboratory pestle and mortar were used to grind botanicals, DEs, and Riverbed Sand. The mean particle size that could be obtained for the Riverbed Sand was 156.8 µm.

In future research, we recommend that the Riverbed Sand be subjected to mechanical grinding to further reduce the particle size and make it closer to that of DE particles. Smaller particles have greater surface to volume ratio, resulting in increased area of contact between particles and insect cuticle, hence causing greater effectiveness in terms of mortality (Korunic, 1997, 1998).

Silica (SiO_2) is the basic material that adsorbs epicuticular wax, leading to desiccation and death in DE-treated insects (Ebeling, 1971; Korunic, 1998; Athanassiou and Steenberg, 2007). It appears that the larger size of Riverbed Sand particles may have offset the benefits of its high Silica content, thereby greatly reducing its effectiveness against *R. dominica* and *S. oryzae*. It is also possible that the silica in sand, being crystalline in nature, having been derived from quartz (Subramanyam and Roesli, 2000), is not as insecticidal as amorphous silica in Bularafa DE, leading to its poor performance in the present

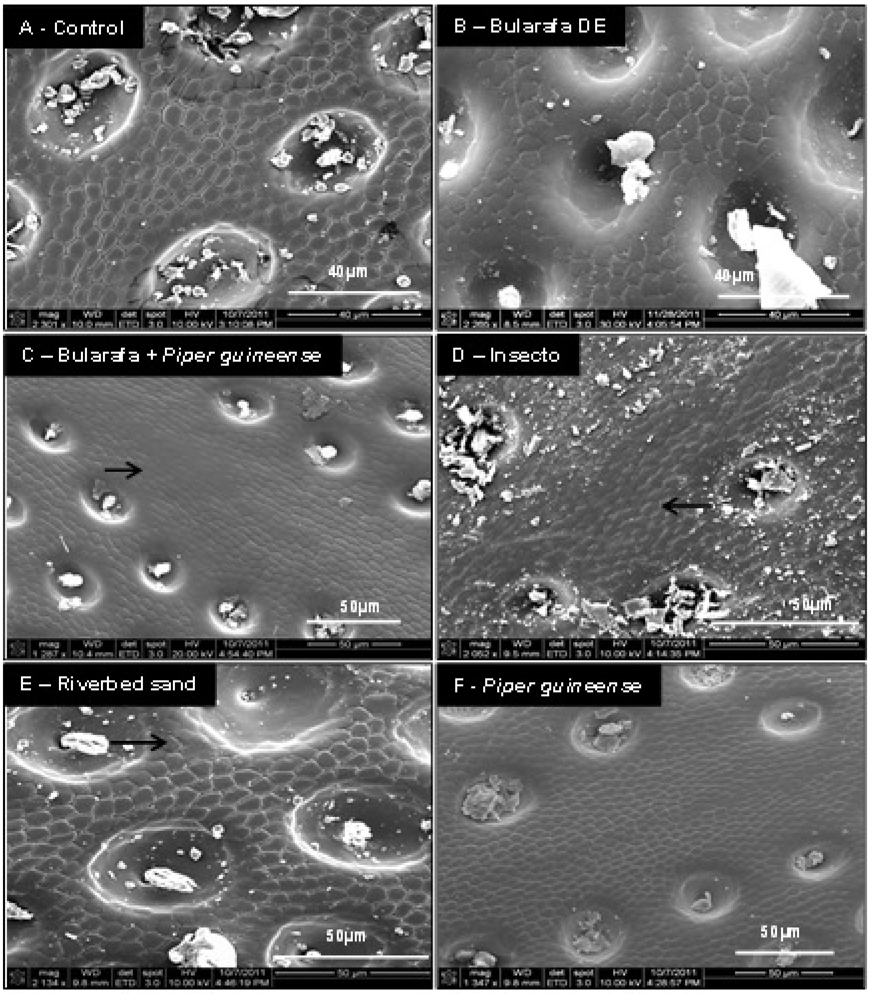

Figure 5. Micrographs of *S. oryzae* cuticle showing that all treatments involving Bularafa DE, Insecto, or *P. guineense* caused markedly more abrasion of the cuticle compared to the control and Riverbed Sand treatments.

study. According to Korunic (1997), silica content is just one of a number of factors that determine the efficacy of a particular abrasive material. A possible way of increasing the efficacy of Riverbed Sand may be to grind it more finely to the size of Bularafa DE particles. Its efficacy can also be enhanced by increasing the dose rate (Golob *et al*., 1982). Golob *et al*. (1982) treated maize with washed sand at the rates of 1:1, 1:2, 1:4, and 1:8 by volume and found that the dose effect significantly restricted infestation of the commodity and the effectiveness was dose related. They argued that sand was added on a volume basis because its high density precluded effective mixing when added on a weight basis. However, they did not grind their sand as was done in our study. They only passed the sand through a sieve with apertures of 1.4 mm.

Based on data from this study, even a mean particle size of 156.8 µm did not substantially increase mortality due to Riverbed Sand. Grinding to a much smaller size may achieve the desired increase in mortality of the insects. The Riverbed Sand could also be combined with *P. guineense* and *D. tripetala* to increase its effectiveness. Besides the direct effects of botanicals in causing insect mortality, the reason why their combination with Riverbed Sand was found effective may be because they block the inter-grain spaces thereby inhibiting pest movement and development (Golob and Webley, 1980).

Mineralogical data (Tables 2 and 3) indicate that Riverbed Sand has slightly higher silica (82.21%) levels than Bularafa DE (80.98%). Tables 2 and 3 also show how levels of the different elements and compounds in Riverbed sand and Bularafa DE differ or are similar. Korunic (2013) and other references cited therein, provide information on how the levels of elements and compounds shown in Tables 2 and 3 relate to health, safety, and regulatory aspects. For example, DEs registered as insecticides generally have less than 6% crystalline silica; in some countries, it is less than 1% (Korunic, 2013). Crystalline silica has been shown to be carcinogenic if inhaled (Anon, 1987).

Micrographs of *S. oryzae* cuticle showed that all treatments involving Bularafa DE and Insecto caused markedly more abrasion of the cuticle compared to the control and Riverbed Sand treatments (Fig. 5). This explains the mode of action of Bularafa DE and Insecto and their greater effectiveness against *S. oryzae*. These observations confirm reports that silica in DEs kills insects through adsorption and abrasion of cuticular wax, that leads to desiccation (Athanassiou and Steenberg, 2007). The fact that there was marked abrasion of insect cuticle in the *P. guineense* treatment may indicate that removal of cuticular wax plays a role in how botanical powders kill insects. The poor abrasive capacity of Riverbed Sand against *S. oryzae* may be attributed to the non- uniformity and large size of its particles compared to the particles of the two DEs used.

CONCLUSION

This study supports the use of Nigeria-derived DEs and biopesticides for stored-product insect pest management since they reduce insect survival and breeding. DE can be used singly or combined to treat seed grain in Nigeria to ensure that they are protected against *R. dominica* and *S. oryzae*. Based on data from this study, research needs to be conducted to determine the lowest concentrations of D. *tripetala* and *P. guineense* combined with Nigeria-derived DEs to adequately enhance the effectiveness of these DEs against *R. dominca*, *S. oryzae*, and other stored-product insect pests.

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