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EFFICACY OF DIATOMACEOUS EARTH (PROTECT-IT) ON MANAGEMENT OF COWPEA BEETLE (*CALLOSOBRUCHUS MACULATUS* (COLEOPTERA: CHRYSOMELIDAE)) INFESTING STORED BAMBARA GROUNDNUT (*VIGNA SUBTERRANEA*)

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Abstract
Laboratory studies was conducted at Entomology Laboratory of University of Maiduguri under ambient laboratory conditions to assess the efficacy of diatomaceous earth (DE) against C. maculatus infesting bambara groundnut. The bioassays were conducted on treated seeds of Bambara groundnut variety Farin ngangala. C. maculatus adults was bioassayed on 50g seed samples using CRD replicated three (3) times at the following dose rates; DE (250, 500, 750, 1000, 1500 mg/kg of seeds) The effects of treatments on seed germination was conducted on freshly treated seeds and at 90 days after treatment (DAT). The results showed that DE applied at 1000mg/kg and 1500mg/kg completely suppressed ovipostion, adult emergence, damage and weight loss. DE only affected germination at the highest dose rate of 1500mg. It was concluded that DE could be used to manage C. maculatus populations in stored bambara groundnut.

Keyword: Diatomaceous earth; Cowpea; Oviposition; Mortality; Germination

Introduction
Bambara groundnut, (*Vigna subterranea* L. (Verdcourt)), is a leguminous crop of the order Fabales, family Fabaceae and sub family, Faboidea, with creeping stems and branching just above ground level. The seeds are variously colored from white to cream, red, black or brown, sometimes mottled; blotched or striped (Bamshaiye *et al*., 2011). The seeds are used in many types of foods, some of which are an important part of human diets. Mature, dry seeds are boiled and eaten as a pulse. Seeds can be milled to make flour. Ripe seeds are very hard and must be cooked for longer than those of other legumes. Like other legume plants, *V. subterranea* is a good soil fertilizer and a good rotation crop. It does not require any additional fertilizer. It is generally intercropped

with cereals (maize, sorghum, and pearl millet), other pulses (cowpea, groundnut), root and tuber crops, or vegetables. Cream-coloured seeds are often preferred to red and black seeds, because they are less bitter and take less time to cook. Large seeds are preferred to smaller ones, especially for use as a snack. Smaller seeds are ground into flour for use in various recipes. The processing of *V. subterranea* results in by- products (shells of offals) that can be fed to animals. Seeds can also be fed to animals if surplus to human requirements. The leafy shoots are used as fodder (Brink *et al.,* 2006). The seeds are used for food and beverage because of their high protein content and for digestive system applications. The entire plant is known for soil improvement because of nitrogen fixation. According to Mazahib *et al*. (2013), high carbohydrate (65%) and relatively high protein (18%) content as well as sufficient quantities of fat (6.5%) make the Bambara groundnut a complete food. According to Bamashiye *et al*. (2011), Bambara groundnut seeds have been found to be richer than peanuts (groundnuts) in essential amino acids such as isoleucine, leucine, lysine, methionine, phenylalanine, threonine and valine. This is an important trait for the potential of bambara groundnut to be used to complement foods lacking in these essential amino acids. The fatty acid content is predominantly linoleic, palmitic and linolenic acids, (Minka and Bruneteau, 2000). The major bruchid species that infest bambara groundnut in storage are: *Callosobruchus maculatus* Fabricus, *C. subinnotatus* Pic, *C. chinensis* Linnaeus and *Zabrotes subfasciatus* Bohemian (Ayamdoo *et al.,* 2013). Of these, *C. maculatus* is the major storage pest of Bambara groundnut seeds (Ayamdoo *et al*., 2013; Magagula and Maina, 2012). They are field to store agricultural pests of Africa and Asia (Beck and Blumer, 2014) and are the most destructive on account of their shorter life cycle (Maina and Lale, 2004). The weevil can cause as much as 99% yield or weight loss in susceptible grain legumes.

The damage results in quantitative and qualitative losses. To combat these debilitating storage pests, farmers and traders in west Africa use storage structures and other available materials like earthenware pots, gourds, mud silos, jute sacks, bagco bags, metal drums, plastic containers and local granaries for grain storage. These are most often, integrated with synthetic pesticides (Aviara *et al*., 2013 and Ayamdoo *et al*., 2013). However, the unabated use of toxic synthetic chemicals has given rise to problems of toxicity, pest resurgence and elevation of secondary pests, development of pesticide resistant populations, deleterious effects on population of non–target organisms, residues in food chain, high costs of most of the chemicals, contamination of the environment, non–availability and the falsification and adulteration of pesticides (Bloch, 2012; Grzywacz and Leavett, 2012).

In this effect, the increased awareness and concern for biorational alternatives, which are effective, economically sound, feasible, and user friendly, have gained an important position in the protection of stored products. They are safe, effective, and relatively simple prevention and control methods available to manage populations of stored-product insect pests without the use of chemical insecticides. The use of biorational approach provides practical economical and safer ways to managing stored-product insect pests. These approaches are either directly take advantage of key aspects of the pest's biology to eliminate or manage pest populations through manipulation of the physical and biological environments of the target species. Control of stored-product insects by desiccation can be facilitated by treatment of infested commodity and spaces with diatomaceous earth (DE). DE represents the fossilized silicon dioxide skeletons of diatoms, which are unicellular aquatic algae. DE kills insects following contact exposure by absorbing the hydrocarbons from their cuticles, which causes dehydration and ultimate death (Korunic, 1998). The activity of DE is increased under low humidity and higher temperatures. An enhanced DE was developed that utilizes added silica gel, a finer and more homogenous source of silicon dioxide (Korunic and Fields, 1998). DE is nontoxic to vertebrates and is even a common food additive and food-processing agent with the designation GRAS (generally regarded as safe). The efficacy of DE varies significantly among its geographic source locations where it is mined, so users must follow label instructions closely to ensure control (Korunic, 1998). Application of DE at effective rates to an entire grain mass can cause a significant loss in bulk density, thus lowering the quality and value of the treated grain (Korunic *et al*., 1996); care should be taken to use minimal effective rates or to treat problem areas only (e.g., the top or bottom layers of the grain mass). The objective study was to evaluate the efficacy of Diatomaceous earth on management of *Callosobruchus maculatus* infesting stored Bambara groundnut.

Materials and method

Study Site

The study was conducted at the Entomology Laboratory of the Department of Crop Protection, Faculty of Agriculture, University of Maiduguri. All experiments were conducted under ambient laboratory conditions. Temperature and relative humidity ranged from 28 to 34°C and 22 to 58% respectively. The study was conducted during the period of March – June.

Source of the Insects

Cowpea Bruchid; *Callosobruchus maculatus* adults were collected from cultures maintained on cowpea in the laboratory and was used to establish new cultures on Bamabara groundnut. The insects were then reared for two generations on Bambara groundnut for the experiments. Culturing entailed placing a hundred unsexed adult insects onto 300 g of sterilized Bambara groundnuts seeds placed in 1-liter capacity jar for seven days and then sieved out and discarded. The resulting F_1 generation aged 1 – 2 days old were used for the experiments.

Handling of Bambara Groundnut Seeds

The seeds, after acquisition, were cleaned and placed in deep freeze refrigerator for 2 weeks to kill all life stages of the insect that might be present. The seeds were conditioned to room temperature for at least ten (10) days before commencement of the experiment.

Source of diatomaceous earth (DE)

Diatomaceous Earth was provided by the Department of Crop Protection, University of Maiduguri, from samples obtained from Diatom Research Consulting Incorporation, Canada.

Bioassay Procedures for Determination of Efficacy of Diatomaceous Earth against *C. maculatus.*

The bioassays were conducted on treated seeds of the variety *Farin ngangala*. The choice of application rates were based on published literature, largely on studies arising from control of pest infesting stored cowpea. The application rates used in the present study were: D.E (250, 500, 750, 1000, 1500 mg/kg of seeds)

Grain treatment, bioassay and experimental design

Fifty grams of *Farin ngangala* cultivar seeds were placed in a 150 ml jar and the appropriate treatments were applied on the seeds. The jars were shaken vigorously to ensure proper mixing after which they were infested with newly emerged (1 – 24 h old) adult bruchids from the culture. Five pairs of the newly emerged adult *C. maculatus* were introduced per jar. The jars were covered and then arranged on the laboratory bench using CRD. Each treatment combination (insecticidal material x dose) was replicated three (3) times. The adults were allowed to oviposit for seven (7) days after which they were sieved out and discarded.

Observation and data collection

Egg counts were carried out seven (7) days post treatment. This was done by random draw of 20 seeds from each jar in each concentration per treatment. The seeds were observed with a magnifying glass (x 100) and the number of eggs on the seeds was counted. The number of adults produced in each jar was counted forty (40) days after infestation. The percentage damage and percentage weight loss was determined by randomly taking 50 seeds from each jar, and then separated into holed (damaged) and whole (undamaged) grains. The seeds in each category were counted and the numbers used to calculate the percentage damage:

$$\text{Percentage seed damage (SD) (\%)} = \frac{\text{Number of damaged grains}}{\text{Total number of grains}} \times 100$$

For percentage weight loss, the grains in each of the above categories were weighed, and the weights used to calculate the percentage weight loss according to Golob *et al*. (1982).

$$\text{Weight loss (\%)} = \frac{(Und) - (Dnu)}{U(nd + nu)} \times 100$$

Adult emergence inhibition rate

Adult emergence inhibition rate (AEIR) was calculated according to Kabir (2011) as:

$$AEIR\% = \frac{Ne - Na}{Ne} \times 100$$

Hatching rate

Hatching rate was calculated as:

$$\frac{\text{Number of emerged } F_1 \text{ progeny}}{\text{Number of eggs produced}} \times 100$$

Determination of Treatment Effect on Seed Germination

Germination test was conducted according to the methods described by the International Seed Testing Association, 2015. Two germination tests were conducted. The first was carried out at the beginning of the experiment with freshly treated seeds. Whereas the second test was performed on treated seeds described earlier and infested with the insects ninety (90) days after treatment (DAT). From each treatment combination (insecticidal material x dose), ten (10) seeds were selected randomly from each jar and placed on moistened plastic Petri dishes, lined with Whatman's No. 1 filter paper. The petri dishes were labeled and arranged on a laboratory bench using CRD for seven days. There were four replicates per treatment combination. Germinated seedlings were counted after seven (7) days. And percentage germination was calculated using the formula.

$$\text{Germination percentage} = \left[\frac{\text{Number of seed germinated}}{\text{Total number of seeds in each petri dish}}\right] \times 100$$

Data Analysis

The data were subjected to analysis of variance (ANOVA) using statistical software (Statistic 8.0). Differences in mean values of treatment were separated using Tukey Kramer's honestly significant difference (HSD) at 5% level of probability. Data on germination test were subjected to T- test to determine any differences between the freshly treated seeds and those treated and stored for 90 days.

Table 1: Effect of diatomaceous earth on ovipostion, adult emergence, hatching rate and inhibition rate of *Callosobruchus maculatus* on Bambara groundnut seeds

Application rate (mg/kg)	Oviposition	Adult Emergence	Hatching rate (%)	Inhibition rate (%)
0.0	193.7 ± 9.8[a]	144.3±4.7[a]	74.7±1.8[a]	-
250	33.7 ±2.3[b]	13.7± 4.1[b]	39.4± 9.9[b]	90.5
500	22.7 ± 4.3[bc]	7.7 ±2.7[bc]	32.0± 5.7[b]	94.7
750	11.0 ±4.6[bc]	2.0±0.6[bc]	20.0± 2.9[bc]	98.6
1000	2.0 ±1.0[c]	0.0± 0.0[c]	0.0 ± 0.0[c]	100

1500	0.0 ±0.0c	0.0± 0.0c	0.0 ± 0.0c	100
F	235	422	33.5	-
P	<0.0001	<0.0001	<0.0001	-

*Means within a column followed by same letters are not significantly different from one another (Tukey Kramer's HSD test)

Effect of diatomaceous earth against *C. maculatus* and seed germination of Bambara groundnut.

The effect of diatomaceous earth on ovipostion, adult emergence hatching rate and inhibition rate of *C. maculatus* on stored Bambara nut at different dose rates is shown on Table 1. Compared to control, there was a drastic decrease in the number of eggs even at the lowest dose rate 250g. Oviposition further decreased with increase in dose rate as low as 0.0 in the highest dose. The result shows that there was significant difference P=<0.0001 between dose rates and control. Treatment also showed significant effect P=<0.0001 on adult emergence. Adult emergence reduces with increase in dose rate up to 0.0 at dose rate 1000g and 1500g. The results on hatching rate and progeny inhibition showed significant difference between dose rate and control, there was 0.0 % hatching rate and 100% inhibition rate at dose rates 1000g and 1500g due to 0% adult emergence. Dose rates varied significantly with the control, hatching rate decreased with increase in dose rare while inhibition rate increased with increase in dose rate.

Fig 1: Effect of DE on percentage damage and weight loss of stored Bambara groundnut caused by Callosobruchus maculatus

Effect of diatomaceous earth against *C. maculatus* on percentage seed damage and weight loss of Bambara groundnut.

Percentage damage and weight loss decreased with an increasing dose rate (Fig 1), with decrease of 0% at dose rates 1000 and 1500mg/100g.

Table 2: Effect of diatomaceous earth on seed germination of bambara groundnut

Application rate (ml/kg)	% germination 0 days after treatment	% germination 90 days after treatment	T-test
0.0	100.00a	100.00a	Nd
250	100.00a	100.00a	Nd
500	100.00a	100.00a	Nd
750	95.00ab	100.00a	0.0917
1000	92.50bc	98.75a	0.0154
1500	88.75c	93.75b	0.0917
F	17.5	12.0	-
P	<0.0001	<0.0001	-

*Means within a column followed by same letters are not significantly different from one another (Tukey Kramer's HSD test)

Nd – not determinable

Effect of diatomaceous earth on seed germination of Bambara groundnut

The effect of diatomaceous earth on Germination rate of Bambara groundnut at 0 DAT showed that treatment did not differ with control at 250, 500 and 750g; 750g and 1000g did not vary; and 1000g and 1500g do not vary with each other. At 90 DAT dose rates did not vary with each other except the highest dose. T-test showed no significant difference P=<0.0001 between germination at 0 DAT and 90 DAT at dose rate 0.0, 250mg and 500mg (Table 2).

Discussion

The findings from this study indicated that Protect-It can be used successfully as a protectant against adults of Cowpea bruchid *Callosobruchus maculatus*. Regardless of the synergism of other factors, the effect of DEs is dose-dependent (Fields & Korunic, 2000; Athanassiou *et al*., 2003; Stathers *et al.*, 2004; Mahdi & Khalequzzaman, 2006), as in the case of residual insecticides used as grain protectants. **The overall efficacy depends upon different factors such as type and concentration of DE, grain moisture content, temperature, insect species, insect density and type of grain commodity (Korunic and Fields, 2006). Among these factors, temperature and relative humidity plays an important role in determining the efficacy of DE against stored insect pest (White and Loschiavo, 1989).** However, the dose rate is more important in the case of inert dusts, given that the presence of dust in seed highly affects the physical properties of seed (Korunic *et al.*, 1996). In addition, dust formulations that are effective at high application rates (Subramanyam & Roesli, 2000). In the present study, the rates of 1500mg/kg produced high mortality levels, 100% was achieved in most of the cases examined. However, given that *C. maculatus* cannot survive at application rates higher than 1000mg/kg and are effective against other stored-grain beetle species (Fields & Korunic, 2000), higher dose rates or longer exposure intervals are needed to achieve 100% mortality for adults of this species. Moreover, this insecticide has a long persistence after treatment (Kabir and Lawan, 2016). Fields and Korunic (2000) have confirmed the effectiveness of several DE-based formulations on adults of *Tribolium castaneum*. As proven in this work, *C. maculatus* express susceptibility to DE due to morphological, physiological and ecological characteristics.

The efficacy of diatomaceous earth on the mortality of insect pests of stored products is usually affected by several factors among which stands out the temperature (Chanbang *et al*., 2007). Generally, the increase in temperature favors the increase in the effectiveness of this product by stimulating the movement of insects within the grain mass, providing an increased contact of them, with the diatomaceous earth (Vayias *et al*., 2009). In addition, the insects have higher respiration rates at higher temperatures and consequently the greater water loss via spiracles promoting desiccation (Zachariassen, 1991). However, it was shown in some studies that the insect mortality can vary between species (Vayias *et al*., 2009).

Increased exposure time is highly important for DE efficacy, since surviving individuals may disperse from the treated substrate and colonize untreated parts of the product mass (Subramanyam and Roesli, 2000). This fact must be seriously taken into account in cases of partially treated grain masses with DE, such as the surface treatment in grain bulks, when DE in the surface is used alone, as a barrier to infestation (Korunic and Mackay, 2000). Earlier studies have shown that stored grain insect pests can be controlled by commercially available DE formulations. For example, Kavallieratos *et al*. (2005) used DEs Insecto and SilicoSec on eight different grain commodities, at 750 mg/kg and recorded mortality after 14 days of exposure ranging from 63 to 97%. Similarly, Vardeman *et al*. (2006) reported that Protect-It at the same exposure found that 400 mg/kg gave 85% adult mortality for *R. dominica*. However, our findings are consistent with the results reported in other studies (Fields and Korunic, 2000; Athanassiou and Kavallieratos, 2005). Athanassiou *et al*. (2006) reported 100% mortality of the exposed *R. dominica* adults after 14 days in wheat. However, **longer exposure interval suppresses the progeny emergence in the treated substrate provided the dry condition prevails (Athanassiou *et al.*, 2005). Also, Paula (2001) reported that the higher dose rate was negatively correlated with the production of F_1 of *Sitophilus* spp. The present trial undoubtedly supports the statements of other researchers as there was less progeny at high dose rates and longer exposure intervals, which practically prevented damage and weight loss.**

Conclusion

Conclusively, DE proved to be effective in managing *C. maculatus* on stored bambara groundnut. The treatments significantly reduced oviposition and progeny emergence, which varies significantly with control. Similarly, treatments decreased damage and weight loss significantly compared to the untreated control. Treatments increased effectiveness with increase in dose rate. All treatments did not significantly reduce germination both at 0 DAT and at 90 DAT except in control treatment.

Recommendations

It is recommended that DEs should be adopted by farmers as alternative for synthetic insecticides, as they have proven effectiveness in the management of *C. maculatus* on stored Bambara nut. DE could be used at 1000 mg/kg. The use of DE as a safe control method against stored pests is highly recommended. The results obtained indicate the potential of non-chemical method development.

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