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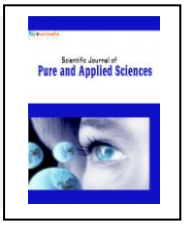

Original article

Effect of raw diatomaceous earth and plant powders on *Callosobruchus subinnotatus* (Pic.) infesting stored bambara groundnut seeds

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ABSTRACT

Experiments were conducted on the efficacy of raw DE and plant powders (*Jatropha curcas* seed and leaf powders of *Eucalyptus* and *Ricinus*) for their insecticidal potential against *Callosobruchus subinnotatus* infesting stored bambara groundnut seeds. The treatments consisted of the raw DE, plant powders, actellic dust (2%) as check and an untreated control. Thirty adults 0-3 days old were used to infest 100 g bambara groundnut seeds (cv. Ka'aro). Raw DE was used at 0.3, 0.6 and 0.9g/100g seeds and plants powders at 0.5, 1.0, and 2 .0g/100g seeds each replicated three times in completely randomized design. Adult mortality was recorded from 24 to 96 hours; progeny emergence as well as percentage seed damage and viability were assessed. The result revealed that, raw DE at 0.9g recorded significant ($P<0.05$) mortality of 78.81% and JSP at 2.0g gave 63.52 % mortality both after 24 h of exposure. Higher mortality was observed for higher dosage of all treatments after 96 h. Progeny production was significantly ($P<0.05$) inhibited in treated seeds. The results therefore suggested that, raw DE and plant powders are promising alternatives to synthetic pesticides against *C. subinnotatus* in bambara groundnut seeds.

1. Introduction

Bambara groundnut (*Vigna subterranea* L. Verdc.) is an indigenous grain legume grown mainly by subsistence farmers in drier parts of sub-Saharan Africa. In much of Africa, bambara groundnut is the third most important legume after peanuts and cowpea (Sellschop, 1962). It can thrive on poor soils with little rainfall as well as produce substantial yields under better conditions (Brough and Azam-Ali, 1992). Bambara groundnut is a rich source of protein (16-25 %) and its seeds are valued both for their nutritional and economic importance (Coudert, 1982).

Bambara groundnut seeds are most often infested simultaneously by two species of bruchids: *C. subinnotatus* (Pic.) and *Callosobruchus maculatus* (F.); *C. subinnotatus* is restricted to bambara groundnut in West Africa (Haines, 1991). These bruchids commence infestation in the field once bambara groundnuts have been harvested and left to dry (Prevett, 1966). Infestation levels in the fields are usually low but, it increase rapidly during storage (Caswell, 1980). Seeds suffer quantitative and qualitative losses as a result of damaged by *C. subinnotatus*, thereby reducing the dietary protein level, lower income and germination ability (Oparaeke and Bunmi, 2006).

The control of storage insects including *C. subinnotatus* relies on the use of synthetic insecticides (Zettler and Cuperus, 1990). There are however major setback to the use of synthetic insecticides including the risk to the user, high cost of procurement, effects on non-targeted as well as development of pest resistant strains and toxic residue in the food crops (Lale, 2002). The above therefore have necessitated the search for safe and environmental friendly alternative crop protectants such as diatomaceous earth and botanical insecticides (Subramanyam and Roesli, 2000; Isman, 2006). DEs are fossilized remains of unicellular algae called diatoms; it largely composed of amorphous silicon dioxide which is non-toxic to mammals, does not break down rapidly and does not affect the end use quality (Subramanyam and Roesli, 2000). Several DE formulations are now registered and commercially available for use as grain protectants in Australia, Brazil, Canada, Croatia, China, Germany, Indonesia, Japan, Philippines, Saudi Arabia, United Arab Emirates and USA; and are generally effective against stored product insects (Athanassiou *et al*., 2006). Many studies have been documented on the efficacies of commercial DE formulation. However, there is scarcity of published work on raw DE. Although all DE affect insects in the same way, there are considerable variations in efficacy among different formulations (Kabir *et al*. 2010). The present work is aimed at evaluating the efficacy of raw DE and plant powders in the control of *C. subinnotatus* infesting stored bambara groundnuts.

2. Materials and methods

This study was conducted in Agricultural Biology Laboratory, Department of Crop Science, Usmanu Danfodiyo University, Sokoto. Sokoto is located in the Dry sub-humid agro-ecological zone (Latitude $13^0$01'N and Longitude $5^0$15'E). This agroecological zone is characterized by rainy seasons which starts between mid-May to early June and reaches peak in August. Dry season starts in mid-October and ends in late April. The coldest months are November to January which is characterized by dry harmattan. The monthly average temperature varies from 15^0 C to 45^0C during the year and the annual rainfall ranges from 500-700 mm unimodal rainfall pattern (Ojanuga, 2006).

2.1. Culturing of C. subinnotatus

C. subinnotatus were collected from infested bambara groundnut seeds from the Kara market in Sokoto and the identity of the insect was confirmed at Insect Museum, Department of Crop Protection, Ahmadu Bello University, Zaria. Bambara groundnut seeds (cv. Ka'aro) were obtained from farmers in Tambuwal, Sokoto, Nigeria. The seeds were initially sterilized in a Gallenkamp hot air oven at 80^oC for 4 hours before usage (Allotey and Azalekor, 2000). Cultures were maintained in 1- L glass jars containing 200 adult bruchids per 500 g of bambara groundnut seeds in the laboratory under ambient conditions at 26-33^oC and 41-56% relative humidity. The experiments were also conducted under similar conditions as described above.

2.2. Diatomaceous earth

Diatomaceous earth (DE) was obtained from the Department of Geology, University of Maiduguri, Nigeria. This was supplied in a form of crude, soft chalky rock. This was later milled finely in the laboratory using pestle and mortar and these were sieved using 0.20 mm sieve to obtain fine powder. It has tapped density of 312g/l, pH-9.2

and the mineral composition is as follows SiO_2 - 38.04%, Al_2O_3 - 12.6%, CaO - 26.5%, Na_2O - 11.6%, K_2O - 9.3%, FeO - 0.9%, ZnO - 0.33%, CuO - 0.18%- MnO - 0.55%.

2.3. Plant materials

Fresh leaves of Eucalyptus *camaldulensis* Dehnh, *Ricinus communis* (L.) and seeds of *Jatropha curcas* (L.) were sourced from the vicinity of Usmanu Danfodiyo University, Sokoto (Main campus) and identified at the Botany unit of the University. Voucher specimens were prepared and deposited in the herbarium of the University for reference. The plant materials were air dried in the shade and later ground to powder using pestle and mortar. The powders were further sieved using 0.20 mm sieve to obtain fine powder. The powders were packed in air tight bottles and stored in a deep freezer at 5^0C prior to use. Pirimiphos-methyl (Actellic dust) bought from a commercial store was used as check.

2.4. Bioassay

Treatments (raw DE and plant powders) were applied to 100g bambara groundnut seeds in 500 ml capacity glass jars at the doses 0.3, 0.6, 0.9 g and 0.5, 1.0, 2.0g, respectively. Actellic dust at 2.0g was used as check and untreated served as control. The jars with their contents were manually shaken to ensure thorough admixture of the bambara groundnut seeds with the treatments. Later 30, 0-3 days-old mixed sex adults of *C. subinnotatus* were introduced to the treated and untreated seeds in the glass jars. The jars were covered with perforated lids to facilitate ventilation and prevent escape of the insects. The treatments were arranged in a completely randomized design and replicated three times on the laboratory bench.

2.5. Data collection

2.5.1. Mortality

Mortality was assessed 24, 48, 72 and 96 hours after treatment application. The number of dead insects in each jar was sieved and counted during each assessment and insects were considered dead if they did not respond to touch with a pin.

2.5.2. F_1 progeny emergence

The live insects were allowed to mate and oviposit for 3 days after the last mortality assessment of 96 hours. Later adults in both treated and control jars were removed after 7 days. Based on the life cycle of the adult insects, F_1 progeny started emerging 32 days after introduction, the number of emerged F_1 progeny was counted daily up to 39 days after introduction to avoid overlapping of generations. Percentage reduction in progeny production or inhibition rate was determined using Aldryhim's (1995) formular

$$\%\,\text{reduction in progeny} = \left[\frac{\text{Number of progeny in control-Number of progeny in treatment}}{\text{Number of progeny in control}}\right]\times 100$$

2.5.3. Seed damage and weight loss

Ninety days after introduction, seeds in all the treatments were separated into holed (damaged) and whole (undamaged) to assess the level of damage. The seeds in each category were counted and the numbers used to calculate the percentage damage:

The percentage seeds damage were calculated according to Enobakhare and Law – Ogbomo (2002):

$$\%\,\text{seed damage} = \left[\frac{\text{Number of damaged seeds}}{\text{Total number of seeds}}\right]\times 100$$

The percentage seeds weight loss was calculated according to Baba-Tierto (1994):

$$\%\text{Weight loss} = \left[\frac{\text{Initial weight of control sample-Final weight of sample}}{\text{Initial weight of control sample}}\right]\times 100$$

2.5.4. Seed viability assay

Ninety days after, the seeds were tested for viability. From each jar, 10 seeds were randomly taken and placed in Petri dishes lined with moistened filter paper. These were left on the laboratory bench at room

temperature for 5 days after which percentage germination was recorded. Each treatment was replicated three times. The germination percentage was calculated according to Ogendo *et al*. (2004) as follows:

$$\text{Viability index (\%)} = \left[\frac{\text{Number of seed germinated}}{\text{Total number of seeds in each petri dish}} \right] \times 100$$

2.6. Statistical analysis

Mortality counts were corrected by using Abbott's (Abbott's, 1925) formula. Before the analysis, adult mortality, seeds damaged, seeds weight loss, seeds viability and inhibition rate data were arcsine transformed, while progeny data were transformed using square root transformation in order to meet the assumptions of ANOVA. The transformed data were subjected to ANOVA using the GLM procedure of statistical software (Statistix 8.0). After analysis, data were back-transformed by squaring the sine of the number. Significant difference between means were compared using Tukey – Kramer (HSD) test at P=0.05 (Zar, 1984).

3. Results

There was significant difference (P<0.05) between treatments in adult mortality (Table 1). Raw DE at highest dose rates of 0.9g caused higher adult mortality (78.81%) which is statistically similar to Actellic dust at 2.0g (67.05%) and the mortality of *C. subinnotatus* adults exposed to plant powders was generally low 24 hours after exposure. However, 96 hours after exposure mortality ranged between 47.38 and 100% (Table 1). The results suggested that mortality increased with an increase of exposure periods and dose rates.

The mean number of F_1 progeny emergence in untreated control was significantly (P<0.05) higher than other treatments (Table 2.). In the treated seeds, progeny productions varied according to the treatment and are statistically similar. Progeny production was lowest from seeds treated with Actellic dust at 2.0g/100g of bambara groundnut (0.3 adults). The adverse effect of the treatments suppressed F_1 emergence and drastically reduced their development at all doses tested (Table 2).

Table 1
Mean mortality of adult *C. subinnotatus* on bambara groundnut seeds treated with raw diatomaceous earth and plant powders

Treatments	Dose(g/100g seeds)	Post treatment Percentage (%) mortality			
		24	48	72	96
DE	0.3	30.58±3.1^{cd}	61.24±11.9^{cde}	70.87±12.8^{bcde}	72.97±13.8^{bcd}
	0.6	61.17± 2.0^{b}	84.99±0.0^{abc}	96.19± 2.1^{ab}	100.00±0.0^{a}
	0.9	78.81± 2.0^{a}	92.42±3.7^{ab}	100.00±0.0^{a}	100.00±0.00^{a}
JSP	0.5	24.70±1.2^{de}	63.74±3.3^{cd}	79.73±5.0^{bcd}	89.51±3.5^{ab}
	1.0	42.35±3.1^{c}	72.49±1.2^{bcd}	83.53±6.6^{abc}	93.40±1.3^{ab}
	2.0	63.52±2.3^{b}	81.24±3.7^{abc}	84.89±4.2^{abc}	93.51±3.4^{ab}
ELP	0.5	17.64±2.3^{e}	31.24±4.9^{ef}	43.03±7.9^{de}	51.33±3.4^{cd}
	1.0	32.93±2.0^{cd}	51.32±7.4^{def}	68.34±10.8^{cde}	72.62±12.1^{bcd}
	2.0	42.34±5.1^{c}	48.74±6.9^{def}	81.00±4.3^{bc}	86.98±5.7^{abc}
RLP	0.5	15.29±2.0^{e}	21.68±5.6^{f}	36.69±5.0^{e}	47.38±2.6^{d}
	1.0	32.93±2.0^{cd}	45.21±8.5^{def}	65.81±7.9^{cde}	80.92±6.2^{abcd}
	2.0	42.34±1.2^{c}	70.02±2.1^{bcd}	83.53±2.5^{bc}	92.08±2.2^{ab}
Actellic dust	2.0	67.05±4.2^{ab}	97.77±1.1^{a}	100.00±0.0^{a}	100.00±0.0^{a}
Control (Untreated)	0.0	0.00±0.0^{f}	0.00±0.0^{g}	0.00±0.0^{f}	0.00±0.0^{e}

Each value is a means (± SE) of three replicates. Mean within a column followed by the same superscripts are not significantly different: Tukey- Kramer HSD test at P = 0.05. DE-Diatomaceous Earth: JSP- *Jatropha* seed powder: ELP- *Eucalyptus* leaf powder: RLP-*Ricinus* leaf powder.

Table 3 shows percentage seed damage and weight loss as a result of feeding by *C. subinnotatus*. DE and plant powders at higher dosage gave protection against *C. subinnotatus* than the untreated control. Percentage seed damage varies from 9.68 % in DE 0.9g to 50.89 % in the untreated control and less than 2 % in Actellic dust. Weight loss of 17.00 % was recorded from seed treated with RLP 0.5g followed by ELP 0.5g (19.00 %) and untreated control (40.80%). The present finding revealed that there was significant weight loss on untreated control as a result of activity of *C. subinnotatus* (Table 3).

Table 2

Mean number of *C. subinnotatus* F_1 adults' emergence and inhibition rate on bambara groundnut seeds.

Treatments	Dose (g/100g seeds)	Mean number of F_1 adults	Inhibition rate (%)
DE	0.3	15.00 ± 2.5^{b}	89.01 ± 0.3^{b}
	0.6	13.66 ± 1.2^{b}	86.48 ± 2.2^{b}
	0.9	13.66 ± 0.8^{b}	87.68 ± 0.7^{b}
JSP	0.5	19.66 ± 5.2^{b}	82.28 ± 4.6^{b}
	1.0	12.66 ± 0.8^{b}	88.58 ± 0.7^{b}
	2.0	10.66 ± 0.8^{b}	90.38 ± 0.7^{b}
ELP	0.5	14.33 ± 1.4^{b}	87.08 ± 1.3^{b}
	1.0	14.33 ± 1.2^{b}	86.48 ± 0.5^{b}
	2.0	10.66 ± 0.8^{b}	90.38 ± 0.7^{b}
RLP	0.5	14.66 ± 1.4^{b}	89.48 ± 0.7^{b}
	1.0	11.66 ± 0.8^{b}	86.78 ± 1.3^{b}
	2.0	10.66 ± 0.8^{b}	90.69 ± 0.3^{b}
Actellic dust	2.0	0.33 ± 0.3^{c}	99.69 ± 0.3^{a}
Control (Untreated)	0.0	111.00 ± 11.3^{a}	0.00 ± 0.0^{c}

Each value is a means (± SE) of three replicates. Mean within a column followed by the same superscripts are not significantly different: Tukey- Kramer HSD test at P = 0.05. DE-Diatomaceous Earth: JSP- *Jatropha* seed powder: ELP- *Eucalyptus* leaf powder: RLP-*Ricinus* leaf powder

Table 3

Effect of raw DE and plant powders on percentage seed damaged and seed weight loss after of three months of storage.

Treatments	Dose (g/100g seeds)	% Seed damaged	% Seed weight loss
DE	0.3	25.36 ± 1.6^{bc}	15.80 ± 0.8^{bc}
	0.6	17.10 ± 1.1^{def}	10.00 ± 0.7^{def}
	0.9	9.68 ± 0.5^{g}	6.0 ± 0.3^{f}
JSP	0.5	27.11 ± 0.6^{cd}	16.40 ± 1.0^{bcde}
	1.0	21.78 ± 2.9^{bc}	14.00 ± 2.1^{b}
	2.0	14.47 ± 2.0^{efg}	9.0 ± 1.5^{ef}
ELP	0.5	32.53 ± 0.7^{b}	19.00 ± 0.5^{b}
	1.0	23.91 ± 0.6^{cd}	15.40 ± 0.5^{bcd}
	2.0	16.99 ± 1.4^{def}	10.40 ± 0.5^{cdef}
RLP	0.5	27.61 ± 1.5^{bc}	17.40 ± 1.3^{b}
	1.0	20.61 ± 0.7^{cde}	14.00 ± 1.9^{bcde}
	2.0	12.01 ± 0.7^{fg}	7.60 ± 0.5^{f}
Actellic dust	2.0	1.66 ± 0.3^{h}	1.0 ± 0.2^{g}
Control (Untreated)	0.0	50.89 ± 1.3^{a}	40.80 ± 1.2^{a}

Each value is a means (± SE) of three replicates. Mean within a column followed by the same
Superscripts are not significantly different: Tukey- Kramer HSD test at P = 0.05. DE-Diatomaceous Earth: JSP- *Jatropha* seed powder: ELP- *Eucalyptus* leaf powder: RLP-*Ricinus* leaf powder

Table 4
Viability of seeds treated with raw DE and plant powders

Treatments	Dose (g/100gseeds)	% Seed viability
DE	0.3	66.66±3.3^{ab}
	0.6	60.00±5.7^{ab}
	0.9	66.66± 8.8^{ab}
JSP	0.5	60.00±11.5^{ab}
	1.0	56.66±3.3^{ab}
	2.0	73.33±3.3^{a}
ELP	0.5	66.66±8.8^{ab}
	1.0	80.00±5.7^{a}
	2.0	63.33±8.8^{ab}
RLP	0.5	53.33±6.6^{ab}
	1.0	80.00±5.7^{a}
	2.0	63.33±3.3^{ab}
Actellic dust	2.0	76.66±3.3^{a}
Control (Untreated)	0.0	33.33±3.3^{b}

Each value is a means (± SE) of three replicates. Mean within a column followed by the same superscript are not significantly different: Tukey- Kramer HSD test at P = 0.05. DE-Diatomaceous Earth: JSP- *Jatropha* seed powder: ELP-*Eucalyptus* leaf powder: RLP-*Ricinus* leaf powder

The percentage germination over a period of five days is presented in Table 4. There was no significant difference (P > 0.05) between some treatments in the germination of bambara groundnut seeds when compared with untreated control.

4. Discussion

The raw DE and plant powders tested showed great potential as grain protectants against adults *C. subinnotatus* and their efficacy increased with increasing doses and exposure period. This finding was consistent with Kabir *et al*. (2010, 2011) which reported that raw DE was effective against *Rhyzopertha dominica* (F.) and *Tribolium casteneum* Herbst at higher dose rates and two exposure period. Mvumi *et al*. (2008) found that African DEs applied at higher dose rates showed significantly high mortality of *Sitophilus zeamais*, *T. casteneum* and *R. dominica* after 7, 14 and 28 days exposure period. Furthermore, the study demonstrated that different plant powders can be used in the control of *C. subinnotatus* in stored bambara groundnuts. Among the plant powders tested, *Jatropha* seed powders at 2.0g/100g seeds caused 63.52% mortality on *C. subinnotatus* after 24 hours exposure, which is comparable to that of Actellic dust at the same rate of 2.0g/100g seeds with 67.05 % mortality. Similarly, *Eucalyptus* leaf powder and *Ricinus* leaf powders applied at the higher dosage of 2.0g/100g seeds caused 86.98% and 92.08% mortality of *C. subinnotatus,* 96 hours after treatment (Table 1). Insecticidal property of any plant material would depend on the active constituents of the plant products. The active constituent in these plant powders appears to be responsible for their insecticidal properties against *C. subinnotatus*. Umar (2008) reported that leaf, bark and wood powders of *J. curcas* applied at 0.5, 1.0 and 2.0g/20g cowpea seeds against *C.maculatus* reduced oviposition from 8-30%. Araya and Emana (2009) reported that the botanical powders applied at high dosages caused 97 % oviposition inhibition and significantly reduced emerging F_1 progeny by *Z. subfasciatus*. Mortality of *C. subinnotatus* due to the treatments is dose dependent and gradual as longer exposure period are needed to achieve effective control.

The emergence of progenies in treated seeds could be explained by the delayed lethal action of DEs on insects. This finding agrees with Arthur and Throne (2003) who reported that few progenies emerged in treatments where 100% mortality was recorded. Similarly, Tapondjou *et al*. (2002) reported that few progenies were produced on seeds treated with botanical powders.

The reduction in F_1 progeny emergence in the treated seeds could be due to increased adult mortality, reduction of oviposition, ovicidal activity and larvicidal activity. These findings are similar to Ofuya (1990) and Tapondjou *et al*. (2002), which reported that oviposition inhibition property of botanical powders on adult

bruchids in terms of weakening of adults by powder treatments, made them laid fewer eggs and killing the larvae hatching from eggs laid on grains. The reduction in adult emergence as a result of raw DE and plant powders suggest that *C. subinnotatus* development was adversely affected on seeds treated with these treatments than the control. Significant reduction in damage on seeds treated with diatomaceous earth and plant powders were observed, which indicates the higher potentials of these materials. Furthermore, DE at 2.0g/ 100g seeds had 9.68 % seed damaged suggesting good protection compared to plant powders.

The viability test of bambara groundnut seeds treated with diatomaceous earth and plant powder against *C. subinnotatus* demonstrated that the materials have no visible effect on the germination ability of the treated seeds except that some of the treatments were infected by moulds which resulted in reduced germination percentage from 33.33 to 80.00 %. Stathers *et al*. (2000) reported diatomaceous earth products did not have negative effects on seed germination. Araya and Emana (2009) also reported that botanical plant powders had no effect on the germination capacity of haricot beans.

5. Conclusion

In conclusion, this study revealed that the raw DE can provide acceptable level of control of *C. subinnotatus*. However, their efficacy varies with dose, exposure time and grain types. Furthermore, it is evident from this study that all the plant powders tested have the potential of being used as biopesticides. Higher dosage of the leaf and seed powders could be more effective since it is expected to contain more active components. Further investigations are required on the potentials of combining this raw DE and plant powders in order to enhance their activities and achieve effective level of protection on stored bambara groundnuts and other agricultural produce. In addition it will reduce the bulkiness of the powders when used for control of pests in bags or in storage bins since their potential as protectants against stored product insects appears great.

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