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# Insecticidal evaluation of some tropical leaf powders for the protection of stored Bambara groundnut against *Callosobruchus subinnotatus* (Pic) (Coleoptera: Chrysomelidae)

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**Abstract**
Plant powders prepared from three tropical plant species: *Platycerium stemaria* (P. Beauv.) Desv., *Andrographis paniculata* (Burm Fil.) Nes – GBIF and *Oleandra distenta* Kunze were tested in the laboratory under constant conditions at 30±2 °C and 60 – 65% r. h. for their ability to protect Bambara groundnut against *Callosobruchus subinnotatus* Fabricus in storage. Three levels of treatment concentrations, 0, 10 and 20 g per 150 g of Bambara groundnut were applied for evaluation of adult weevil mortality, suppression of egg laying and weight loss of Bambara groundnut due to *C. subinnotatus* attack. Treated and untreated Bambara groundnut seeds were infested with *C. subinnotatus* under prevailing storage conditions and replicated three times using Completely Randomized Design (CRD). The results showed that both *P. stemaria* and *O. distenta* leaf powders at 20 g per 150 g seeds of groundnut caused higher mortalities of adult *C. subinnotatus* at 24, 48 and 72 hours respectively. *O. distenta* at same concentration of 20 g per 150 g seeds evoked greater inhibition of *C. subinnotatus* egg laying for 8 weeks of treatment. However, the lowest percentage weight loss of Bambara groundnut was evoked by *O. distenta* at 20 g per150 g seeds. The efficacy of the powders on the beetles was dose-dependent with higher doses providing greater protection of the Bambara groundnut. This research proved that the plant powders were effective in killing the insect pests and protecting the seeds, and could therefore be recommended for stored products pest management programmes.

**Keywords:** *Callosobruchus subinnotatus*, Bambara groundnut, damage, Botanical Pesticide

## Introduction
Bambara groundnut, *Vigna subterranea* (L) Verdc. is the third most important legume indigenous to Africa, and grown at low levels very extensively across the semi-arid sub-Saharan Africa for food, feed, starch and other industrial products [1-4]. Most people in Nigeria may not be conversant with the name 'Bambara groundnut' as the local name is commonly used and forms most parts of some families' daily meal. Locally, it is called "Okpa" (Igbo), "Epa-Roro" (Yoruba) and "Kwaruru or Gurjiya" (Hausa) [5-7]. Nigeria is regarded as the largest producer of the legume crop with a mean production of 0.1 million metric tons, Burkina Faso with a production of 44,712 metric tons is next while Niger Republic comes third with a capacity of 30,000 metric tons [2, 8-11]. However, despite the massive production of the legume in Nigeria, it is relatively underutilized compared with major cash crops, and has been associated with small- scale subsistence farming with women being the major producers [12, 13]. Bambara groundnut has the potential to serve as an ingredient for food fortification due to its affordability, versatility, nutritional quality and sensory acceptability [14, 4]. It has nutrient composition of 64.4% carbohydrate, 23.6% protein, 6.5% fat, and 5.5% fibre and rich in minerals and phytochemicals. [15, 12, 16, 7, 4]. recently reviewed studies on the application of Bambara groundnut flour in a number of products including snacks and pastries, breakfast cereal and pasta. In Nigeria, especially in the East, Bambara groundnut is used in traditional preparations of various recipes. The seeds are roasted, pulverized and used in preparing soup [17]. The popular "Okpa" (steamed Bambara groundnut pudding) which is made from the flour and red palm oil plays an important role in contributing to dietary protein and vitamin A intake among school children [18]. In addition to human consumption and folder for livestock, Bambara groundnut is used in traditional medicine as a remedy for many ailments in humans and livestock [19, 20].

It is also used as a legume in fixing atmospheric nitrogen and contributes to soil fertility [21]. Stored crop products are perishable biomaterials especially in the tropics where high temperature and humidity accelerates spoilage. Post-harvest losses and quality deterioration of the legume is majorly caused by the insect pest, *Callosobruchus subinnotatus* (Pic) [22, 23]. Synthetic insecticides commonly applied are generally accepted as effective but hazardous to non-target organisms and the environment due to their indiscriminate and massive usage [24- 26]. In recent years, researchers have been focusing on naturally existing plants to crop protection to stem the trend of food shortage as a result of infestation [27- 29]. Insecticides from botanical origin are more biodegradable and have with a few exceptions some pure compounds, and low mammalian toxicity [30-32]. This present paper reports findings of pesticidal activity of some indigenous plant species on beetle mortality, egg hatchability and legume weight loss due to *C. subinnatatus* insect pest attack.

## Materials and Methods

### Study Area

The study was conducted at the Teaching and Research Laboratory of the Department of Crop Science, University of Calabar (Latitude 4.95° N, Longitude 8.32° E) which is located in the Niger Delta region of Southern Nigeria. The area has a bimodal rainfall with a three-year annual average of 4000 mm and average temperature of 28 °C and a relative humidity that ranges from 65 to 79%.

### Collection and Preparation of Bamabara Groundnut

Bambara groundnut used for this experiment were purchased in an unshelled form from a food stuff market in Obudu, Cross River State (Latitude 6.6694° North and Longitude 9.167° East). The seeds were decorticated manually and damaged seeds as well as other debris carefully removed. The grain colour is cream with brown eye, moderate size and very smooth in texture. The clean whole seeds were then fumigated with one phostoxin tablets put in air tight container to kill off any existing storage insect pests, mainly adult *C. subinnatatus* and their eggs, according to the method of [33]. Thereafter, the seeds were brought out of the container and spread in a shaded well ventilated place for 48 hours to ensure that they were free from the fumigant residues. The seeds were finally transferred into fresh containers and kept at – 4 °C inside a fridge for four days to avoid re-infestation and ensure the killing of any insect left in the container [27, 34].

### Processing of Plant Materials

The plant materials from *Platycerium stemaria* (P. Beauv) Desv, *Oleandria distenta* Kunze and *Andrographis paniculata* (Burm fil.) used for insecticidal activity against *C. subinnotatus* were all collected from Calabar and identified to species level [35]. The plant leaves were cleaned, chopped into smaller pieces, weighed and shade- dried for 3 weeks. They were fined ground in a mortar with pestle and sieved into powder using 1mm mesh [36]. Plant powders were preserved in polythene bags and stored in the refrigerator at 4±2 °C until ready for use.

### Rearing of Experimental Insects

Culture stock of *C. subinnotatus* were sieved from an infested Bambara groundnut seeds in the laboratory and subjected to proper taxonomic identification. A culture of the insect was then massively reared by placing 20 pairs of freshly emerged *C. subinnotatus* into 150 g of clean uninfested Bambara groundnut seeds in four separate transparent plastic containers of volume 16.00 x 18.00 x 13.00 $cm^3$ each. The containers had their covers drilled with holes of diameter 9.00 cm and then covered with nylon mesh number 10 (sieve size 2 mm). The containers were observed for a period of three to four weeks under prevailing laboratory conditions with temperature ranging between 23 – 35 °C and relative humidity of 65 – 80%. After 28 days culturing, freshly emerged (0 – 24 hrs.) adult insects were sieved out using a sieve of 2 mm and used for infestation.

### Application of plant powders

Plant powders were evaluated for the control of *C. subinnotatus* infesting stored Bambara groundnut. Disinfected Bambara groundnut seeds were weighed and placed into transparent test plastic containers. Powders of each plant were measured out and applied at three levels of 0.0, 10.0 and 20 g per 150 g of Bambara groundnut seeds. The controls consisted of the untreated. Five pairs (five males and five females) of freshly emerged (0 – 24 hours) *C. subinnotatus* adults were introduced into each of the well labeled test plastic vessels containing 150 g of seeds. Test plastic vessels had their covers perforated and covered with nylon mesh to facilitate aeration and confinement of bruchids, and were vigorously shaken except the control, to ensure proper mixing of seeds and treatment powders. Each treatment was replicated three times and laid out in a Completely Randomized Design (CRD) and kept for 28 days.

### Effect of Plant Powders on *C. subinnatatus*

Observations were made on each treatment for adult insect mortality and recorded in all the test containers at 24, 48 and 72 hours post treatment. Dead insects were removed and live ones returned to their respective vessels. After 7 days, all insects dead or alive were sieved out and percentage insect mortality calculated using the formula.

$$\%\ Mortality = \frac{INLI - FNLI}{INLI} \times 100$$

Where

$INLI$ = Initial number of life insect
$FNLI$ = Final number of life insect

### Effect of Plant Powders on Oviposition and Progeny Emergence of *C. subinnotatus*

At 14 days post treatment, 20 seeds of Bambara groundnut were randomly picked from each test vessels to ascertain the number of eggs laid in them. The seeds were later returned to their vessels and thereafter progeny emergence, first and second filial generations ($F_1$, $F_2$) taken at 30 and 60 days after infestation respectively. All newly emerged adults were sieved out on each occasion and counted. It was already established that within these aforementioned periods, all $F_1$ and $F_2$ generations would have emerged.

### Effect of Plant Powders on Weight loss of Bambara groundnut seeds due to *C. subinnotatus* infestation

At the 8th week (2 months) post treatment, an evaluation of the weight loss of the seeds due to *C. subinnatatus* infestation

was conducted using the formula.

$$\% \text{ Weight loss} = \frac{\text{initial weight of grains-final weight of grains}}{\text{initial weight of grains}} \times 100$$

**Data Analysis**

The data collected were transformed using the square root transformation or used as collected. Analysis of Variance (ANOVA) was carried out and the treatment means showing significant differences, separated using SAS – SNK (Student Neumann Keuls Test at 5% (SAS, 1990).

**Results**

Table 1 shows the efficacies of *P. stemaria*, *O. distenta* and *A. paniculata* leaf powders to kill *C. subinnotatus* infesting Bambara groundnut seeds in storage. *P. stemaria* and *O. distenta* powders at 20 g per 150 g Bambara groundnut seeds evoked greater mortalities of adult *C. subinnotatus* at 24, 48 and 72 hours respectively but was not significantly ($P$>0.05) different from that of the remaining plant powder at lower levels and the control. Table 2 presents the effect of leaf powders of test plants on insect's oviposition. There was no significant difference ($P$>0.05) amongst treatments on oviposition in $F_2$ progenies, but a significant difference was observed on $F_1$ progeny emergence amongst the treatments. The leaf powders of *O. distenta* at 10 g per 150 g Bambara groundnut seeds recorded a significantly ($P$<0.05) lower $F_1$ than the other plant powders at different levels and the control. *P. stemaria* powder came next but at a higher level of 20 g per 150 g of seeds.

The result also revealed that at $F_2$, there was no significant difference ($P$>0.05) among all the treatments, although *A. Paniculata* at 20 g had higher $F_2$ numbers than the other plant powders and the untreated. *O. distenta* at 20 g per 150 g Bambara groundnut seeds recorded the least oviposition effect and the third lowest in F1 progenies. The effect of leaf powders on weight loss of Bambara groundnut seeds is shown in table 3. The result revealed that there was no significant ($P$>0.05) difference amongst the treatments although *O. distenta* at 20 g per 150 g of Bambara groundnut evoked the least weight loss.

**Table 1:** Effect of Plant Powders on Mortality of *C. subinnotatus* on Bambara groundnut

| Treatment Sources | Concentration (g/150 g seed) | Mean Mortality (hour) | | |
|---|---|---|---|---|
| | | 24 | 48 | 72 |
| **Control** | 0.0 | 2.33$^{d}$ | 5.22$^{b}$ | 6.44$^{c}$ |
| *Platycerium stemaria* | 10.0 | 3.00$^{c}$ | 4.67$^{c}$ | 5.33$^{d}$ |
| *Platycerium stemaria* | 20.0 | 4.00$^{a}$ | 5.00$^{b}$ | 5.33$^{d}$ |
| *Oleandra distenta* | 10.0 | 3.67$^{ab}$ | 5.33$^{b}$ | 7.33$^{b}$ |
| *Oleandra distenta* | 20.0 | 3.67$^{ab}$ | 6.33$^{a}$ | 8.00$^{a}$ |
| *Andrographis paniculata* | 10.0 | 1.67$^{e}$ | 4.33$^{c}$ | 4.67$^{e}$ |
| *Andrographis paniculata* | 20.0 | 3.33$^{bc}$ | 4.33$^{c}$ | 5.33$^{d}$ |
| SE (±) | | 0.1 | 0.13 | 0.12 |

**Table 2:** Effect of Plant Powders on Oviposition and Progeny Emergence of *C. subinnotatus*

| Treatment Sources | Concentration (g/150 g seed) | Mean number of egg laid | Mean Progeny | |
|---|---|---|---|---|
| | | | **$F_1$** | **$F_2$** |
| **Control** | 0.0 | 10.00$^{b}$ | 2.55$^{b}$ | 4.54$^{c}$ |
| *Platycerium stemaria* | 10.0 | 6.00$^{c}$ | 1.10$^{d}$ | 2.77$^{d}$ |
| *Platycerim stemaria* | 20.0 | 7.00$^{c}$ | 0.91$^{d}$ | 1.73$^{e}$ |
| *Oleandra distenta* | 10.0 | 9.00$^{b}$ | 0.82$^{e}$ | 3.27$^{d}$ |
| *Oleandra distenta* | 20.0 | 1.00$^{d}$ | 0.94$^{d}$ | 0.67$^{f}$ |
| *Andrographis paniculata* | 10.0 | 9.67$^{b}$ | 2.27$^{c}$ | 7.25$^{b}$ |
| *Andrographis paniculata* | 20.0 | 28.67$^{a}$ | 3.53$^{a}$ | 8.91$^{a}$ |
| SE (±) | | 0.57 | 0.06 | 0.18 |
| | | | Sign. (5%) | |

Means followed by same alphabet F1 are not significant different at 5% level of probability

**Table 3:** Effect on percentage weight loss of Bambara groundnut due to *C. subinnotatus*

| Treatment Sources | Concentration (g/150 g seed) | Mean Percentage Weight loss |
|---|---|---|
| **Control** | 0.0 | 6.40$^{b}$ |
| *Platycerium stemaria* | 10.0 | 3.40$^{d}$ |
| *Platycerim stemaria* | 20.0 | 2.13$^{e}$ |
| *Oleandra distenta* | 10.0 | 3.53$^{d}$ |
| *Oleandra distenta* | 20.0 | 1.82$^{e}$ |
| *Andrographis paniculata* | 10.0 | 5.10$^{c}$ |
| *Andrographis paniculata* | 20.0 | 9.20$^{a}$ |
| SE (±) | | 0.19 |

**Discussion**

[26, 37] both stated that the use of plant derived products could have an excellent advantage over synthetic pesticide as a cost-effective and environmentally sustainable substitute for protecting food in storage against insect pest attack. In this investigation, the three plant powders tested for biological activities demonstrated low toxicity against test insect pest. *P. stemaria* at 20 g per 150 g of Bambara groundnut in 24 hours and *O. distenta* in 48 and 72 hours at same 20 g evoked higher insect mortality, but their effects were not significantly different from the remaining plant powder and the control. This result is however at variance with the work of [38] who

reported that the leaves of *Moringa oleifera* killed adult *Callosobruchus maculatus* at higher concentration. Similarly, [39] reported that *Withania somnifera* (leaf stem, fruit and root extracts) applied at 5% and 10% caused significantly higher mortality of *C. chinensis* infesting *Vigna radiata*. However, [40] showed that powder extracts of *Lantana camara*, *Tephrosia vogelii* and *Azadirachta indica* were not effective against adult *P. truncatus* and that mortality of insect was dependent upon the concentration of powder applied and duration of contact with treated maize grains. [41] reported that pulverized orange rind at six different doses from 0.5 – 30 g had no significant effect on mortality of adult *S. zeamais* after 45 days of observation. However, higher mortalities of storage beetles were recorded in treatments with lemon grass and Eucalytus products [42- 45] and *Acmela oleracea L. camera*, *Lavender officinale* [46]. The low toxicity of the leave powders against *C. subinnotatus* in this investigation could be attributed to the poor contact between powders and the insects as a result of the settlement of powders at the bottom of the plastic test vessels and given that the insects did not settle down but rather moved around and stuck on the walls of the containers.

Low toxicity levels of neem and other plant powders against *Sitophilus zeamis* had earlier been reported and low efficacy attributed to the settling of powder particles at the bottom of containers[47, 48], a phenomenon observed in this present study. It was also observed in this study that there was significant difference in emergence of adult insects reared on both treated and untreated seeds. *O. distenta* powder was found to be the most effective in affecting the emergence of test insects. At 10 g per 150 g of groundnut, it had significantly ($P$<0.05) lower $F_1$ than the other powders at different levels and the control. This result is in agreement with the report of [49] that 20 g plant powder of *Striga hermonthica* added to 500 cowpea seeds reduced oviposition and egg hatch in *C. maculatus*.

All the plant products were found effective in reducing weight loss, but there was no significant difference amongst them even though *O. distenta* at 20 g per 150 g of grain recorded the least weight loss. This result is not consistent with the report of [50] who observed that there was significant reduction in the weight loss of corn seeds affected by *S. granarius* treated with the extract of *S. sesban*. Leaves of *A. indica* and *C. nigricans* have been reported to have a negative effect on *C. maculatus* thus resulting in a higher weight loss[51]. Furthermore, it is thought that increase in larval density resulted in larval competition which subsequently affected food availability, larval mortality, development time and adult size. This probably may have accounted for the reduced weight loss of grain per individual insect pest.

The relatively severe oviposition suppression observed, suggests that the powders of *O. distenta* have larvicidal action which prevented the emergence of large numbers of $F_1$ compared with the other powders [27]. reported that the emergence of *C. subinnotatus* in seeds treated with *Piper guineense* powder was reduced up to 100 per cent. Similarly, [52] reported that neem seed powder drastically reduced egg laying in female *S. oryzae* from 154 in the untreated control to only 9 and 3 with neem powder doses at 0.5 and 1.0 per 20 g maize grains respectively [53]. reported related findings on maize treated with *A. oleracea* and *L. camara*, where 3 and 5% of the plant powders per 50 g of grains significantly suppressed adult weevil emergence compared to 1% powders and controls. The effectiveness of these plant powders in controlling insect pest during storage is probably a reflection of their insecticidal active ingredients. Some of the insecticidal activities of the plant powders used in this study can impair oxygen supply to the insect as well as physiological development disruption of the various stages of insect pests [53]. Finally, the degree of weight loss and damage done on Bambara groundnut by *C. subinnotatus* after 8 weeks in storage is suggestive that the insect pests would prefer to avoid grains treated with any of the plant products.

## Conclusion

The use of plant extractives from *P. stemaria, O. distenta and A. paniculata* investigated in this study could be a reliable means of controlling pest attacks on Bambara groundnut during storage, which is capable of reducing wastage and damage. The local availability and effectiveness of these plant materials especially *O. distenta* could be encouraging and a possible means of ensuring a steady supply of good quality Bambara groundnut. Farmers could also be encouraged to expand on the existing cultivation of these plants, thereby boosting agriculture and their finances. Further investigation is however advocated to identify the optimum concentration and active ingredients of these plant materials for effective control of *C. subinnotatus* and other insect pests on stored Bambara groundnut with minimum application technology and optimal consumer acceptability.

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