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TOXICITY OF *Piper guineense* SCHUM AND THONN AND Chlorpyrifos POWDERS FOR THE CONTROL OF *Callosobruchus subinnotatus* (Pic.) ON STORED *Vigna subterranea* (L.) Verdcourt IN GOMBE STATE, NIGERIA.

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ABSTRACT

A study was conducted with aim of determining toxicity effects of *Piper guineense* and chlorpyrifos powders on adult mortality of *Callosobruchus subinnotatus*, progeny emergence (F_1), damage and weight loss, germination of Bambara nuts. Study was carried out at Botany laboratory, Gombe State University, Gombe State, Nigeria. *P. guineense* and chlorpyrifos were mixed at 0.0g and 0.8g concentration with 20g Bambara nuts inside 1000ml plastic containers. Experiment was conducted in randomized complete block design in three replicates. Ten pairs of adult *C. subinnotatus* unsexed were introduced into treatments nuts and stored for 7days. Bruchid mortality, progeny emergence, nut damage and weight loss, and germination were assessed. Data obtained were subjected to analyses of variance, significant differences of treatment mean were separated using New Duncan's multiple range test at 5% probability level. *Piper guineense* and chlorpyrifos powders had comparable toxicity effects, causing 100% *C. subinnotatus* adult mortality between 5-7 days of treatment. *P. guineense* and chlorpyrifos powders proved effective as lower *C. subinnotatus* progeny emergence was observed compared with control. Progeny emergence under control was 93.42% and 100% higher than nuts treated with *P. guineense* and chlorpyrifos, respectively. *P. guineense* and chlorpyrifos did not cause significant reduction in seed germination. Study recommends *P. guineense* is a reliable organic material that could offer protection to Bambara nuts against *C. subinnotatus*.

Keywords: Bambara nut, *Piper guineense*, Chlorpyrifos, *Callosobruchus subinnotatus*

INTRODUCTION

Bambara nut grows well anywhere groundnut (peanut) grows and so is vastly present from Kwara state and throughout the Northern parts of Nigeria including Gombe (Abba *et al.*, 2018). Bambara nut (*Vigna subterranea* (L.) Verdc.) is an indigenous legume crop, cultivated by subsistence farmers throughout sub-Saharan countries. Research findings indicate that the crop has great nutritional and agronomic potential, but it remains scientifically neglected (Mubaiwa *et al*., 2018). The seeds are used for food and beverage because of their high protein content (Nwadi *et al*., 2019) for digestive system applications. In West Africa, the nuts are eaten as a snack, roasted and salted, processed into cake, or as a meal, boiled similar to other beans (Afolabi *et al*., 2018).
Many researchers claim that Bambara nut is an underutilized legume (Adzawla *et al*., 2015; Bonthala *et al*., 2016; Karunaratne *et al*., 2015; Suhairi *et al*., 2018). However, it appears that this assertion is no longer valid, because there is recent advancement in the use of the grain in various applications. Furthermore, the Bambara nut seems to have attained a better status because there are evidences in the literature where the crop has been used to enrich a variety of foods used in complementary feeding which represents a good source of insoluble dietary fibre. Diedericks and Jideani (2015) produced acceptable white bread enriched with insoluble dietary fibre, which was isolated from Bambara nut. Bambara nut has also been reportedly used in enriching staple maize gruel called ogi. Afolabi *et al*., (2018) fortified ogi with maize, millet, or sorghum with Bambara nut flour (Bambara nut [40%] and maize, millet, or sorghum [60%] and reported 100% nutrient improvement in the protein content of the ogi.
The plant can grow under extreme drought conditions. Nutritionally, it is considered a complete food because of its reasonably high protein content (9.60-40%; Ogundele *et al*., 2017; Oyeyinka *et al*., 2019; Okudu and Ojinnaka, 2017) and it has a good balance of the essential amino acids (Yao *et al*., 2015). Traditionally, the grain has been reportedly used in the production of vegetable milk (Brough *et al*., 1993; Murevanhema and Jideani, 2013), low fat yoghurt (Falade *et al*.,2015), value added snacks e.g bread, biscuits, doughnuts, meat analogue, fura, fufu, pasta (Oyeyinka *et al*.,2018), and a puree for infant feeding (Oyeyinka *et al*.,2017).
However, in spite of its usefulness and traditional values, Bambara nut, is reported to be threatened by the devastating activities of stored products pests, notably *Callosobruchus subinnotatus* Pic. (Mbah-Omeje, 2019).
Recently, the use of synthetic insecticides such as carbamates, organophosphate has reduced and interest has been shifted toward environmentally friendly and less toxic insecticides of plant origin (Mbah-Omeje, 2019). Synthetic insecticides can cause pests resistance, resurgence and development of resistance by insects. In the light of the above, alternative consideration could be sourced from use of indigenous preservatives locked in plants as bio-constituents (Yusuf *et al*.,2006; Bamaiyi *et al*.,2006; Mundi *et al*.,2012).
Maina *et al*., (2011) reported that the severity of damage caused by *Callosobruchus subinnotatus* is higher starting from the third month of Bambara groundnut seeds storage and reports that the longer the storage term is, the more vital the damage is because of the increase spawning and adult

emergence of *C. subinnotatus*. Damage by bruchids in the field before harvest has rarely been reported. Baoua *et al*., (2015) reported losses of 61.8% for Bambara groundnuts and 83.9% due to *C. maculatus* and *C. subinnotatus* in Niger after 7 months of storage without treatment.

Studies have shown that green plants act as a reservoir for inexhaustible source of innocuous pesticides, which are non-toxic, and easily biodegradable than synthetic chemicals (Joana and Gungula, 2010). These ethno-botanical constituents like anthraglycosides, phlobatanin, tannins, reducing sugars, flavonoids, alkaloids, saponins, coumarins, phenols, carboxylic acids, terpenes etc. have conferred specific characteristics and properties to plants which could be considered for use as insect pests antifeedants or repellents (Sathish *et al*., 2013).

Recently, particular interest has been focused on the use of natural plant products because they are available locally, cheap, less hazardous and environmentally friendly as well as safe and easy to handle. Moreover, botanical pesticides are biodegradable thereby leaving no residual toxicity to man. However, little information exists on the insecticidal properties of *Piper guineense* (Schum. and Thonn) (Piperaceae) for the control of *C. subinnotatus* in stored Bambara nut, hence the aim of the study is to evaluate the efficacy of contact toxicity of *Piper guineense* and Chlorpyrifos powders for the control of *Callosobruchus subinnotatus* against Bambara nut.

Materials and Methods

Description of the Study Area

Location of the study

This study was conducted at the Botany laboratory of the Department of Botany, Gombe State University, Gombe State, Nigeria (Fig. 1). The duration of the study was from July to October, 2019.

Plant Collection

The Bambara nuts used for the study was obtained from Gombe State Agricultural Development Project, Gombe, Gombe State, Nigeria and this was firstly cleaned and disinfested by keeping at -5^{0}C for 7 days (at Tumfure where there is uninterrupted power supply) to kill all hidden infestations. This is because all the life stages, particularly the eggs are very sensitive to cold (Koehler, 2003). The disinfested Bambara nuts was then placed inside a Gallenkamp oven (Model 250) at 40^{0}C for 4 hours (Jambere *et al.*, 1995) and later air dried in the laboratory to prevent mouldiness (Adedire *et al.*, 2011) before they were stored in plastic containers with tight lids disinfested by swabbing with 90% alcohol.

Insect Culture

Callosobruchus subinnotatus was obtained from insectory laboratory where the culture was reared on disinfested Bambara nuts, *Vigna subterranea* (L.) Verdc., at ambient temperature of 28±2^{0}C and 75±5% relative humidity.

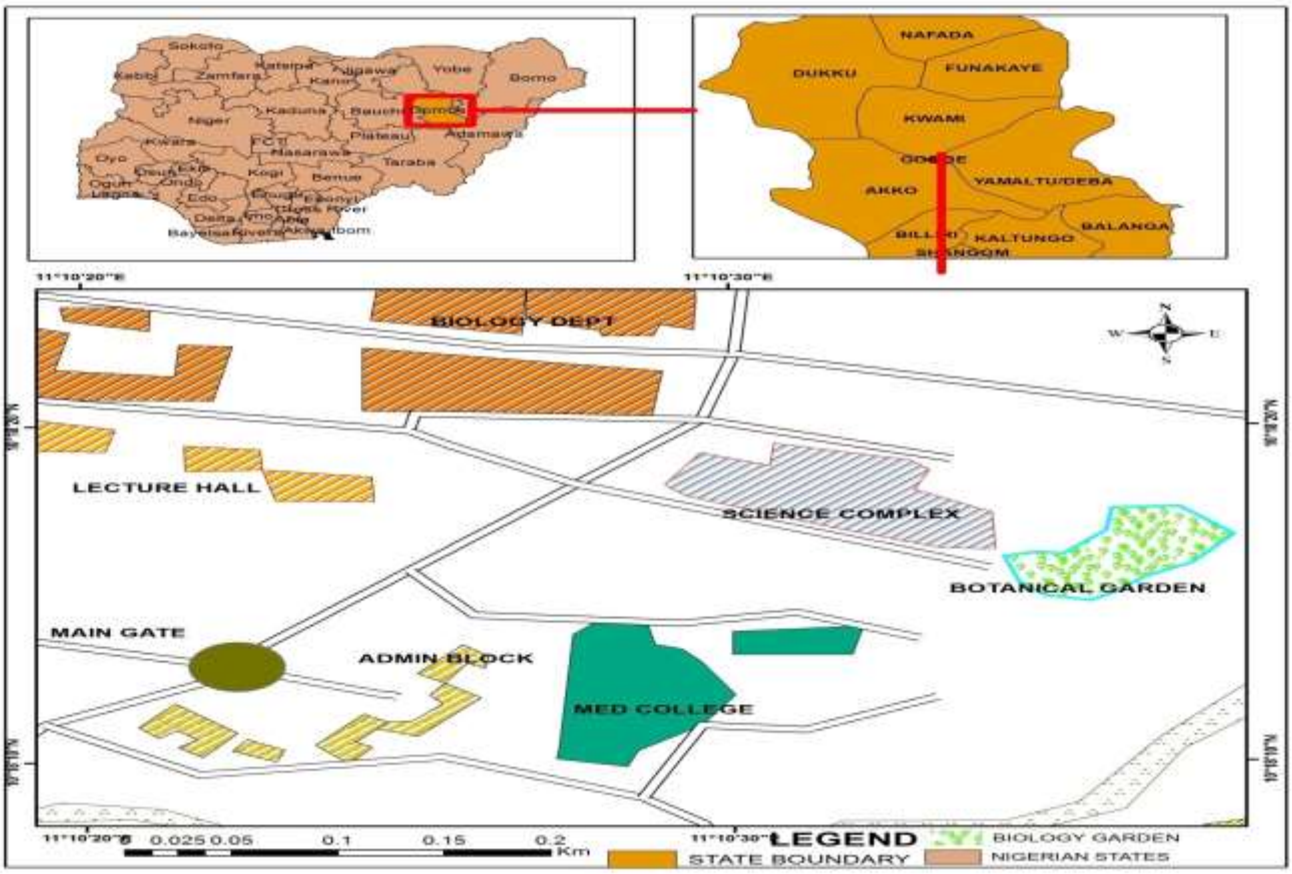

Figure 1: Gombe State University showing the location of the Science complex (Botany laboratory).
Source; Elisha (2018).

Plant Material

The plant material used in the present study was *Piper guineense seeds.* This material was sourced fresh from Gombe State Agricultural Development Project, Gombe State, Nigeria. The seeds were sun dried for 3days. The cleaned dried seeds were pulverised into fine powder using a blender (Supermaster ®, Model SMB 2977, Japan). The powder was further sieved to pass through $1mm^2$ mesh. The powder was packed in plastic containers with tight lids and stored in a refrigerator at 4^0C prior to use.

Effects of *Piper guineense* powder and Chlorpyrifos powder on Adult Mortality of *C. subinnotatus.*

The methodology of Ileke and Bulus (2012) was adopted. The experiment was set up in a Randomized Complete Block Design (RCBD) and each treatment was duplicated three times. Ten pairs of adult *C. subinnotatus* unsexed (2 to 3 days old) were introduced into the treated and control (Idoko and Adebayo, 2011; Udo, 2011). Bruchid mortality was assessed every 24 hours for one week after treatment (Ashamo, 2007).

Effects of *Piper guineense* powder and chlorpyrifos powder on progeny emergence

The experiment one above was observed for F1 *Callosobruchus subinnotatus* adult emergence. The number of F1 adults that emerged from each replicate were counted and recorded (Parugrug and Roxas, 2008).

Assessment of damaged and weight loss of the treated Bambara nuts

Damage assessment was carried out on treated and untreated nuts after F1 *Callosobruchus subinnotatus* adult emergence using weighing balance (FAO, 1985). Samples of 15 Bambara nuts were randomly taken from each plastic container and the number of damaged nuts (nuts with characteristic holes) and undamaged nuts were counted and weighed. Percentage weight loss and percentage damaged respectively was calculated using the formula (Baba – Tierto, 1994).

$$\%\ \text{Nut weight loss} = \frac{\text{Initial weight of nut} - \text{Final weight of nut}}{\text{Initial weight of nut}} \times 100$$

$$\%\ \text{Nut damage} = \frac{\text{Number of damaged nuts}}{\text{Total number of nuts}} \times 100$$

Effects of *Piper guineense* powder and chlorpyrifos powder on the germination of the treated Bambara nuts

Ten Bambara nuts were randomly selected from each treatment after F_1 adult emergence and planted on moistened cotton wool in a Petri dish. Germination count was taken on the 5^{th} day (Adedire *et al*., 2011). Nut germination was thereafter determined and expressed as percentage of total nuts planted as follows:

$$\%\ \text{Germination} = \frac{\text{Number of nuts that germinated}}{\text{Total number of nuts planted}} \times 100$$

Statistical Analysis

Data were subjected to analysis of variance. Where significant differences existed, treatment means were separated using the New Duncan's Multiple Range Test 5% probability level (Zar, 1984).

RESULTS AND DISCUSSION

Table 1, Presents the effects of *Piper guineense* and chlorpyrifos powder on adult mortality of *C. subinnotatus*. The data shows 1, 2, 3 and 4 days after treatment, highest adult mortality was recorded with chlorpyrifos powder with values of 10.0% each day, followed by *Piper guineense* powder with values of between 3.42 to 7.92% while control (no treatment) recorded the lowest value of 3.0 to 3.67%. After 5, 6 and 7 days of treatments, powders of Chlorpyrifos and *Piper guineense* had statistically higher values of adult mortality of 9.17 to 10.0% while the control had lower values of between 4.00 to 9.33% each day. Generally, adult *Callosobruchus subinnotatus* mortality increased with increase in days of treatment for *Piper guineense* and lower compared to *Piper guineense* in the control treatment but the values were constant with chlorpyrifos powder from day 1 to day 7 with (10%). This may probably be because of the biological activity of *P. guineense* which could be imputed to the presence of chavin and piperine (Ashamo, 2007).

Table 1: Effects of *Piper guineense* and Chlorpyrifos powders on Adult Mortality of *C. subinnotatus*.

	Days after treatment (DAT)						
Treatment	1	2	3	4	5	6	7
Piper guineense powder	3.42^b	5.50^b	6.58^b	7.92^b	9.17^a	9.67^a	10.00^a
Chlorpyrifos powder	10.00^a	10.00^a	10.00^a	10.00^a	10.00^a	10.00^a	10.00^a
Control (Negative)	3.00^c	3.00^c	3.00^c	3.67^c	4.00^b	8.00^a	9.33^a
Mean	5.47	6.17	6.50	7.19	7.71	9.22	9.77
S. E	1.00	1.80	2.00	1.5	1.43	1.23	1.00

Means followed by the same alphabetic along the column are not different from one another at 5% probability level according to NDMR test.

In the present study Table 1 shows that *Piper guineense* powder had toxicity of comparable effect to that of Chlorpyrifos powder causing 100% adult mortality of bruchid within 7 days of application while control showed lowest adult mortality as from 2 days till 7days. This is because Chlorpyrifos powder is an organophosphate which degrades relatively quickly once applied on Bambara nut. It also takes about 24hours for the active amount of the chemical to decrease by 50% on the Bambara nut. This effect that is similar to the report of Adedire and Lajide (2003), Ileke and Bulus (2012), that extract of *Piper guineense* adversely affected the survival of *Callosobruchus subinnotatus*. This result also validated the reports of Asawalam and Emosairue (2006); Ashamo (2007) that *P.guineense* and Chlorpyrifos powders caused 100% mortality of adult *callosobruchus* *subinnotatus* within 7 days of application. These authors established that the biological activity of *P.guineense* could be attributed to the presence of chavin and piperine, an unsaturated amide (Lale, 1992).

Ofuya and Dawodu (2002) also reported the effectiveness of *P. guineense* powder against *C. subinnotatus*. Several authors have postulated that the powder of *P. guineense* may also cause physical abrasion on the cuticle of bruchids with a resultant loss of body fluids or blockage of spiracles (Ofuya, 2001, Ofuya and Dawodu, 2002; Asawalam and Emosairue 2006., Asawalam *et al*., 2007; Kabeh and Jalingo, 2007). The ability to significantly cause adults mortality of *C. maculatus* inhibiting oviposition and eventual suppression of F_1 progeny emergence can be attributed to contact toxicity of the powders.

Table 2: Effects of *Piper guineense* and Chlorpyrifos powders on progeny emergence of *Callosobruchus subinnotatus*

Treatment	Progeny Emergence
Piper guineense powder	1.58^{b}
Chlorpyrifos powder	0.00^{b}
Control (Negative)	24.00^{a}
Mean	8.53
S. E	1.00

Means followed by the same alphabetic along the column are not different from one another at 5% probability level according to NDMR test.

Table 2 shows the number of *Callosobruchus subinnotatus* adults (F_1) that emerged from *P.guineense* and Chlorpyrifos powders treated Bambara nuts were significantly lower than in the control treatment. Moreover, the number of adults that emerged from treated nuts decreased with increase in concentration of plant products. This is because it shows the efficacy of *P. guineense* and Chlorpyrifos powders on Bambara nut against *C. subinnotatus*.Chlorpyrifos powder had the least number of emerged adult (0.00b), *P.guineense* powder was next with (1.58b) and control had the highest number of emerged adult with value (24.00a). This result is consistent with the work of Kemabonta and Falodu (2013) on post – harvest grain protectants against *Sitophilus oryzae*. Similar effects of plants as insect protectant have also been observed in treatment of cowpea and maize weevils (Adedire and Ajayi, 1996; Olotuah *et al*., 2007; Benson *et al.,* 2019).

Table 3: Assessment of damaged and weight loss of the treated Bambara nuts

Treatment	BNut Damage	BNutWL
Piper guineense powder	5.95^{b}	0.78^{b}
Chlorpyrifos powder	0.00^{b}	0.00^{c}
Control (Negative)	53.03^{a}	7.28^{a}
Mean	19.66	3.20
S. E	1.00	1.00

Means followed by the same alphabetic along the column are not different from one another at 5% probability level according to NDMR test.

Table 3 shows the effect of treatment on nut damage weight loss, chlorpyrifos powder was found to give the lowest value of nuts damaged, closely followed by *P. guineense* powder whereas control statistically showed value of (53.03a) nuts damaged. By implication, powder of *Piper guineense* and Chlorpyrifos was significantly more effective in preventing nut damage by *C. subinnotatus*.

From this study, it is evident that *P. guineense* and chlorpyrifos powders displayed some potential of antifeedants, food poison, contact poisons, and repellency. Therefore, it strongly suggests the possibility of using this plant extract as toxicants, repellents and food poisoning agents against *C. subinnotatus*. Since nut damage and weight loss are related, the higher the nut damage the higher the weight loss and vice versa. It can be inferred from the results that the low weight loss observed can be attributed to the low nut damage recorded. This is because as the number of live insects reduced in the nuts in storage, the amount of damage caused to the nuts also reduced as there is less feeding. Hence the lower weight loss observed. This agrees with the report of Abdulmalik *et al*., (2018) which indicated that the extent of damage during storage depends upon the number of emerging adult insects during each generation and the duration of each life cycle. Nuts that had more *C. subinnotatus* emergence were more seriously damaged. This result is consistent with the works of Abdulmalik *et al*., (2018).

Table 4: Effects of *Piper guineense* and Chlorpyrifos powders on the germination of the treated Bambara nuts

Treatment	No of Seeds sown	Seed germination (%)
Piper guineense powder	10	72.80
Chlorpyrifos powder	10	83.30
Control (Negative)	10	66.70
Mean		74.26
S. E		7.80

Means followed by the same alphabetic along the column are not different from one another at 5% probability level according to NDMR test.

Table 4 shows the effect of these plant products on seed viability (seed germination) after storage showed result, it is apparent that *Piper guineense* and chlorpyrifos powders in addition to protection of stored grain, maintained viability of the treated nuts and may thus be used for the storage of grain of Bambara nuts reserved for planting. This finding is in consistent with the work of Adedire *et al*., (2011). Adebisi *et al*., (2016) also observed that seeds of sesame dressed with *Piper guineense* powders increased seed longevity by 55 to 57% and 3 to 8% over synthetic (Apron plus) and control treatments for a period of 240 days, respectively. Another observation from this research is that plant materials that acted as contact poisons were found to be active in suppressing growth or development of insects. This result is consistent with the works of Arannilewa *et al*., (2006).

SUMMARY AND CONCLUSION

The study reveals that *Piper guineense* powder had toxicity of comparable effect to that of chlorpyrifos powder causing 100% adult mortality of *Callosobruchus subinnotatus* between 5 and 7days of application.

For progeny emergence, *Piper guineense* and chlorpyrifos powder proved very active as significantly lower *C. subinnotatus* (F_1) emergence was lower than the control. The percentage of progeny emergence recorded under the control was 93.42% and 100% higher than nuts treated with *piper guineense* and chlorpyrifos, powders, respectively.

Highest Bambara nut damage occurred with control treated nuts but comparable lower nuts damage was detected with *P. guineense* and chlorpyrifos powders. The percentage damage under control was 88.78 and 100% higher than values obtained with the *Piper guineense* and chlorpyrifos powders, respectively.

Chlorpyrifos powder was most effective as neither damage and weight loss were observed, closely followed by *P. guineense* treatment while control had both highest nut damage and nut weight loss.

It is apparent that powders of *P. guineense* and chlorpyrifos in addition to the protection of stored nuts, did not cause significant reduction in seed viability (seed germination) of treated nuts.

This study concluded that the efficacy of *Piper guineense* plant extract powder in offering protection to Bambara nuts against *C. subinnotatus* has revealed its potential as a reliable source of insecticides of plant origin. It has considerable potential as stored product insecticide.

RECOMMENDATION

Piper guineense could be used as biopesticides against *C. subinnotatus* in the storage of Bambara nuts.

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CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

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