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INSECTICIDAL ACTIVITY OF PIPER GUINEENSE (SCHUMACH AND THONN) SEED OIL AGAINST CALLOSOBRUCHUS MACULATUS (F.) (COLEOPTERA: CHRYSOMELIDAE) IN STORED COWPEA SEEDS

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ABSTRACT

Post-harvest losses due to insect pest infestation in storage constitute a challenge to sustainable food security and economic losses to farmers throughout the world. Use of synthetic chemicals have been the most effective means of reducing losses in storage. Unfortunately, these toxicants have adverse effects in humans and the environment. There is renewed search for safer plant products as alternatives. In this study, we assessed the potential use of Piper guineense seed oil in the control of cowpea bruchid, Callosobruchus maculatus (F.) infestation on stored cowpea seeds. Adult mortality, oviposition rate, F1 progeny emergence, cowpea seed damage and seed germinability were the indices considered in the assessment under laboratory conditions. The setup of the experiment was completely randomized design (CRD). Statistical analysis of the data was by analysis of variance (ANOVA). Adult C. maculatus mortality was dose-dependent with higher dosages inducing 100% mortality. The number of eggs laid on the treated seeds were significantly (P<0.001) reduced. Progeny emergence and feeding damage were completely inhibited at higher dosages. The seed oil has no adverse effect on cowpea seed viability. The results indicated that P. guineense seed oil has protectant potential against insect pest damage in storage and a good alternative to synthetic insecticides in the control of insect pest of stored products.

1. INTRODUCTION

Cowpea, *Vigna unguiculata* (L. Walp), is an annual crop in the family Fabaceae, grown successfully in extreme environments for its edible seeds and pods. The seeds are important food crop, high in protein content and source of revenue in tropical countries (Langyintuo *et al*. 2003; Adedire *et al*. 2011).

Storage insect pests are responsible for substantial damage in stored seeds, such as dry weight losses, reduced nutritional qualities, seed viability reduction and low market value. The extent of losses vary according to insect species. *Callosobruchus maculatus* (Fabricius) is a principal insect pest of stored cowpea seeds and can cause significant reduction in quality and quantity of seeds within 3-5 months of storage (IIeke *et al*. 2012). The insect is a cosmopolitan field-to-store pest as its infestation of cowpea often begins in the field while the mature pods dry (Murdock *et al*. 2003) and multiplies very rapidly in storage where it causes very high losses (Murdock *et al*. 2003; Ojo *et al*. 2013). Losses are mainly due to the consumption of cowpea seed cotyledons by larvae, resulting in reduced seed weight, increased seed perforation and decreased seed germination (Deshpande *et al*. 2011). Effective control measure is of paramount importance in order to avert adverse consequences due to insect pests damage to stored products and ensure steady food supply for the teeming world population.

Synthetic insecticides have been the major means of controlling insect pests largely due to their quick action and long lasting effect. However, apart from their high cost, there is growing concern for the use of synthetic insecticides because of some associated risks, such as environmental persistence, decimation of beneficial fauna, carcinogenic effect, insect resistance and other health problems (IIeke *et al*. 2012). These problems associated with the use of synthetic insecticides have necessitated the search for safer, cost-effective, readily available and biodegradable alternative insecticides. The use of plant materials with insecticidal properties will be of immense help to resource poor small-scale farmers since they are locally available, biodegradable, safe and cost effective (Echo *et al*. 2012; Shiberu 2013; Nzelu and Okonkwo 2016). Plant products such as powders, ashes and oils with insecticidal, anti-feedant and repellent properties have been identified and shown to be useful in the control of various insect pests (Raja *et al*. 2001; IIeke *et al*. 2012; Jayakumar 2010; Emeasor *et al*. 2017).

Piper guineense (Schumach and Thonn), the West African Black pepper, is a climbing perennial plant of the family Piperaceae. The edible seeds contain piperine, resins, alkaloids, tannins, saponins, terpenes, flavonoids, steroids, phenols and essential oils (Nwankwo *et al*. 2014; Besong *et al*. 2016; Ukpai *et al*. 2017). The seed oil is commonly used as aromatic in the beverage and pharmaceutical industries (Rehn and Espig 1991). The pungency of the black pepper is due to the presence of 5-8% piperine (Besong *et al*. 2016). *Piper guineense* is also known to have antioxidant, anticonvulsant, antibacterial, anti-inflammatory, hepatoprotective, fertility, larvicidal and aphrodisiac properties (Echo *et al*. 2012; Okoye and Ebeledike 2013; Nwankwo *et al*. 2014; Memudu *et al*. 2015; Besong *et al*. 2016; Ukpai *et al*. 2017). Some studies have shown the effectiveness of *Piper guineense* seed powder in the control of insect pests of some vegetable crops, catfish and stored grains (Fasakin and Aberejo 2002; Ibekwe *et al*. 2014; Ukpai *et al*. 2017; Benson *et al*. 2019). In this study, we sought to evaluate the efficacy of *Piper guineense* seed oil for the control of *C. maculatus* infestation on stored cowpea seeds.

2. MATERIALS AND METHODS

2.1. INSECT CULTURE

Cowpea seeds were obtained from the Eke Awka Market, Anambra State, Nigeria and taken to the laboratory. The infested seeds were placed in a plastic container and covered with muslin cloth which was held in place with rubber bands in order to allow sufficient air into the container, till the emergence of adults. Emerged *C. maculatus* adults of both sexes were transferred into another plastic container containing sterilized clean cowpea seeds and maintained at 28 ± 2ºC, 60 ± 5% R.H. and light/dark 12:12 hour cycle as culture for subsequent use. The culture was kept undisturbed to produce enough progeny used for the experiments.

2.2. PREPARATION AND EXTRACTION OF SEED OIL FROM PIPER GUINEENSE SEEDS

Dried seeds of *Piper guineense* were similarly procured from the same Eke Awka Market, Anambra State, Nigeria. The dried seeds were ground into fine powder using a stainless electric grinder and sieved (mesh size: 1mm^2). The oil extraction was carried out using n-hexane solvent. *P. guineense* seed powder of 250g was weighed into a beaker and 400ml of n-hexane added. The mixture was stirred, left on the bench for 6h and subsequently, filtered using filter paper (Whatman No. 1). The powder was repeatedly washed and filtered using 200ml of n-hexane for 3 times. The filtrate was then transferred into Soxhlet Extractor maintained at 68.7ºC by means of a heating mantle for evaporation and collection of the n-hexane. Further, the oil extract was dried in an oven (95-100ºC) for 1h. The seed oil extract was stored in air-tight bottle and kept at room temperature. This served as stock solution.

2.3. BIOASSAY

2.3.1. COWPEA SEEDS

Experimental cowpea seeds were properly sieved and hand-picked to ensure that only wholly and uninfested seeds were used. The seeds were also sterilized in electric oven at 50°C for 4h to disinfest them thoroughly and allowed to cool for 1h before use. All bioassay tests were carried out under ambient laboratory conditions, similar to the insect culture; 28 ± 2 °C and 60 ± 5% R.H. and 12h:12h light: dark regime.

2.3.2. TOXICITY OF PIPER GUINEENSE SEEDS OIL ON ADULT CALLOSOBRUCHUS MACULATUS

Twenty grammes (20g) of the cowpea sample were weighed separately into sterilized 100ml plastic jars. Serial dilutions containing 200, 400, 600, 800 and 1000µl of the oil in acetone (Analytical grade), corresponding to 1, 2, 3, 4, and 5% concentration, respectively, were prepared. The treated seeds were vigorously shaken to ensure proper coating of the seeds and kept for 1-2h to allow the acetone evaporate completely. Controls without oil treatment (0%) were included. Subsequently, twenty freshly-emerged *C. maculatus* adults (1-2 days old) of both sexes were introduced into each jar which was covered with a muslin cloth and held firmly with a rubber band to allow aeration and prevent exit or entry of insects. Each treatment level was repeated four (4) times and arranged in a completely randomized design on a workbench in the laboratory. The experiment was observed daily for 3 consecutive days and the number of dead insects in each jar was counted every 24h and recorded. Percent bruchid mortality was determined using the standard formula:

$$\% \text{ mortality} = \frac{\text{Number of dead insects}}{\text{Total number of insects}} \times 100$$

2.3.3. EFFECT OF PIPER GUINEENSE SEEDS OIL ON OVIPOSITION, F_1 ADULT EMERGENCE AND ASSESSMENT OF SEED DAMAGE

At the end of the mortality record, all insects were removed. The number of eggs laid on treated seeds (Ts) and control seeds (Cs) were recorded and the average number was calculated per treatment. After the egg count, the experimental set up was left undisturbed till the emergence of F_1 adults from day 30 post treatment. The number of F_1 adults from the control (AC) and treated seeds (At) were recorded. The percentage reduction (PR) in F_1 progeny was calculated as follows:

$$PR = [(Ac-At)/Ac] \times 100$$

The number of damaged cowpea seeds (seeds with characteristic emergence holes) in both treated and untreated sample and undamaged seeds were counted. The number of damaged seeds were expressed as a percentage of the total number of seeds in each jar.

2.3.4. SEED GERMINATION TEST AFTER TREATMENT

Viability of the treated seeds was carried out at the end of experiment using sterile glass Petri dishes containing Whatman No.1 filter paper (10.9cm diameter). Ten randomly selected seeds from each treatment, including the control (0%) were taken from each jar for germination test. The seeds were moistened daily (10ml of distilled water). The set-up was exposed to light on the laboratory workbench. The number of seeds that germinated was recorded after four days and the percentage germination was subsequently determined according to the following formula:

$$\% \text{ seed germination} = \frac{\text{Number of germinated seeds}}{\text{Total number of seeds}} \times 100$$

2.4. STATISTICAL ANALYSIS

For the toxicity test, data obtained were transformed to percentage. Abbot's formula was used to adjust for treatment mortality after applying mortality in control treatment (Abbott 1925). Data obtained from parameters were subjected to analysis of variance (ANOVA), using SPSS software package. Furthermore, the mean separation was carried out using the Least Significant Difference (LSD) at 5% level of significance.

3. RESULTS

3.1. ADULT CALLOSOBRUCHUS MACULATUS MORTALITY

The bioactivity of the *P. guineense* seed oil against adult *C. maculatus* is presented in Table 1. All dosages of the oil significantly (P<0.001) caused mortality to the beetle compared to the control, irrespective of dosage. There was dose-dependent mortality response by the insects in the treated seed, with oil treatments of 800µl/20g and above, inducing 100% mortalities of the bruchid within 72h.

Table 1: Effect of *P. guineense* seed oil on adult mortality of *C. maculatus*

Dosage (µl/20g) (Concentration %)	% mortality
Control (0.0%)	5.533 ± 2.767a
200 (1.0%)	58.333 ± 17.342b
400 (2.0%)	72.233 ± 18.375c
600 (3.0%)	89.600 ± 10.400d
800 (4.0%)	100.000 ± 0.000e
1000 (5.0%)	100.000 ± 0.000e

Means ± SE followed by different letters are significantly different at the 0.05 level.
Mortality was significant at P<0.001 by Fisher's F-test.
SE: standard error.

3.2. EFFECT OF THE SEED OIL ON OVIPOSITION, ADULT EMERGENCE AND SEED DAMAGE BY CALLOSOBRUCHUS MACULATUS

The mean number of eggs laid by female *C. maculatus* was significantly (P<0.001) reduced among the different oil dosages compared with the control treatments (Table 2). The least number of 0.6 eggs/20g were laid on seeds treated with 1000µl (5.0%)/20g oil which was not significantly (P>0.05) less than number of eggs laid on the treated seeds with lower dosages. All dosages elicited over 70% reduction in oviposition in the treated seeds compared with the control (Table 2). Thus, the oil appeared to be effective in reducing the egg laying capacity of the insect pest, indicating oviposition deterrent effect.

The number of progeny produced by *C. maculatus* in untreated seeds and those treated with lower oil concentration were significantly (P<0.001) different (Table 3). The F_1 adult emergence from the control treatment was significantly (P<0.001) higher than the later. Emergence was significantly (P<0.001) reduced from 13.75 in the control treatment to less than 3.00 in lower dosage of oil treatments. No progeny emerged from seeds treated with 600µl/20g and above, indicating effective protection.

A similar observation was made on the extent of seed damage by *C. maculatus* in the oil treated seeds. There were significant (P<0.001) differences in the reduction of seed damage by the insect at lower dosages compared with the control (Table 3). There was no damage recorded in the seeds treated with 600µl/20g and above. The level of seed damage (6.100±0.665) in the untreated seeds was significantly (P<0.001) higher than the lower dosage applications (200 and 400µl/20g, respectively). The oil treatments, irrespective of dosage, proved superior to control and showed significant protectant potential in storage.

Table 2: Oviposition deterrent activity of *P. guineense* seed oil against *C. maculatus*

Dosage (µl/20g) (Conc. %)	No. of *C. maculatus* eggs	% Reduction in oviposition
Control (0.0%)	32.325 ± 3.529a	-
200 (1.0%)	7.175 ± 1.927b	77.80
400 (2.0%)	5.175 ± 0.512bc	84.00
600 (3.0%)	3.500 ± 0.576bcd	89.17
800(4.0%)	1.850 ± 0.352bcde	94.28
1000 (5.0%)	0.600 ± 0.170bcde	98.14

Means ± SE followed by different letters are significantly different at the 0.05 level.
Number of eggs laid was significant at P<0.001 by Fisher's F-test.
SE: standard error.

Table 3: Production of F_1 progeny and seed damage by *C. maculatus* on oil treated seeds

Dosage (µl/20g) (Conc. %)	No. of F_1 progeny	% damage
Control (0.0%)	13.750 ± 1.109a	6.100 ± 0.665a
200 (1.0%)	3.000 ± 1.780b	1.525 ± 0.909b
400 (2.0%)	0.750 ± 0.479bc	0.625 ± 0.361bc
600 (3.0%)	0.000 ± 0.000c	0.000 ± 0000c
800 (4.0%)	0.000 ± 0.000c	0.000 ± 0.000c
1000 (5.0%)	0.000 ± 0.000c	0.000 ± 0.000c

Means ± SE followed by different letters are significantly different at the 0.05 level.
All indices were significant at P<0.001 by Fisher's F-test.
SE: standard error.

3.3. EFFECT ON SEED GERMINATION

There was no adverse effect of the oil on seed viability compared with the control treatment (Table 4). The highest germination percentage (85%) was recorded in the seeds treated with 1000µl/20g seed oil. This was then followed by 800, 600 and 400µl/20g oil treatments, which achieved 75.00, 62.50 and 50.00 percent germination, respectively.

Table 4: Effect of P. guineense seed oil on germination of cowpea seeds

Dosage (µl/20g) (conc. %)	% germination
Control (0.0%)	10.00 ± 4.10a
200 (1.0%)	25.00 ± 6.50ab
400 (2.0%)	50.00 ± 4.10c
600 (3.0%)	62.50 ± 6.30cd
800 (4.0%)	75.00 ± 8.70de
1000 (5.0%)	85.00 ± 2.90e

Means ± SE followed by different letters are significantly different at the 0.05 level.

All indices were significant at $P<0.001$ by Fisher's F-test.
SE: standard error.

4. DISCUSSION

Storage insect pest infestation results in seed weight loss, quality deterioration and low market value of cowpea. Effective control of stored product pests is very important for sustainable food supply for the teeming world population and sustainable economic growth. The use of plant extracts as alternative to synthetic insecticides has generated renewed interest in entomological research. Seed oils are commonly used in insect pest control due to their effectiveness against virtually all life stages of insects (Adedire 2002).

Efficacy of any grain protectant is attributed to its ability to induce mortality of adult and/or immature stages of pests, confirmed by lack of progeny generation and seed perforation. In this study, *P. guineense* seed oil demonstrated high toxicity to the cowpea bruchid and adult mortality increased as the concentration of oil increased. The 100% mortality recorded at higher dosage applications indicated potential for *P. guineense* seed oil against storage insect pests. However, the precise mode of action for the bioactivity of the *P. guineense* seed oil against bruchid is not very clear. The toxic effect could result from contact action as evidenced by inability of the insects to right themselves, motionlessness after gentle touch with dissecting pin, and interference by blockage of spiracles of the insects, resulting in asphyxiation and subsequent death. Moreover, insecticidal property of any plant material would depend on the active constituents of the plant material. The potency of *P. guineense* has been attributed to piperine, the main active principle in the edible seed, which acts as a neurotoxin in insects (Scott *et al*. 2008). In addition, bioactivity of most insecticidal-active plant extracts is often associated with the volatile phytochemical compositions such as phenols, alkaloids, flavonoids and terpenes, which penetrate into insects and interfere with physiological functions (Rubabura *et al*. 2014).

The ability of *C. maculatus* to oviposit on oil treated cowpea seeds was impaired. Reduction in oviposition increased with the increase in dosage of each treatment. However, all the oil treatment levels showed high oviposition deterrent on cowpea seeds, irrespective of dosage, compared with the untreated seeds. The oil may have induced physiological and behavioral changes in the insects, thereby lowering their oviposition potential. It is also possible that the *P. guineense* seed oil may have affected the bruchid locomotion which led to inability of insects to mate freely. Similar oviposition suppression were reported for neem seed oil (*Azadirachta indica*); Nutmeg oil (*Myristica fragrans*), sandbox seed oil (*Hura crepitans*), Jatropha seed oil (*Jatropha curcas*) and melon seed oil (*Citrullus colocynthis*) (Lale and Mustapha 2000; Adedire 2002; Adedire and Ajayi 2003; Boateng and Kusi 2008; Nzelu and Okonkwo 2016). Furthermore, the black pepper oil effectively reduced the emergence of *C. maculatus.* The reduction and/or complete inhibition of F_1 emergence could be attributed to the fewer number of eggs laid, and probably the inability of the eggs to hatch. This observation implies that *P. guineense* seed oil offer a good storage treatment against *C. maculatus*. Some studies have also shown that plant-derived extracts such as *Zanthoxylum zanthoxyloides*, *Azadirachta indica*, *Chenopodiu ambrosioides*, *Ocimum kenyense*, *Capsicum frutescens* and *Citrullus colocynthis* inhibit/suppress F_1 progeny emergence in insects (Obeng-Ofori *et al*. 1997; Ogunwolu *et al*. 1998; Tapondjou *et al*. 2002; Govindan and Jeyarajan 2008; IIeke *et al*. 2013; Nzelu and Okonkwo 2016). In addition, pulverized plant material from *P. guineense* was reported to inhibit egg hatchability and adult emergence of *Dermestes maculatus* in smoked catfish (*Clarias gariepinus*) during storage (Fasakin and Aberejo 2002).

Grain damage to the embryo by the feeding bruchids impairs germination and poor grain quality for human consumption due to reduction in the amounts of carbohydrates and proteins (Allotey and Oyewo 1993; Rahma and Talukder 2006). Interestingly, in this study, the inhibition of progeny emergence was strongly related to the *P. guineense* seed oil, with 100% seed protection. *P. guineense* seed oil did not affect cowpea seed viability and quality, even at higher concentrations. This result is consistent with previous studies with *P. guineense* seed powder (Asawalam and Emosairue 2006) and other plant products: *Jatropha curcas* powder (Umar 2008), *Ocimum suave* leaf oil (Ojianwuna *et al*. 2014) and *Citrullus colocynthis* melon seed oil (Nzelu and Okonkwo 2016) which reported that plant-derived products generally do not affect the viability of stored cowpea or maize seeds.

5. CONCLUSION

This study has demonstrated the insecticidal, ovicidal and behaviour-modifying properties of *P. guineense* seed oil against *C. maculatus* in stored cowpea seeds. The seed oil did not adversely affect cowpea seed viability. These observations indicate the enormous potential for the use of edible *P. guineense* seed oil as protectant against stored product insect pests. It can be locally sourced and is cheap. Thus, its application as grain protectant would offer safer alternative to synthetic insecticides, especially at small-scale farmers level.

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CONFLICT OF INTEREST

The author have declared that no competing interests exist.

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