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Ameliorative potentials of two plant extracts against loss in cowpea seeds infested with *Callosobruchus maculatus* (F) (*Coleoptera: Chrysomelidae*)

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Abstract
Ameliorative potentials of ethanolic extracts of fruits of *Piper guineense* and *Syzygium aromaticum* in the reduction of quality loss in *Callosobruchus maculatus* infested stored cowpea *Vigna spp.* was investigated. Laboratory bioassays for oviposition, emergence and weight loss were conducted using cowpea seeds preserved with the extracts of *P. guineense* and *S. aromaticum*. The extracts were prepared by measuring 0.5g, 1.0g and 2.0g of grinded plant powders into 5cm^3 of ethanol. The filtrates were then used to treat 10g of cowpea seeds in kilner jars. These concentrations translated into 5%, 10% and 20% of extracts treatment, respectively. Ten pairs of sexed beans weevil were introduced into each treatment jar and four replicates were set for each treatment including control. The experiment was set for forty-two (42) days. Data obtained were analyzed using descriptive statistics of mean, standard deviation, percentage and bar charts. Mean were compared and separated using Analysis of Variance (ANOVA) and Least Significance Difference (LSD), respectively. The result showed that both extracts significantly (P<0.05) reduced oviposition between 27.3-76.8%, emergence between 79.0-100% and weight loss by 90.6-100%. The extracts were able to reduce crude protein loss by 100%, Ash content by 76% and oil extract by 100%. All mineral contents were also preserved by 100%. The study showed that these plant extracts were efficient in preserving the quality of stored cowpea seeds.

Keyword: Ameliorative, weight loss, amino acid, ovipostion, emergence, feeding scars

Introduction
Cowpea, *Vigna unguiculata* (L) walpers belong to the family fabaceae, an important edible legume crop in many parts of the world. It is use as human food due to its high protein content [1] and also as life stock feed to make silage and hay. Cowpea production is affected by insect pest and disease infestations which lead to economic losses. Insect damage is the major constraint to cowpea grain production. The principal post-harvest pest of cowpea in Nigeria which causes substantial quantitative and qualitative losses is *Callosobruchus maculatus* [2, 3]. The insect can cause damage of legume seeds up to 100% during storage [4].
Management of cowpea seed storage pests has mainly relied heavily on the use of chemical insecticides. However most of the small-scale farmers have not adopted these new techniques due to financial and technical reasons. Insecticides also had negative impacts on the environment, human and non-target organisms. Therefore, there is a need to develop cheap, safe and easy methods of protecting stored cowpeas against cowpea weevil. Many authors have reported insecticidal effect of aromatic plant products against a broad range of pests [5, 6]. Aromatic plants contain a variety of volatile oils which have insecticidal, anti-feedant and repellent effects on insect pests. Examples of such products are clove, *Syzygium aromaticum* and black pepper, *Piper guineese*. The chemical repellency hypothesis state that non-host plant odors repel herbivores by disrupting their ability to locate or feed on the host plant [7]. These plant products produce odours that are believed to repel weevils.
The objective of this study was to investigate the efficacy of crude extracts of *P. guineese* and *S. aromaticum* in reducing weevils' oviposition, adult emergence and weight loss of stored cowpea infested with bruchids, *C. maculatus* under laboratory conditions.

Materials and Methods
The experimental site
The experiment was carried out in the biology laboratory, Federal College of Education Kontagora, Niger state, Nigeria.

The insects
Adult cowpea weevils were collected on infested red cowpea variety from the seed multiplication unit. The stock culture of *C. maculatus* was raised by placing 100 unsexed adults in two liter jars half full of disinfected red cowpea seeds. Muslin cloth was used to cover the top of the jars to prevent weevils from escaping. These parent weevils were allowed to mate for seven days under laboratory condition (25-28 ^{o}C and 75-85% relative humidity) and lay eggs, after which they were removed. A day after emergence, the insect was sexed by the examination of their elytra patterns. Females are usually maculated with four elytra spots while males are plain with less distinct spots.

Cowpea seeds
The cowpea seed used for the experiment were gotten from a farmer on the farm at Salka town Niger State, Nigeria. Seeds were removed from their pods and the seeds were checked to ensure that they were not infected by visual observation for presence of egg or any suspicious material.

Plant products
The two plant products, clove and black pepper used as treatment for this study were purchase from old market Kontagora, Niger state of Nigeria. Each plant product was grounded with mortal and pistil separately and sieve into fine particle. Each sieved plant product was stored in a 100ml plastic jar.

Heating of cowpea seeds
Clean red beans seeds used for the experiment were heated at a temperature of 40 ^{o}C for five minutes in an oven to destroy any weevil eggs. This was subsequently removed and allowed to cool for about 30 minutes and kept in a big container for safe keeping before setting up the experiment.

Weighing of cowpea seeds
Cowpea seeds were measured with a digital analytical traveler balance (Model; Traveler TA302) of maximum capacity of 300 ± 0.01g manufactured by OHAUS Corporation. 10 grams of beans was measured into each container and the weight of the container was also noted.

Extract preparation
0.5g, 1.0g and 2.0g of crude powder of both plant products were measured each into 5ml of ethanol solution. The ethanol and powder were left over night and were subsequently filtered with Whatman No 4-filter paper to obtain the crude extract. Extract prepared from 0.5g, 1.0g and 2.0g of crude powders translated into 5% w/v, 10% w/v and 20% w/v concentration, respectively.
10g of beans were poured into each extract containing petri-dish and turn with spatula so that the extract will evenly coat the beans and the beans was left to dry up for 30 minutes. Each plant extract and concentrations have four replicates. Also, four replicate containers with 10g of beans without extract were used for control.

Introduction of the bean's weevil
After all the measurement has been done 20 sexed beans weevil (10 male and 10 female) were introduced into each container and cover with the muslin sheet. The muslin sheet is meant to provide the insects with ventilation as well as prevent them from escaping from the container. The container is arranged in the wire nettle cage and kept in the laboratory for observation.

Data collection
Anti-feedant, oviposition, emergence and bean weight loss were recorded. The anti-feedant activity was elucidated by using feeding scars. The feeding scars were determined after 3 weeks of the experiment, feeding scars are the evidences that weevils have feed on the bean. Ovipositions are the weevil eggs lay on the beans. The number of feeding scars and oviposition were counted. The number of emergences was also noted under each concentration after 6 weeks of the experiment. The whole seeds per replicate were re-weighed after the experiment and the new weight was subtracted from the original weight at the start of the experiment to determine the weight loss of cowpea seed.

Data analysis
Descriptive statistics of mean and standard deviation were used to present oviposition, emergence, feeding scars, weight loss and nutritional loss. Inferential statistics of analysis (ANOVA) was used to test the significance in all these parameters across treatment and concentration. The mean was separated using Least Significance Difference (LSD).

Results
Table 1 shows the effect of *P. guineense* and *S. aromaticum* on oviposition, the result revealed that, *P. guineense* at 5% w/v concentration reduced oviposition in *C. maculatus* by 44.1%, at 10% w/v concentration by 56.3%, while at 20% w/v concentration by 70.9%. Also *S. aromaticum* at 5% w/v concentration reduced oviposition in *C. maculatus* by 27.3%, at 10% w/v concentration by 57.9%, while at 20% w/v concentration by 76.8%. The finding shows that extracts of *P. guineense* and *S. aromaticum* caused significant reduction in the ovipostion of *C. maculatus*.

Table 1: Effect of *P. guineense* and *S. aromaticum* extracts on oviposition of *C. maculatus.*

Concentration	***P. guineense* (% reduction)**	***S. aromaticum* (% reduction)**
5% (w/v)	43.80 ± 15.80^{a} (44.10) ↓	57.20 ± 25.59^{a} (27.30) ↓
10% (w/v)	34.20 ± 10.50^{b} (56.30) ↓	33.00 ± 9.42^{b} (57.90) ↓
20% (w/v)	22.80 ± 4.92^{c} (70.90) ↓	18.20 ± 7.02^{c} (76.80) ↓
Control	78.33 ± 6.55^{e}	78.33 ± 6.55^{e}
LSD	9.27	12.47

Mean in the same column followed by the same letter are not significantly (p<0.05; ANOVA, LSD).

Table 2 shows the effect of *P. guineense* and *S. aromaticum* on emergence, the result revealed that, *P. guineense* at 5% w/v concentration reduced emergence in *C. maculatus* by 79.0%, at 10% w/v concentration by 90.2%, while at 20% w/v concentration by 100%. Also *S. aromaticum* at 5% w/v concentration reduced emergence in *C. maculatus* by 80.5%, at 10% w/v concentration by 100%, while at 20% w/v concentration by 100%. The experiment shows that extracts of *P. guineense* and *S. aromaticum* caused significant reduction in the emergence of *C. maculatus*.

Table 2: Effect of *P. guineense* and *S. aromaticum* extracts on the emergence of *C. maculatus*.

Concentration	*P. guineense (% reduction)*	*S. aromaticum (% reduction)*
5% (w/v)	14.60 ± 14.29^{a} (79.0) ↓	13.60 ± 16.95^{a} (80.5) ↓
10% (w/v)	6.80 ± 5.71^{b} (90.2) ↓	0.00 ± 0.00^{b} (100.0) ↓
20% (w/v)	0.00 ± 0.00^{bc} (100.0) ↓	0.00 ± 0.00^{b} (100.0) ↓
Control	69.67 ± 11.00^{e}	69.67 ± 11.03^{d}
LSD	8.27	8.78

Mean in the same column followed by the same letter are not significantly (P<0.05; ANOVA, LSD).

Table 3 shows the effect of *P. guineense* and *S. aromaticum* on quantity (weight) preservation of *cowpea*, the result revealed that, *P. guineense* at 5% w/v concentration lead to quantity preservation of *cowpea* by 90.8% weight preservation, 10% w/v concentration by 99.6%, while at 20% w/v by 100%. *S. aromaticum at* 5% w/v concentration lead to 92.2% quantity preservation, 10% w/v concentration by 99.5%, while 20% w/v by 100%. The finding shows that the extracts of *P. guineense* and *S. aromaticum* significantly preserve the quantity or weight of cowpea seeds.

Table 3: Effects of *P. guineense* and *S. aromaticum* extracts on quantity (weight) loss of cowpea protected against *C. maculatus*.

Concentration	*P. guineense (% retention)*	*S. aromatcum (% retention)*
5% (w/v)	9.50 ± 0.79^{a} (90.8)	9.57 ± 0.78^{a} (92.2)
10% (w/v)	9.96 ± 0.05^{ab} (99.6)	9.95 ± 0.04^{ab} (99.5)
20% (w/v)	10.00 ± 0.00^{ac} (100.0)	10.00 ± 0.16^{ac} (100.0)
Control	4.98 ± 0.57^{e}	4.98 ± 0.57^{e}
LSD	0.94	0.95

Mean in the same column followed by the same letter are not significantly (P<0.05; ANOVA, LSD).

Discussion

The study shows that the extracts of clove, *S. aromaticum* and black pepper, *P. guineense* seeds has insecticidal properties and can be used for the control of *C. maculatus.* Clove and black pepper seed extracts therefore possess the properties such as toxicity to adult, reduction of oviposition, ovicidal activity required for the control of insect pests feeding on grains and legumes [6]. The result obtained in this study shows that extracts of clove and black pepper seeds were toxic to beans weevil, *C. maculatus.* At the highest concentration of 20% w/v, oviposition of weevils was suppressed by 70.9% and 76.8% respectively with *P. guineese* and *S. aromaticum* respectively, thus producing significant reduction in oviposition. This agrees with the work of [8], which reported that seeds treated with clove and black pepper could suppress F_1 progeny production in *C. maculatus*, which may imply ovicidal action of these extracts.

The extracts also significantly reduced emergence of *C. maculatus*. At a concentration of 20% w/v, *P. guineese* and *S. aromaticum* extracts were able to completely stop emergence of adult weevils. This is similar to the report of [16] which found, that the powder of *P. guineense* significantly reduced adult emergence of adult *Sitophilus zeamais* (mots) when compared with control [10]. also observed reduced emergence in rice weevils when rice grains were treated with hexane and methanol clove extracts. Moreover, the quantity or weight of cowpea stored with *P. guineese* and *S. aromaticum* extracts at 20% w/v concentration were 100% retained. This shows that these extracts of caused significant quantity preservation of cowpea. This is similar to the report of [11] who reported significantly higher maintenance of weight in cowpea treated with powder of *Vernonia amygdalina.* Quantity preservation of cowpea seeds can be attributed to the antifeedant and ovicidal properties of both *P. guineese* and *S. aromaticum.*

These lethal properties of these plant products have attributed to their component chemicals. The suppression of F_1 progeny emergence in *P. guineense* treated beans could be associated with the ability of its component piperine to distort the insect physiology [12]. The odour of the extract volatile compounds is believed to block the spiracular tracheal respiration of the insects leading to suffocation and subsequently death [13, 14]. Clove was earlier reported to decrease in oviposition when its essential oil was tested against females *Zabrotes subfasciatus* [15]. This effect can be due to the chemical components especially eugenol.

Conclusion

This study showed that the seed extracts of black pepper, *P. guineense* and clove, *S. aromaticum* are effective in protecting cowpea seeds in the store against *C. maculatus*. Moreover, farmers can easily prepare and apply them to protect their grains in the store. The used of these plants' products would be effective and sustainable, especially considering that these plants are easy to grow.

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