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Laboratory evaluation of five botanicals as protectants against cowpea bruchid *Callosobruchus maculatus* F. (Coleoptera: Bruchidae) on stored cowpea

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ABSTRACT

Cowpea (*Vigna unguiculata* L. Walp) is an important legume grown primarily in the tropics. Cowpea bruchid *Callosobruchus maculatus* is a major pest of stored cowpea in Nigeria. A laboratory study was conducted to evaluate the effectiveness of some indigenous botanical powders and one essential oil extract as grain protectants against *C. maculatus* at Agronomy Laboratory, Michael Okpara University of Agriculture Umudike in 2013. The treatments applied were 1.5 g powder each of tumeric rhizomes, garlic bulb, sand paper leaves, bitter kola seeds, ginger rhizomes, 0.5 ml of ginger rhizome essential oil and a control. The experiment was laid out in Completely Randomized Design (CRD) with four replicates. Adult mortality, progeny emergence, percentage grain damage, percentage weight loss and germination percentage were assessed. The result obtained showed that all the botanicals gave protection to the stored cowpea seeds compared with the control. Botanical extracts did not affect percentage stored cowpea weight loss ($P > 0.05$). All the botanicals significantly ($P < 0.05$) reduced the grain damage of cowpea seeds by *C. maculatus* and F1 progeny emergence. The total number of *C. maculatus* adults that emerged from untreated control progressively increased with time of exposure compared to the other treatments. Significantly higher germination percentage was recorded in the various treatments with botanicals compared with the control which recorded 35%.

Keywords: Botanicals, *Callosobruchus maculatus*, control, damage, mortality.

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INTRODUCTION

Cowpea (*Vigna unguiculata* L. Walp) is a member of the family Fabaceae. It is a food and animal feed crop grown in the semi-arid tropics covering Africa, Asia, Europe, United States and Central and South America (Asante et al., 2001). Huge losses of between 20 and 50% have been reported on stored cowpea due to attack by cowpea weevil, *Callosobruchus maculatus* F. and sometimes the loss could be complete accounting for 100% loss (Udo and Harry, 2013). Cowpea bruchid also attacks chickpeas (*Cicer* sp.), lentils (*Lens* sp.), garden peas (*Pisum* sp.) and mungbeans (*Vigna* sp.) (Cope and Fox, 2003). The damage is distinctive. Larvae feed and develop inside the seed and when adults emerge they leave a neat circular exit hole. Each adult consumes approximately 25% of the seed from which it develops. Heavy infestation causes the commodity to heat up. This results in loss of quality and mould growth.

The larvae bore into the pulse grains which become unsuitable for human consumption, viability for replanting, or for the production of sprouts (Rahman and Talukder, 1996). They are important pests of pulse crops in Asia and Africa under storage conditions (Ogunwolu and Idowu, 1994; Okonkwo and Okoye, 1996; Mulatu and Gebremedhin, 2000; Ajayi and Lale, 2001; Tapondjou et al., 2002). Serious problems of genetic resistance by insect species, pest resurgence, residual toxicity, phytotoxicity, vertebrate toxicity, widespread environmental hazards and increasing costs of application of the presently used synthetic pesticides have necessitated research for effective, biodegradable pesticides (Weaver et al., 1991; Rahman and Talukder, 1996).

This awareness has created worldwide interest in the development of sustainable and safe alternative strategies, which include biorational control using plant derivatives against agriculturally important insect pests. Plant-derived materials (botanical insecticides) are more readily biodegradable. Some are less toxic to mammals, may be more selective in action, and may retard the development of resistance. Their main advantage is that they may be easily and cheaply produced by farmers and small-scale industries as crude, or partially purified extracts (Rahman and Talukder, 1996). In recent times, considerable efforts have been directed at screening plants in order to develop new botanical insecticides as alternatives to the existing insecticides. According to Rahman and Talukder (1996) mixing stored grains with leaf, bark, seed powder, or oil extracts of plants reduced oviposition rate and suppressed adult emergence of bruchids, as well as reduced seed damage rate.

The objective of this study was to evaluate the efficacy of *Zingiber officinale* essential oil and powders of five medicinal plants (*Curcuma longa, Allium sativium, Zingiber officinale, Garcinia kola* and *Ficus exasperata*) against *C. maculatus* in stored cowpea seeds.

MATERIALS AND METHODS

Experimental location

The experiment was carried out at Crop Science Laboratory of the Department of Plant Health Management, Michael Okpara University of Agriculture, Umudike, Abia State, Nigeria.

Insect culture

Infested cowpea seeds were purchased locally from Umuahia main market in Abia State. The weevils (cowpea bruchid) were cultured by weighing 1000 g of the infested cowpea in a plastic bucket that was covered with a muslin cloth and held tight with a rubber band to avoid escape of the insects. The muslin cloth was used to ensure adequate aeration and prevent entering of other insects. The insects were cultured for 105 days in Crop Science Laboratory, Michael Okpara University of Agriculture, Umudike, Abia State, Nigeria. The insect culture was carried out under ambient environmental conditions.

Collection of uninfested cowpea seeds

Uninfested and susceptible cowpea grains were purchased locally from Umuahia Main Market, in Abia State. The seeds were graded manually to obtain uniform sized seeds and were cleaned of broken seeds and debris. Fifty grams of the cowpea grain samples were placed into 200 ml plastic vials and covered with white muslin cloth.

Collection of plant materials

Rhizomes of *Curcuma longa* and *Zingiber officinale* were collected from National Root Crop Research Institute in Abia State, Nigeria, while bulb of *Allium sativum* and seeds of *Garcinia kola* were purchased locally from Umuahia Main Market. Fresh and matured leaves of *Ficus exasperata* were collected from its natural habitat around Michael Okpara University of Agriculture, Umudike Eastern farm. The leaves were air dried for 7 days in the laboratory. The leaves, rhizomes, bulbs and seeds were pulverized using a crown manual hand blender and were sieved using a 600 µm mesh sieve to obtain uniform particle size. The powdered materials (Table 1) were kept in air-tight containers, labeled, and stored at room temperature ready for use in the experiment. The botanical powders were utilized within 5 days. The extraction of the essential oil of *Z. officinale* was carried out at Biochemistry Laboratory, National Root Crops Research Institute Umudike using soxhlent extractor apparatus.

Application of plant materials

Each of the plastic vials containing 50 g of uninfested cowpea seeds was treated with the plant powders (Table 1) at the rate 1.5 g and oil at the rate 0.5 ml each by mixing thoroughly to ensure adequate contact with the grains. Prior to the introduction of the insect into treated grains, the insects were picked individually using entomological forceps. Twenty adult *C. maculatus* were introduced into each treatment. The top of the plastic vials were covered with white muslin cloth and held with rubber bands. The treatments were arranged in Completely Randomized Design (CRD) with four replications.

Adult mortality test

The mortality of adult weevils was recorded after 7 days for 48 days. Insects that failed to respond to three probing using blunt dissecting probe were assumed dead and were included in the counts (Udo et al., 2010).

$$(\%)\ \text{mortality} = \frac{\text{No of dead insects}}{\text{Total no of insects}} \times 100$$

The F1 progeny emergence, the percentage grain damage, percentage weight loss and germination percentage were also assessed as follows:

The F1 progeny emergence test

The newly emerged adult weevils (F1 progeny emergence) were recorded on weekly basis on day 28, day 35, day 42 and day 49 from each treatment replicate.

Percentage grain damage

Percentage grain damage was assessed after 2 months of introduction of adult *C. maculatus* to each experimental plastic vial. Number of grains with characteristic hole was recorded following the general formula as follows:

$$(\%)\ \text{grain damage} = \frac{\text{No of perforated grains}}{\text{Total no of grains counted}} \times 100$$

Table 1. Plants to be evaluated for insecticidal properties.

Common name	Scientific name	Family	Part used
Turmeric	*Curcuma longa*	Zingiberaceae	Rhizomes
Ginger	*Zingiber offincinale* Rosc.	Zingiberaceae	Rhizomes
Garlic	*Allium sativum* L.	Alliaceae	Bulb
Sandpaper leaf	*Ficus exasperata*	Moraceae	Leaves
Bitter kola	*Garcinia kola*	Guttiferaceae	Seeds

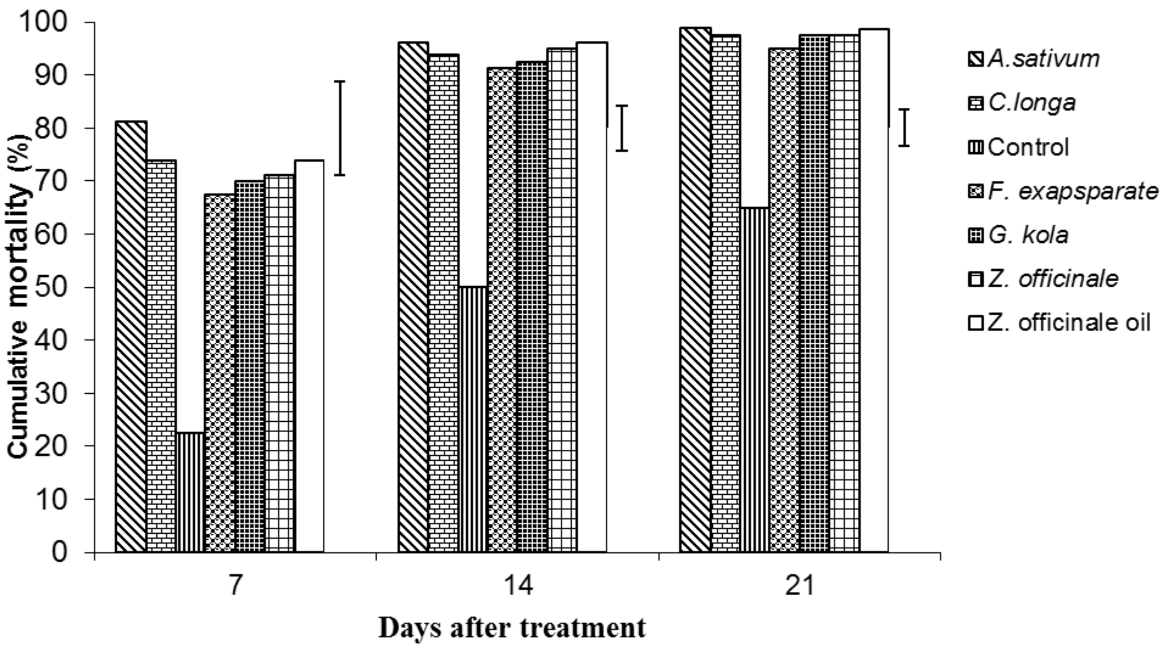

Figure 1. Cumulative percentage adult mortality of *Callosobruchus maculatus* treated with botanical powders and oil. Vertical bars represent LSD0.05.

Percentage weight loss

Percentage weight loss was calculated using the following formula (Fekadu et al., 2012):

$$(\%) \text{ weight loss} = \frac{\text{Initial weight} - \text{final weight}}{\text{Initial weight}} \times 100$$

Germination percentage

Ten healthy grains were visually selected from the experimentally treated grains from each plastic vial. The selected grains were soaked in distilled water for 3 h. There after the seeds were placed in petri dishes containing moistened filter paper (Whatman no.1 filter paper). The petri dishes were kept in the laboratory at room temperature. The number of geminated seeds from each petri dish was counted and recorded from the 5^{th} day to the 10^{th} day after soaking. The percentage germination was computed as follows:

$$\text{Germination } (\%) = \frac{\text{No of seeds germinated}}{\text{Total no of grains sampled}} \times 100$$

Statistical analysis

Data obtained were subjected to analysis of variance (ANOVA) procedure. Significant treatment mean were separated using Least Significant Difference (LSD) at 5% level of probability.

RESULTS AND DISCUSSION

Effects of plant powders and extract in the mortality of adult *C. maculatus* at weekly basis after treatment

The result of mortality of *C. maculatus* is given in Figure 1. Results of this study showed that percentage of adult mortality was significantly higher at first week of application of the botanicals than that of second week after treatment. Percentage mortality progressively increased with time of exposure. The treatments were highly significant in the mortality of adult *C. maculatus.*

At 7 DAT, there was significant difference in mortality in all the treatments and control but *A. sativum* recorded significantly high mortality at 81.2% while mortality of the

Table 2. Percentage weight loss (g) of infested cowpea seeds treated with botanical powders and oil.

Treatments	Days after treatment		
	14	28	42
A. Sativum	0.12	0.22	0.39
C. longa	0.17	0.25	0.42
F. exasperata	0.22	0.39	0.57
G. kola	0.75	0.78	0.99
Z. officinale	0.12	0.22	0.39
Z. officinale oil	0.12	0.22	0.39
Control	0.30	0.47	0.74
LSD (0.05)	0.99	0.55	0.54

treatment with *C. longa* and *Z. officinale* oil was not significantly different from each other. At 14 DAT, the mortality of *C. maculatus* was significantly reduced. The treatments were not significantly different from each other. The control showed significant mortality at 50%.

At 21 DAT, the treatments and control were significantly different from each other. However, the least *C. maculatus* adult mortality occurred in the control treatments for the 7 DAT, 14 DAT and 28 DAT. The result showed that cowpea seeds treated with plant powders and *Z. officinale* oil caused a significant mortality within 7 DAT. This result also showed that effectiveness of the powder reduced with time. Ofuya et al. (2010) proved that fumigation of pods with crushed bulbs of *A. sativum* and *A. cepa* showed a toxic effect to *C. maculatus.*

Effect of botanical powders and *Z. officinale* oil on damage and weight loss of cowpea seeds, at two weeks interval

Weight loss of stored cowpea seeds was not significantly affected by the treatments. The result for weight loss of the cowpea seeds is presented in Table 2. At 28 DAT, mean weight losses in the different treatments were not significantly different from each other. However, mean weight loss of *G. kola* (0.78%) and control (0.47%) were higher compared to those of *A. sativum* (0.22%), *C. longa* (0.24%), *Z. officinale* (0.22%) and *Z. officinale* oil (0.22%). At 42 DAT, weight loss was significantly high in *G. kola* (0.99%) and control (0.74%) with least significant weight loss in *A. sativum* (0.39%). However, weight loss for all the treatment increased as the number of days increased.

Botanical extracts significantly affected percent grain damage of stored treated seeds. Percent damage of treated seed was significantly different from the control (48.7%). Apart from the control, *F. exasperata* recorded high grain damage (39.8%) while *A. sativum* recorded the least grain damage (21.2%) (Table 3). The damage caused to control was due to low mortality and high progeny emergence implying that the insect numbers

Table 3. Percentage grain damage of cowpea seeds infested by *C. maculates.*

Treatments	Percentage grain damage
A. Sativum	21.2
C. longa	24.4
F. exasperata.	39.8
G. kola	25.7
Z. officinale	28.5
Z. officinale oil	25.8
Control	48.7
LSD (0.05)	10.89

Table 4. *C. maculatus* progeny emergence from cowpea seeds treated with botanical plant powders at different time interval (days).

Treatments	Days after treatment			
	14	28	42	49
A. Sativum	4.75	8.25	4.50	2.50
C. longa	5.25	8.25	5.50	3.00
F. exasperata	8.25	13.00	11.00	6.50
G. kola	5.50	4.75	4.25	2.50
Z. officinale	5.75	3.75	3.50	3.00
Z. officinale oil	5.00	3.75	4.00	3.25
Control	16.25	24.25	23.00	14.00
LSD (0.05)	3.64	4.14	3.87	2.83

were increasing thereby causing more damage on grain. There was a significant reduction in damage caused by the weevils to treated cowpea seeds compared with the control and that is why, the weight loss of treated samples was low compared with the control. There was a corresponding reduction in the number of exit holes in treated cowpea seeds as a result of limited contact of the weevil with the treated seeds especially those treatments that contain oil such as *C. longa, A. sativum, Z. officinale* and *Z. officinale* oil. The results of this study is similar to the work of Musa et al. (2013) who showed that adult emergence and seed weight loss were insect population dependent.

Effects of botanical powders and *Z. officinale* oil on F1 progeny emergence at different time intervals.

The result for progeny emergence is presented in Table 4. At 28 DAT, the progeny emergence in control was significantly high (16.25) followed by *F. exasperata* (8.25), with A*. sativum* recording the least significant progeny emergence (4.75). At 35 DAT, F1 progeny emergence was significantly affected by botanical extract treatment but control had the highest population (24.25) followed by *F. exasperata* (13.00). At 35 DAT, F1 progeny populations significantly increased in *A. sativum*

Table 5. Mean percentage germination of cowpea seeds treated with botanical powders and oil.

Treatments	Mean percentage germination
A. Sativum	62.5
C. longa	65.0
F. exasperata	50.0
G. kola	62.5
Z. officinale	62.5
Z. officinale oil	65.0
Control	35.0
LSD (0.05)	23.42

and *C. longa* treated seeds while *G.kola, Z. officinale* and *Z. officinale* oil F1 progeny reduced with the *Z. officinale* powder and oil recording low progeny emergence (3.75).

At 42 DAT, F1 progeny emergence population was high among botanical extract treated seeds compared to control but *F. exasperata* was significantly different from other treatments. *A. sativum* and *C. longa* populations significantly reduced with *Z. officinale* powder recording the least significant progeny emergence (3.50) (Table 4). At 49 DAT, F1 progeny emergence was much lower in each treatment including the control but *F. exasperata* was significantly different from other treatments with the *A. sativum* and *G. kola* having the least significant progeny emergence (2.50). Oviposition by *C. maculatus* was reduced in all treatment levels except in the control treatments; the reason must have been due to high mortality of the adult *C. maculatus* observed in the treated cowpea seeds. This agrees with reports that besides causing mortality of grain weevils, plant powders and oils also impair oviposition and progeny emergence (Udo and Harry, 2013). The result of this investigation is in agreement with the findings by Musa et al. (2009) that mixing leaf powders of *V. amygdalina* and *O. gratissimum* at equal proportion (50:50) caused 60.0% mortality of adult bruchid, reduced oviposition and suppressed adult emergence.

Effects of plant powders and oil on germination of cowpea seeds at 49 days after treatment

The result of germination of the cowpea seeds is presented in Table 5. Germination percentage in the treated seeds was highly significant. The treated seeds recorded higher germination percentage while the control had the least germination percentage (35%). This result showed that the weevil attack altered the germination of the control treatments. It also showed that plant materials tested against *C. maculatus* did not show any adverse effect on germination capacity of the cowpea seeds. Asawalam and Dioka (2012) also found that powders of *C. longa* rhizomes and *D. tripetala* leaf, fruit and bark can be utilized in protecting stored cowpea from *C. maculatus* infestation since they are cheaper and safe for humans.

CONCLUSION AND RECOMMENDATIONS

There is abundant evidence of poor insecticide education and abuse in Nigeria. In most villages in Nigeria, farmers apply overdoses of insecticides to effect rapid and immediate kill of the insect pests of cowpea. The current result revealed that most of the botanical powders and *Z. officinale* oil was effective and had insecticidal activity similar to synthetic chemicals for the control of *C. maculatus*. The treatments significantly achieved high mortality of the adult *C. maculatus* and significantly reduced weight loss due to its ability to inhibit oviposition by adult *C. maculatus* and hatchability of oviposited eggs hence the significantly lower number of progeny that emerged from the treatment with botanicals.

Therefore, these botanicals can be used as an alternative to synthetic chemicals for the control of *C. maculatus* in stored cowpea. Moreover, the local availability of these botanicals makes it easy for small holder farmers and reduces the cost of cowpea seed production. Also these botanicals are environmentally friendly and provide food safety in terms of replacing the more dangerous toxic synthetic insecticides.

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