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COMPARATIVE BIOCIDAL ACTIVITIES OF SOME CRUDE PLANT SPECIES POWDERS AGAINST THE COWPEA WEEVIL (CALLOSOBROCHUS MACULATUS (F.)(COLEOPTERA: BRUCHIDAE)

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ABSTRACT
Callosobruchus maculatus is one of the most important pests of cowpea in storage causing severe economic damage to the grain. This study investigated the efficacies of three plant materials (*Azadirachta indica, Calotropis procera and Chromolaena odorata*) leaves against the cowpea weevil. Concentrations of 0.1, 0.25 and 0.5g of the plant powders were used on 10g of grains with 10 adult weevils in each and a Control (untreated) in triplicates. The results showed significant ($P < 0.05$) negatively effects of the plant materials on the survival of *C. maculatus* at the highest concentration. In all trails, mean daily mortality in adult *C. maculatus* were significantly($p<0.05$) increased. All plant powder type were effective but concentration-dependent, with *C. procera* recording significantly ($P < 0.05$) higher mortality at the various concentrations while *C. odorata*, elicited the least mean daily mortality. The lowest LD_{50} (0.63g) was obtained with *C. procera*. These plants materials were found to also affect the egg-laying capacity of *C. maculatus*. Treatment with *C. odorata* recorded significantly($P<0.05$) higher number of eggs laid at all concentrations, though the egg-laying capacity was also concentration-dependent; whereas *C. procera* recorded the least number of eggs laid. All the three plants powders tested demonstrated significant insecticidal potency on stored cowpea weevils, with *C. procera* and *C. odorata* showing significantly higher and lower insecticidal potentials respectively. These findings will help in solving problem associated with food security especially with respect to stored produce.

Keywords: Plant Extract, Food Security, Egg Laying Capacity, Mortality.

INTRODUCTION

Cowpea *(Vigna unguiculata)* is a major staple food in many regions of the world. The crop has a huge strategic importance and versatile usage, e.g., as food, as feed for livestock and raw material for industries due its rich protein, iron, calcium, phosphorus and vitamin B contents (Bazzano *et al.,* 2001). The production and storage are faced with numerous constraints, a major one being insect pest infestation in storage (Ajayi & Lal, 2010). Leguminous crops are widely grown in the tropics and subtropics for human food as well as for animal feeds. Examples include Cowpea (*Vigna unguiculata*), Pigeon pea (*Cajanus cajan*), Soya bean (*Glycine max*) and Groundnut (*Arachis hypogea*). They contain good quantities of most nutrients especially protein, vitamins and minerals (Olawuni *et al.*, 2012). Nigeria, Brazil and Niger Republic are among the major producers of cowpea and it accounts for over 70% of world crop (Onyido *et al.*, 2011). Africa produces 75% of the world's crop and Nigeria is responsible for about 58% of the entire Africa's production (Onyido *et al.*, 2011). Nigeria alone produces about 900,000 tonnes of cowpea annually, grown mainly as a secondary crop in association with staples such as maize, sorghum, millet and cassava (Oyerinde *et al.*, 2013). The major method adopted in the realization of this goal is chemical mode; unfortunately, the mode has raised so many secondary problematic concerns such as: resistance development, environmental safety, insect pest resurgence, lethal effects on non-target organisms and toxicity to humans (Kumar *et al*., 2021). The use of bio-pesticides, sourced from plants, is believed to be a way out of these secondary problems (Abdallah *et al*., 2017). To this end, quite a number of plants have been listed as promising source of potent bio-pesticides (Ajayi & Lal, 2010). All the conventional methods available for stored produce insect pest control have drawbacks and hence bio-pesticides seems to be gaining grounds. Unfortunately, no one bio-pesticide has been named as good against *Callosobrochus maculatus* hence making search for same continue (Iloba & Umoetok, 2007). Though the heavy losses from cowpea, *Vigna unguiculata* can be minimized through the use of synthetic chemicals, this method is costly, environmentally unfriendly and could lead to insect pest resurgence, as a result of resistance to insecticides. Bio-pesticides are however, less problematic and may give the desired results. Thus, the interest of this research is to confirm the biological activities in leaves of some plant materials and compare their efficiencies, in a bid to identify plants that may serve as potential source of bio-pesticides.

MATERIALS AND METHODS

Collection and Preparation of plant Leaf Powders

Fresh plant specimens of *Chromolaena odorata, Azadirachta indica* and *Calotropis procera* were collected from their natural habitat in Minna the capital city of Niger State, located within longitude 6°33E and latitude 9°37N (Salihu *et al*., 2017), and taken to Department of Biological Sciences, of the Federal University of technology Minna, for identification, and were given a unique voucher number as follows: FUT/PLB/AST/001 (*Chromolaena odorata*),FUT/PLB/MAL/001 (*Azadirachta indica*) and

FUT/PLB/APO/001 (*Calotropis procera*). The collected leaves of the three plant species were dried separately under the shade on a laboratory bench (Mulungu *et al.*, 2007). Thereafter, the leaves were pulverized using blender, and sieved to obtain fine powders. The pulverized content obtained in each case was kept in polyethene bags and preserved under laboratory conditions, until needed for assay.

Source and Culturing of weevil

Adult *C. maculatus* individuals were obtained with infested cowpea grains from National Stored Product Research Institute in Ilorin. Cowpea were kept in freezer at 4°C for two weeks, then air dried in the laboratory at 27 -29°C for one week, the goal of this activity is to eliminate bean weevil immature stages in the cowpea, thereafter, 200g of the disinfested cowpea grains were measured into five (5) different bottles and in each bottle, 5 pairs of the adult *C. maculatus* were introduced and the bottles were screened with muslin net and secured tightly with rubber band to allow gaseous exchange but prevent escape of the insects. The set ups were maintained under 27-29°C ambient Laboratory conditions for rearing. When eggs were noticed on the grains, the adult weevils were sieved out, following the modified methods of Ouali-Ngoran *et al*. (2014) leaving only the grains in the petri dishes.

Collection and Disinfection of Cowpea Grains

Prior to the experiment, the collected cowpea seeds were refrigerated for 10days at 4°C, to eliminate any form of insect infestation and thereafter allowed to cool at 27-29°C before use (Haine, 1991).

Mortality Test

This was conducted based on the procedures described by Ntonifer *et al.* (2011) with slight modification. 10g of disinfested cowpea grains were weighed into separate Petri dishes, and three different concentrations of leaf powders from the *A. indica, C. procera and C. odorata* plants (0.1, 0.25 and 0.5 g) were separately inoculated into separately Petri dish, containing 10 g of disinfested cowpea, equivalent to 1%, 2.5% and 5% w/w, respectively. All the Petri dishes were vigorously shaken to ensure proper mixing of cowpea grains with plant powder. Control treatment was set along with no plant material powder. Ten adults of the insects were introduced into each Petri dish (Mulungu *et al*., 2007). All the Petri dishes were labeled according to the concentration used and covered with muslin cloth to permit aeration but prevent the escape of insects (Akinwumi *et al*., 2007). Each treatment was replicated three times. The experiment was arranged in completely randomized design. Mortality count of the *C. maculatus* was carried out on a daily basis of post treatment. The dead insects were removed and live ones left until after 10 days when the remaining insects were all removed from the Petri dishes.

Oviposition (Egg laying capacity) Test

After the completion of mortality test, some cowpea grains were selected and stained with acid fuchsin solution to ensure enhanced view of the eggs laid. The number of eggs laid on each grain was observed and counted; this was done on the Control, as well as, all the replicates of each concentration of the treatments with leaf powders (Holloway, 1985).

Progeny Emergence and Developmental Rate Test

The remaining grains in the Petri dishes were allowed to stay and monitored for adult emergence of F1 progeny. The number of F1 progeny produced was counted upon emergence. The rates of development (in days) from egg to adult stage were determined by counting the day interval between egg laying and adults emergence of the insects.

Statistical Analysis

Significant differences between the mean percentage mortalities at different concentrations and between the different leaf powders were analyzed using SPSS 20th version. Analysis of variance (ANOVA), Duncan Multiple Range Test were used to separate the means. Dose response mortality data were analyzed using linear regression analysis to calculate LD_{50} and LD_{90}.

RESULTS

Table 1 highlight results on insecticidal effect of different concentrations of *Chromolaena odorata* on weevil. The mean mortality of adult weevils subjected to different treatments of *C. odorata* after 10 days post-exposure recorded zero mortality between days 1 and 2, however, as the exposure periods (days) increases, weevil mortality increases. The highest weevil mortality was recorded at the 0.5g plant concentration, followed by 0.25g in all the days when there was mortality. The mortality is significantly higher in the treatment with 0.5g plant powder than in the other treatments, especially when compared with the control.

Table 1: Mortality caused by *C. odorata* leaf powder treatment at varying concentrations against the weevils *C. maculatus*

	Plant Powder Concentration (grams)			
Days	0.1	0.25	0.5	Control (0.0)
1	0.00±0.00^{a*}	0.00±0.00^{a}	0.00±0.00^{a}	0.00±0.00^{a}
2	0.00±0.00^{a}	0.00±0.00^{a}	0.00±0.00^{a}	0.00±0.00^{a}
3	0.33±0.33^{a}	1.00±0.00^{b}	1.00±0.58^{b}	0.00±0.00^{a}
4	0.33±0.33^{a}	1.33±0.33^{b}	1.33±0.88^{b}	0.00±0.00^{a}
5	0.67±0.33^{a}	2.00±0.00^{b}	2.00±0.58^{b}	0.00±0.00^{a}
6	0.67±0.33^{a}	2.33±0.33^{b}	2.33±1.20^{b}	0.00±0.00^{a}
7	0.67±0.33^{a}	2.67±0.33^{b}	3.00±0.58^{b}	0.00±0.00^{a}
8	1.33±0.33^{b}	3.33±0.33^{c}	3.67±1.86^{c}	0.00±0.00^{a}
9	2.00±0.58^{a}	4.00±0.00^{b}	5.00±1.00^{c}	0.00±0.00^{a}
10	2.33±0.33^{a}	4.67±0.67^{b}	6.33±1.19^{c}	0.00±0.00^{a}

Number of insect used: 10 per replicate
*Values followed by the same superscript alphabets in the same row, are not significantly different at P>0.05.
Values are presented in Mean±Standard error of mean of 10 replicates

Table 2 shows the results on insecticidal effects of different concentrations of *Azadirachta indica* leaf powder on the weevils for ten days. The adult weevils subjected to different concentrations of *Azadirachta indica* leaf powder record no mortality as at day1 except in the highest concentration (0.5g). In day2, mortalities were recorded in 0.25g and 0.5g treatments while, the treatment with 0.1g started showing mortality from day3. The mortalities increased with duration of exposure to the plant material powder.

Table 2: Mortality caused by *A. indica* leaf powder treatment at varying concentrations against the weevils *C. maculatus*

	Plant Powder Concentration (grams)			
Days	0.1	0.25	0.5	Control (0.0)
1	0.00±0.00a*	0.00±0.00a	0.33±0.33a	0.00±0.00a
2	0.00±0.00a	0.33±0.33a	0.67±0.33b	0.00±0.00a
3	0.33±0.33a	0.67±0.67b	2.00±1.15c	0.00±0.00a
4	1.00±0.58a	1.33±1.33b	3.00±0.58c	0.00±0.00a
5	1.33±0.33a	2.33±0.88b	4.33±0.33c	0.00±0.00a
6	1.67±0.33a	3.00±0.58b	5.00±0.58c	0.00±0.00a
7	2.33±0.33a	4.33±0.33b	6.67±0.33c	0.00±0.00a
8	3.00±0.00a	5.00±0.00b	7.33±0.33c	0.00±0.00a
9	3.33±0.33a	5.67±0.33b	8.33±0.88c	0.00±0.00a
10	4.00±0.58a	6.67±0.33b	9.67±0.33c	0.00±0.00a

Number of insects used: 10 per replicate
*Values followed by the same superscript alphabets on the same row, are not significantly different at P>0.05.
Values are presented in Mean±Standard error of mean of 10 replicates.

For *Calotropis procera* powder treatments, mortality started on day2 in all the concentrations (Table 3). 0.5g had significantly (P< 0.05) highest mortality, as all the introduced weevils had died by the 8th day at this concentration. Therefore, treatment with *Calotropis procera* powder acted most effectively.

Table 3: Mortality caused by *C. procera* leaf powder treatment at varying concentrations against the weevils *C. maculatus*

	Plant Powder Concentration (grams)			
Days	0.1	0.25	0.5	Control (0.0)
1	0.00±0.00a*	0.00±0.00a	0.00±0.00a	0.00±0.00a
2	0.67±0.33b	0.67±0.33b	1.67±0.67c	0.00±0.00a
3	1.33±0.67b	1.33±0.67b	3.67±0.67c	0.00±0.00a
4	1.67±0.88b	2.67±0.88c	4.67±0.88d	0.00±0.00a
5	2.00±0.58b	3.00±1.51c	5.67±0.88d	0.00±0.00a
6	2.67±0.33b	4.33±1.33c	7.33±0.67d	0.00±0.00a
7	3.33±0.33b	5.00±1.00c	9.00±0.00d	0.00±0.00a
8	4.00±0.00b	6.00±1.00c	10.0±0.00d	0.00±0.00a
9	4.67±0.33b	6.67±0.67c	10.0±0.00d	0.00±0.00a
10	5.33±0.33b	7.33±0.33c	10.0±0.00d	0.00±0.00a

Number of insects used: 10 per replicate
*Values followed by the same superscript alphabets on the same row, are not significantly different at P>0.05.
Values are presented in Mean±Standard error of mean of 10 replicates

Table 4 highlighted results on mean daily mortality of *C. maculatus,* caused by the three different plant leaf powders at varying concentrations tested. *C. odorata* recorded significantly (P<0.05) lower mean daily insect mortality at all concentrations while *C. Procera* elicited significantly highest mortalities. *A. indica* show significant moderate mean daily mortality at all concentrations compared to *C. odorata* and *C. procera.*

Table 4: Daily mortality of *C. maculatus* caused by three different plant powders at varying concentration.

Plant powder	Plant Species		
Concentration (grams)	*C. odorata*	*A. indica*	*C. procera*
0.1	0.83±0.24a*	0.83±0.24a	2.57±0.55b
0.25	2.13±0.50a	2.93±0.75ab	3.70±0.81b
0.5	2.47±0.67a	4.73±1.02b	6.20±1.16c
CONTROL (0.0)	0.00±0.00a	0.00±0.00a	0.00±0.00a

*Mean values with the same superscript alphabets, in the row, are not significantly different at P >0.05

The results on lethal dosage of insecticidal effects of the three plant powders on the weevils are presented in table 5. The LD50 and LD90, each, varied considerably among the three plant materials tested, and respectively ranged from 0.63g in *C. procera* to 3.41g in *C. odorata* and 4.08g (*C. procera*) and 7.55g (*C. odorata*). Relative moderate concentrations of 1.56g and 4.42g of *A. indica*, were required to elicit 50 and 90% mortality, respectively, in the *C. maculatus* insects.

Table 5: Lethal dosages of insecticidal effects of the three plant powders on the weevils, *C. maculatus*

Plant Species	LD_{50}	LD_{90}	R^2	Regression equation
Calotropis procera	0.63	4.08	0.9963	y = 11.573x + 42.742
Azadirachta indica	1.56	4.42	0.988	y = 13.953x + 28.266
Chromolaena odorata	3.41	7.55	0.9429	y = 9.6571x + 17.071

Figure 1 shows the relationships between mortality in *C. maculatus* and concentrations of each leaf powder treatment. All the three treatments had high concentration dependent effect on mortality in the *C. maculatus* population tested.

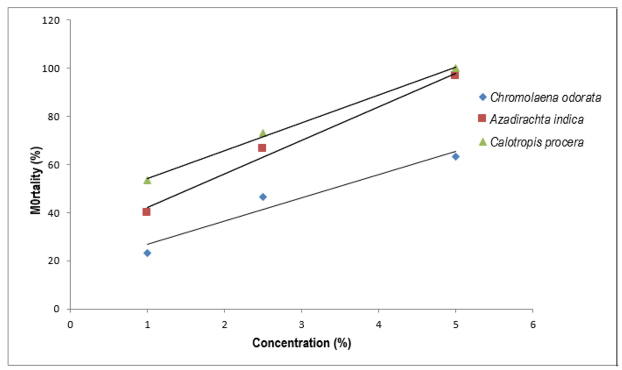

Figure 1: Correlation between mortality in *C. maculatus* and concentrations of each plant powder treatment.

Table 6 shows results of effects of the leaf powders on egg-laying capacity of the insects. The eggs laid by adult female weevils decreased with increase in leaf powder concentration. In all the powder treatments, the insects exposed to 0.1g of *C. odorata* leaf powder laid the highest number of eggs per grain (52.33±0.67) compared with the control experiment insects that laid 62.00±0.00 eggs/grain. Also the numbers of egg laid by adult weevils in the treatment with *A. indica* and *C. procera* were significantly higher (P<0.05), at the concentration 0.1g (46.67±2.60 and 37.33±2.19 eggs/grain respectively) compared with the other concentrations.

Table 6: Effects of the plant powders on egg laying capacity of the weevils, *C. maculatus*

Plant powder	Plant Species		
Concentration (grams)	*C. odorata*	*A. indica*	*C. procera*
0.1	52.33±0.67c*	46.67±2.60b	37.33±2.19a
0.25	48.67±0.88c	44.00±1.53b	27.33±1.45a
0.5	43.67±0.88c	39.33±2.33b	15.67±0.67a
CONTROL (0.0)	62.00±0.00ab	60.00±0.00a	65.00±0.00b

*Values followed by the same superscript alphabet, in the same row are not significantly different at P>0.05.
Values are presented in Mean±Standard error of mean of 10 replicates

DISCUSSION

The results of this study indicated that the three powders tested at varying levels of application were better than the untreated Control in protecting cowpea against *C. maculatus*. The insecticidal activities of botanicals were broad, variable and dependent on different factors like presence of bioactive chemicals which need to be identified, isolate and manufactured in the factory for pest management (Mulungu *et al*., 2007). The mortality rates of the weevils were found to increase with the treatment level and duration of application, that is to say, the mortality was dose dependent and probably the extract might have some bioactive phytochemicals within. This is in agreement with the findings of Al-Moajel (2004), who reported an increase in effectiveness of leaf powder of *L. inermis* with increase in concentration. *Calotropis procera* recorded the most effective insecticidal activity on the weevils followed by *Azadirachta indica* while *Chromolaena odorata* showed the least mortality effects. This may be due to the presence of high contents of alkaloids and phenols in *C. procera* leaf (Rajani *et al*., 2017) as these phytochemicals are known to possess significant insecticidal effects against a broad range of insect pests (Bakavathiappan *et al*., 2012). The results after all the three trials of different concentrations had shown clearly that the tested leaf powders of *C. procera, A. indica* and *C. odorata* affected the oviposition of *C. maculatus* in cowpea. The number of eggs laid by the insects was greatly reduced in samples treated with *C. procera* when compared with the untreated (Control). This is in conformity with the findings of Ahmed *et al.* (2006) who reported that aqueous extract of *C. procera* was effective against survivorship and fecundity in *Henosepilachna elaterii rossi*. There was higher mean number of *C. maculatus* eggs laid on untreated (i.e., Control) when compared to the treated cowpea seeds, and this indicates that the leaf powders of the three plant species tested effectively deterred Oviposition by *C. maculatus* on the cowpea grains. This effect was however, dosage-dependent, in which significantly lower number of *C. maculatus* eggs were laid on cowpeas treated with higher dosages of the leaf powder. More so, significantly, higher adulticidal and ovicidal activities demonstrated by *C. procera* may be due to high reported Alkaloid contents, as it act as protective against consumption by insects and vertebrate herbivores due to its bitterness and toxicities, in addition to other phytochemical components i.e., Tannin, Saponnin, Flavonoid and Alkaloid for *C. odorata, A. indica* and *C. procera* (Begum *et al*., 2010; Udebuaru *et al.,* 2015 and Sushree *et al*., 2017).

Conclusion

The results of this study indicated that leaf powders of *C. procera, A. indica* and *C. odorata*(leaf) applied at varying level were effective against stored cowpea weevil *Callosobrochus maculatus* and, thus may serve as promising source of insecticidal lead agents for the preservation of cowpea and similar grain produce attacked by weevils. However, there is need for phytochemical and histopathological screening of the tested leaf powders, to confirm the safety of such preserved grains for post storage human and/or animal consumption.

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