



Evaluation of the Insecticidal Properties of *Cassia alata* L. Against Cowpea Weevil, *Callosobruchus maculatus* Fab. (Coleoptera: Bruchidae)

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Abstract: A laboratory study was conducted to investigate the efficacy of powder and extract of *C. alata* on *Callosobruchus maculatus* Fab. damage in stored cowpea (*Vigna unguiculata* L.) seeds. The powder and the extract were evaluated on *C. maculatus* for mortality, oviposition and adult emergence effects. The long-term storage of the treated seeds was also investigated. All treatments recorded higher significant ($p < 0.05$) mortality than the experimental control. Highest mortality of 100 % was recorded in seeds treated with *C. alata* extract at the dosage level of 4.0 % v/w while highest mortality of 80.30 % was also recorded in seed treated with 4.0 g of *C. alata* powder. The powder and the extract significantly reduced oviposition and adult emergence in the treated seed. Adult emergence was totally suppressed in seeds treated with 3.0 % v/w and 4.0 % v/w. The powder and the extract also reduced infestation and subsequent damage of the treated seeds. No damage was recorded in seeds treated with 3.0 % v/w and 4.0 % v/w of the extract. The result obtained revealed that extract and powder of *C. alata* were effective in controlling *C. maculatus* and could be recommended as an alternative to synthetic insecticides.

Keywords: *Callosobruchus maculatus*; *Vigna unguiculata*; *Cassia alata*; mortality, oviposition

I. Introduction

Cowpea (*Vigna unguiculata* L. Walp.) is an important staple food crop and essential source of protein in many part of the world, especially in the tropical and subtropical regions. The production and storage of cowpea is majorly faced by the problem of insect pest known as *Callosobruchus maculatus* (Musa, *et al.*, 2009) which infest cowpea seed leading to economic losses as a result of reduction in weight and the presence of emergent holes on the seeds. The extent of damage by *C. maculatus* to pulse seeds is high both qualitatively and quantitatively (Atwal, 1976). *C. maculatus* accounts for over 90 % of the damage caused on stored cowpea seeds by insects (Caswell, 1981). Initial infestation of cowpea seeds occurs in the field just before harvest and the insects are carried into store where the population builds up rapidly (Huignar, 1985).

In an attempt to reduce the losses caused by these insects during storage, several control methods have been employed. Management of storage insect pest relies heavily on the use of chemical insecticides. However, despite the apparent success of these toxic synthetic chemicals, fear of problem of toxicity and chronic poisoning in man (Akunne and Okonkwo, 2006); sudden deaths, blindness, skin irritation pest resurgence in the ecosystem (Lowenberg-Deboer *et al.*, 2008, Omoleye, 2008); development of pesticide resistant population (Golob, 1980); deleterious effects on population of non-target organisms (White and Sinha, 1990); high cost of those chemicals and ozone layer depletion potential (Adedire, 2011), contamination of the environment (Adedire *et al.*, 2011) and non-availability abound.

In the developed countries, conventional fumigation technology is currently being scrutinized for many reasons such as ozone layer depletion potential of methyl bromide and carcinogenic concerns with phosphate (Adedire, 2002). In order to avoid the use of these

dangerous chemical insecticides, attention is currently being focused on the use of natural compounds of plant origin (Ramadhianto *et.al.* 2019, Maimunah *et. al.* 2019) for stored crop protection because they are readily biodegradable, often of low mammalian toxicity and thus pose low danger to the environment (Keita *et al.*, 2001).

The use of plant-derived bio-insecticide such as plant powder and oil that are relatively cheaper and ecologically friendly than the chemical insecticides are being encourage among resource-poor farmers in developing nations (Okosun and Adedire, 2010). This experiment was aimed at determining the effect of powder and extract of *Cassia alata* on cowpea weevils.

II. Methods

The study was conducted in Plant Science and Biotechnology Department Laboratory, Ekiti State University, Ado Ekiti, Nigeria.

2.1. Insect Culture

Parent stock of adult *Callosobruchus maculatus* was obtained from naturally infested cowpea seeds from Oba market, Ado Ekiti, Nigeria. The insects were reared on cleaned, uninfested cowpea seeds at $28\pm 2^{\circ}\text{C}$ and relative humidity of $75\%\pm 5\%$ inside plastic container covered with muslin cloth to disallow the escape of the insect as well as preventing the entry of intruding insects. The culture was maintained by replacing the devoured seeds with new uninfested seeds.

2.2. Collection and preparation test plant powders and extracts

Fresh leaves of *Cassia alata* were obtained from the compound of Ekiti State University Ado-Ekiti, Nigeria. The leaves were air-dried in the laboratory for a period of ten days to prevent it from decaying. The air-dried leaves were pulverized into fine powder using an electric blender. The powders were sieved and passed through 1 mm^2 mesh. Thereafter, some of the powders were measured separately into beakers and packed into thimbles and extracted with 250 mL of ethanol in a Soxhlet apparatus at 60°C . The extract was concentrated by removing the solvent using rotary evaporator. The resulting extract was further concentrated by air-drying by exposing to a slow blowing fan to remove traces of the solvent.

2.3. Bioactivity test of *C. alata* leaf powders on mortality of adult *C. maculatus*

Exactly 50 g of clean and dis-infested Ife brown variety of cowpea seed was weighed and leaf powders weighing 1.0, 2.0, 3.0 and 4.0 g were added to the cowpea seeds in 100 mL volume conical flasks. The cowpea seeds and powders were thoroughly mixed together to enhance uniform spreading of the powders. Untreated cowpea seed was also set up to serve as the control experiment. Twenty teneral adults of *C. maculatus* were introduced into each of the conical flasks and covered with muslin cloth held tightly in place with rubber band. Each of the treated and untreated control was replicated five times and laid in Complete Randomized Design (CRD). Insect mortality was observed at 24 h interval for a period 96 h. After every 24 h, the number of dead insects were counted and recorded. The insects were confirmed dead when there was no response to probing on the abdomen with a sharp pin.

2.4. Bioactivity test of *C. alata* leaf extracts on mortality of adult *C. maculatus*

Exactly 50 g of clean and dis-infested cowpea seed was weighed into 100 mL volume conical flask and treated with 1.0, 2.0, 3.0 and 4.0 % v/w seed extracts. Untreated seed was set

up to serve as the control experiment. The cowpea seeds and the extracts were thoroughly mixed with glass rod to enhance uniform coating of the extract on the seeds. Thereafter, the treated seeds were air-dried for a period of 2 h, after which 20 teneral adults of *C. maculatus* (0-1 days old) were introduced into the conical flask containing the treated and the control experiment. Five replicates were prepared for each treatment and the controls and laid in a Complete Randomized Design (CRD). The numbers of dead beetles were counted and recorded at 24 h interval for a period of 96 h. The insects were confirmed dead when there was no response to probing on the abdomen with a sharp pin

2.5. Bioactivity test of *C. alata* leaf powders on oviposition and adult emergence of *C. maculatus*

Exactly 50 g of dis-infested cowpea seeds were measured into each of 100 mL volume conical flask and *C. alata* leaf powders, each weighing 1.0, 2.0, 3.0 and 4.0 g were added to the cowpea seeds in each of the conical flask. The powder and the seeds were thoroughly mixed together to enhance uniform spreading of the powders on the cowpea seeds. Untreated seed was also set up to serve as the control experiment. Two pairs of newly emerged copulating adult *C. maculatus* were introduced into each conical flask and covered with muslin cloth held in place with rubber band. Each treated and untreated control was replicated five times and laid in Complete Randomized Design (CRD). The set up was left in the laboratory for 10 days after which the insects were removed and the numbers of eggs laid were counted and recorded. Thereafter, the experimental set-up was kept undisturbed in the wooden cage till the emergence of adults. The number of adults that emerged were counted and recorded

2.6. Bioactivity test of *C. alata* leaf extracts on oviposition and adult emergence of *C. maculatus*

Exactly 50 g of dis-infested cowpea seeds were measured into each of 100 mL volume conical flask and *C. alata* leaf extracts, each weighing 1.0, 2.0, 3.0 and 4.0 % v/w were added to the bean seeds in each of the conical flask. The extracts and the seeds were thoroughly mixed together with the aid a glass rod to enhance uniform coating of the extracts on the bean seeds. Untreated bean seed was also set up to serve as the control experiment. Two pairs of newly emerged copulating adult *C. maculatus* were introduced into each conical flask and covered with muslin cloth held in place with rubber band. Each treated and untreated control was replicated five times and laid in Complete Randomized Design (CRD). The set up was left in the laboratory for 10 days after which the insects were removed and the numbers of eggs laid were counted and recorded. Thereafter, the experimental set-up was kept undisturbed in the wooden cage till the emergence of adults. The number of adults that emerged were counted and recorded

2.7. Effect of leaf powders of *C. alata* on grain damage.

Leaf powders weighing 1.0, 2.0, 3.0 and 4.0 g were each added to 50 g clean and dis-infested bean seeds in a transparent plastic container. The container was thoroughly shaken to enhance the spread of the powders on the seeds. Thereafter, 20 adult insects were introduced into the container. A control without any powder was included in the set-up. Each treated and control was replicated five times. Each plastic container was covered with muslin cloth held tightly in place with rubber band to prevent the escape of insects and to facilitate adequate ventilation. The experimental set-ups were kept in a wooden cage in the laboratory for 90 days.

After 90 days the seeds were assessed for seed damage and weight loss. Percentage seed damage was determined using the formula below

$$\% \text{ damage} = \frac{\text{No of seed damaged} \times 100}{\text{Total no of seeds}}$$

2.8. Data analysis

All data obtained were analysed by analysis of variance (ANOVA), while Turkey test was used for ranking the means. Mortality data were first normalized by arcsine transformation before analysis. The values in the tables and text are back-transformed data obtained by squaring the sine of the number.

III. Results

3.1. Toxicity of *C. alata* leaf powders on adult *C. maculatus*

Mortality of *C. maculatus* in powder treated seeds was significantly ($p < 0.05$) different from weevil mortality in untreated seed (Table 1). Adult weevil mortality increased with period of exposure. The highest mortality of 80.30 % was recorded in cowpea seeds treated with 4.0 g of powder at 96 h post treatment and it was significantly different from weevil mortality of 72.15 %, 46.25 %, 32.14 % and 3.12 %.

Table 1. Effects of *C. alata* leaf powders on mortality of adult *C. maculatus*

Treatment (g)	Percentage mortality at hours post-treatment			
	24	48	72	96
Untreated	0.00±0.00 ^d	0.00±0.00 ^e	0.00±0.00 ^e	3.12±1.22 ^e
1.0	12.20±1.22 ^{cd}	16.30±2.34 ^d	22.15±2.19 ^d	32.14±3.13 ^d
2.0	16.30±2.35 ^c	23.20±3.75 ^c	30.75±1.25 ^c	46.25±2.35 ^c
3.0	28.15±1.25 ^b	36.00±1.25 ^b	58.15±1.44 ^b	72.15±1.39 ^b
4.0	36.75±1.31 ^a	48.15±3.15 ^a	62.22±1.42 ^a	80.30±1.25 ^a

Each value is a mean ± standard error of five replicates. Means within the same column follow by the same letter(s) are not significantly different at $p > 0.05$ using Tukey test.

3.2. Toxicity of *C. alata* leaf extracts on adult *C. maculatus*

Weevil mortality in extract treated seeds differed significantly ($p < 0.05$) from weevil mortality in the untreated (control) (Table 2). All treatments by 96 h showed weevil mortality ranging from 58.10 % to 100 %. Weevil mortality increased with length of exposure. The most effective dosage is 4.0 % v/w evoking 100 % mortality by 72 h and 96 h of exposure respectively followed by 3.0 % v/w evoking 72.15 % by 96 h our of exposure.

Table 2. Effects of *C. alata* leaf extracts on mortality of adult *C. Maculatus*

Treatment (% v/w)	Percentage mortality at hours post-treatment			
	24	48	72	96
Untreated	0.00±0.00 ^d	0.00±0.00 ^d	2.50±1.22 ^d	2.50±1.22 ^e
1.0	22.00±3.05 ^{cd}	38.20±2.44 ^c	54.25±2.19 ^c	58.10±3.32 ^d
2.0	26.15±1.33 ^c	42.20±3.16 ^b	60.10±2.02 ^{bc}	62.20±2.12 ^c
3.0	35.24±1.44 ^b	45.15±2.88 ^b	66.05±1.71 ^b	80.30±3.39 ^b
4.0	48.30±2.53 ^a	75.25±2.32 ^a	100.00±1.15 ^a	100.00±1.15 ^a

Each value is a mean ± standard error of five replicates. Means within the same column follow by the same letter(s) are not significantly different at $p > 0.05$ using Tukey test.

3.3. Toxicity of *C. alata* leaf powders on oviposition and adult emergence of *C. maculatus*

The *C. alata* leaf powder effectively reduced oviposition by the weevils (Table 3). The number of eggs laid by the weevils on treated cowpea seeds was significantly lower ($p < 0.05$) than the number of eggs laid on untreated (control) seeds. The percentage adult emergence in the untreated seeds was significantly different from the emergence in the treated seeds

Table 3. Effects of *C. alata* leaf powders on oviposition and adult emergence of *C. Maculatus*

Treatment (g)	No. of eggs l	% adult emergence
Untreated	32.25±1.25 ^a	88.30±3.25 ^a
1.0	28.15±1.44 ^a	34.20±7.50 ^b
2.0	24.35±4.11 ^c	25.15±3.15 ^c
3.0	16.45±3.25 ^d	18.80±6.25 ^d
4.0	14.75±2.24 ^d	15.30±1.33 ^e

Each value is a mean ± standard error of five replicates. Means within the same column follow by the same letter(s) are not significantly different at $p > 0.05$ using Tukey test.

Table 4. Effects of *C. alata* leaf extracts on oviposition and adult emergence of *C. maculatus*

Treatment (% v/w)	No. of eggs l	% adult emergence
Untreated	30.00±1.66 ^a	87.00±3.35 ^a
1.0	15.35±1.40 ^b	10.20±2.45 ^b
2.0	12.10±2.13 ^c	5.00±1.55 ^c
3.0	10.50±0.81 ^d	0.00±0.00 ^d
4.0	10.20±2.11 ^d	0.00±0.00 ^d

Each value is a mean ± standard error of five replicates. Means within the same column follow by the same letter(s) are not significantly different at $p > 0.05$ using Tukey test.

3.4. Toxicity of *C. alata* leaf extracts on oviposition and adult emergence of *C. maculatus*

The *C. alata* leaf extracts effectively reduced oviposition by the weevils (Table 4). The number of eggs laid by the weevils on treated cowpea seeds was significantly lower ($p < 0.05$) than the number of eggs laid on untreated (control) seeds. There was no egg laid in seeds treated with 3.0 and 4.0 % v/w extract dosage and hence, no adult emerged. The percentage adult

emergence in the untreated seeds was significantly different from the emergence in the treated seeds.

Table 5. Effects of *C. alata* leaf powders on long term storage of cowpea seed.

Treatment (g)	Mean no of seed	% seed damage	% weight loss
Untreated	190.25	78.20±3.04 ^a	25.50±1.50 ^a
1.0	192.00	30.15±2.55 ^b	10.25±1.33 ^b
2.0	188.10	20.40±1.12 ^c	8.18±2.25 ^c
3.0	190.20	10.10±2.50 ^d	5.25±1.38 ^d
4.0	189.60	10.05±1.33 ^d	5.00±2.20 ^d

Each value is a mean ± standard error of five replicates. Means within the same column follow by the same letter(s) are not significantly different at $p > 0.05$ using Tukey test.

3.5. Effects of *C. alata* leaf powders on long term storage of cowpea seeds

There was significant reduction in seed damage and weight loss in powder treated seeds compared to the untreated (control) seeds (Table 5). The feeding activity of larvae of *C. maculatus* on the untreated seeds caused significant ($p < 0.05$) reduction in the weight of the seeds compared with the treated seeds. The untreated seeds caused 78.20 % seed damage which is significantly different from seeds treated with 1.0, 2.0, 3.0 and 4.0 % v/w which caused 30.15, 20.40, 10.10 and 10.05 % respectively.

3.6. Effects of *C. alata* leaf extracts on long term storage of cowpea seeds

There was significant reduction in seed damage and weight loss in extract treated seeds compared to the untreated (control) seeds. The seeds treated with 3.0 % v/w and 4.0 % v/w extracts dosage completely prevented infestation and subsequent damage of cowpea seeds for a period of 90 days (Table 6). There were neither weight loss nor seed damages recorded in the aforementioned dosage levels. The seeds treated with 1.0 and 2.0 % v/w dosage also significantly reduced infestation but the percentage damage is 20.20 % and 5.60 % respectively.

Table 6. Effects of *C. alata* leaf extracts on long term storage of cowpea seed.

Treatment (g)	Mean no of seed	% seed damage	% weight loss
Untreated	190.40	80.15±2.10 ^a	24.00±2.22 ^a
1.0	193.20	20.20±1.33 ^b	10.23±1.60 ^b
2.0	192.30	5.60±1.15 ^c	0.00±0.00 ^c
3.0	189.20	0.00±0.00 ^d	0.00±0.00 ^c
4.0	191.10	0.00±0.00 ^d	0.00±0.00 ^c

Each value is a mean ± standard error of five replicates. Means within the same column follow by the same letter(s) are not significantly different at $p > 0.05$ using Tukey test.

IV. Discussion

The use of botanicals to protect seeds and grains from insect pest infestation has been under practice for long (Ukeh, 2008). This present study revealed that extracts and powders of *Cassia alata* are insecticidal against *Callosobruchus maculatus* and can be used to control the bruchid. The result of the present study agrees with the previous study carried out by Musa *et al.* (2000), Moses and Dorathy (2011), Ojo and Ogunleye (2013), Adedire *et al.* (2011),

Obembe (2017), Obembe and Ogunbite (2017) who all reported various plant parts with efficacy against cowpea weevils, *C. maculatus*

The mortality of weevils recorded in this present findings shows that *C. alata* leaf powder and extract have insecticidal properties capable of controlling pest of stored cowpea seeds. The results on the use of leaf powder agrees with the findings of Ojo and ogunleye (2013) who achieved 83.75 % mortality of *C. maculatus* using 2.5 % w/w *Piptadeniastrum afrikanum* leaf powder and that of Deme *et al.*(2019), who reported that leaf powder of *C. alata* proved effective for preserving cowpea seeds from infestation by *C. maculatus*.

The effects of the extracts and powders on the bruchids could be linked with contact toxicity. Most insects breathe by means of trachea which usually opens at the surface of the body through the spiracle. There is likely hood of the spiracle being blocked by the extract thereby leading to suffocation (Adedire *et al.*, 2011). In the same vein the insecticidal potential of the powder may be as a result of contact toxicity coupled with the blockage of the spiracles by the powders.

The powders and extracts of the test plant significantly reduced oviposition in *C. Maculatus* when compared with the untreated (control) cowpea seeds. Also, the extracts inhibited adult emergence at the dosage level of 3.0 % and 4.0 % v/w after 30 days of exposure. The potency of plant powders and extracts in reducing or completely inhibiting oviposition by female bruchids and mortality of the developmental stages has been reported by number of authors (Boeke *et al.*, 2001). The effect of the extract on oviposition in this study could be associated with respiratory impairment which probably affects the process of metabolism and consequently other system on the body of the insect (Osisioogu and Agbakwuru, 1978; Onolemhohem and oigiagbe, 1991). The extracts inhibits locomotion, hence, the bruchids were unable to move freely thereby affecting mating activities. The inability of the eggs to stick to the cowpea seeds due to the presence of extracts also reduced adult emergence arising from egg mortality.

Phytochemical screening of *C. alata* revealed the presence of alkaloid, tannins, saponins, phenol, flavonoids, anthraquinones and cardiac glycoside (Mahmood and Doughari (2008). Karamanole *et al.* (2011) reported that tannins exert their action by combination of mechanisms that include iron cheration and enzyme inhibition. However the exact mechanism behind the observed action of the powders and extracts is not yet known. Dolui *et al.* (2012) reported that tannins combine with protein to inhibit enzyme activity and reduces the availability of protein in haemolymph in insects. Some researchers reported that saponins increases insect mortality, lowers food intake, weight reduction, retardation in development and decrease reproduction (Chaleb, 2010)

The ability of some plant powders and extracts to protect cowpea seeds from damage by insect pest over a long storage period had been tested with positive results. It has earlier been reported that extracts from crude palm kernel and rice bran offered full protection to cowpea seeds from *C. maculatus* infestation for a period of 4 to 5 months (Pereira,1983, Shaaya *et al.*, 1997).

V. Conclusion

The results obtained from this study confirmed that *C. alata* leaf powders and extracts were highly effective in controlling the population of *C. maculatus* in treated seeds and could serve as alternative to synthetic insecticides for use by resource poor farmers. This is because it is cheap, biodegradable and ecologically friendly, among others.

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