

Bioactivity of *Anacardium occidentale* (L) and *Allium sativum* (L) Powders and Oils Extracts against Cowpea Bruchid, *Callosobruchus maculatus* (Fab.) [Coleoptera: Chrysomelidae]

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Abstract

The powders and oils extracts of *Anacardium occidentale* (L.) seeds and *Allium sativum* (L.) bulbs were tested as contact insecticides against the cowpea bruchid, *Callosobruchus maculatus* (Fab.) in cowpea seeds. The powders were incorporated at rates 1, 2.5 and 5/20g of cowpea seeds and acetone extracts of the plants were also applied at 0.5, 1 and 1.5ml per 20g of cowpea seeds to assess contact mortality of adult insect, oviposition, adult emergence and damage assessment. The results obtained showed that at 72 hours; 1, 2.5 and 5/20g of cowpea seeds evoked 63.3, 80 and 100% adult mortality of the cowpea bruchid while the corresponding results for *A. sativum* were 60.7, 73.3 and 100% mortality respectively. All concentration of acetone extracts that were used evoked 100% mortality of *C. maculatus* after 72 hours of post treatment. Complete protection of seeds and complete inhibition of adult emergence in both powders and extracts of *A. occidentale* and *A. sativum* were achieved. The results obtained from this research revealed that powders and extracts of *A. occidentale* seeds and *A. sativum* bulbs were effective in controlling cowpea bruchid, *C. maculatus* in stored cowpea seeds.

Keywords: *Callosobruchus maculatus*, *Anacardium occidentale*, *Allium sativum*, Bioactivity, Biopesticide, Cowpea seed, Weevil Perforation Index

1. Introduction

Cowpea, *Vigna unguiculata* occupy a prominent place in the nutrition of Nigerians because their edible seeds form cheap alternative source of dietary protein (Ofuya, 2001; Ofuya and Adedire, 2004; Adedire *et al.*, 2011). Cowpea seeds are veritable source of minerals and vitamins such as calcium, thamine and riboflavin (Nelson, 1991). Cowpea have therefore been described as “poor man’s meat”.

The major problem facing grains storage in Nigeria and other Africa countries are several insect pests including weevils, beetles and moths, leading to loss in weight and seeds quality (Adedire, 2001; Udo, 2005; Akinkulore *et al.*, 2006; 2009; Adedire *et al.*, 2011). Bruchid especially those belonging to the genus *Callosobruchus* can infest stored cowpea seeds (Ofuya and Bamigbola, 1991) and this insect pest has been recognized as a constraint to food security in African (Markham *et al.*, 1994). Apart from contamination of food grains by dead insects, pupae and larval cocoons, their integument has been found to contain various carcinogenic compound such as ethyl, methyl and methoxy quinines which can not be denatured by boiling or baking (Zehrer, 1980; Adedire *et al.*, 2011).

The effective measures in food pest control is the use of synthetic insecticides, which are very expensive, unavailable at critical period and they are sometimes constitute health hazards to consumers (Adedire, 2001; Ofuya, 2001; Oni and Ileke, 2008; Ileke and Oni, 2011; Oni, 2011). This led to continuous research towards substitution of synthetic insecticides with plant products as a cheaper and eco-friendly safer means of controlling insect pest's infestation of stored cowpea seeds (Adedire *et al.*, 2011). Attention is being given to the use of edible plants materials as grains protectant (Ivbijaro and Agbaje, 1986; Adedire and Lajide, 2003; Akinkulore *et al.*, 2009; Ileke and Oni, 2011) and the tropic is well endowed with these plant species some of which are also used for medicinal purpose (Adedire and Lajide, 2003; Ileke and Oni, 2011)

Garlic, *Allium sativum*, is a cosmopolitan plant. It is an herbaceous biennial plant, characterized by its penetrating fragrance. It is a seasoning for soups and sauces and its oil is a flavoring agent. Medically, the bulb is used for reviving convulsive patients, cure for hemorrhoids and diuretic in Nigeria (Dike and Mbah, 1992). The pesticidal activities of garlic as a repellent, antifeedant, bactericide, fungicide and nematicide have been reported (Graigne *et al.*, 1985; Oparaeke and Dike, 1996). Cashew, *Anacardium occidentale*, fruit consists of a soft, shing, pear-shaped, swollen, juice basal portion commonly known as a 'cashew apple'. The apple is sweet when fully ripe. The seeds are inedible when raw and must be cooked or roasted to drive off the volatile oil before it is opened or shelled (Anon, 1958; Oparaeke and Bunmi, 2006). The medicinal value of these plants has long been known. Therefore, the plants were believed to be safer as biopesticides for stored food protection (Arannilewa *et al.*, 2006; Oparaeke and Bunmi, 2006; Adedire *et al.*, 2011). This led to the present research.

2. Materials and Methods

2.1 Insect cultures

Parent stock of *Callosobruchus maculatus* was obtained from established laboratory culture at Environmental Biology and Fisheries Laboratory, Adekunle Ajasin University, Akungba Akoko, Ondo State, Nigeria. This was reared on disinfested cowpea seeds collected from Seed Unit, Agricultural Development Programme, Ado Ekiti, Ekiti State, Nigeria. The seeds were cleaned of foreign matter and disinfested by keeping in freezer at -5°C for 7 days. This is because all the life stages, particularly the eggs are very sensitive to cold (Koehler, 2003). The disinfested seeds were then air dried in the laboratory to prevent mouldiness (Adedire *et al.*, 2011) before introduction of insects. They were placed in Kilner jars and covered with muslin cloth. The jars were placed in insect rearing cages at ambient temperature of $30\pm 3^{\circ}\text{C}$ and $70\pm 5\%$ relative humidity.

Cowpea, *Vigna unguiculata* seeds used for the experiment were also disinfested as described above before it was stored in plastic containers with tight lids disinfested by swabbing with 90% alcohol.

2.2 Collection of plant materials

The plants materials used in the present study were *Anacardium occidentale* and *Allium sativum*. Cashew nuts were sourced fresh from Ita Merin farm at Igbara-odo Ekiti, Ekiti State, Nigeria and bulbs of Garlic was purchase fresh at Oja Oba market, Igbara-odo Ekiti, Ekiti State, Nigeria. The nuts were sun dried for three days to allow for safe and easy cracking to remove the nuts without crushing them (Adedire *et al.*, 2011). These plant materials were later dried in an open laboratory and ground into very fine powder using an electric blender (Supermaster®, Model SMB 2977, Japan). The powders were further sieved to pass through 1mm^2 perforations (Ileke and Oni, 2011) before it was stored in separate plastic containers with tight lids and stored in a refrigerator at 4°C prior to use.

Acetone extracts of *A. occidentale* and *A. sativum* seeds and bulbs respectively were carried out using cold extraction method. About 250g of *A. occidentale* and *A. sativum* powders were soaked separately in an extraction bottle containing 70% acetone. The mixture was stirred occasionally with a glass rod and extraction was terminated after 72 hours. Filtration was carried out using a double layer of Whatman No. 1 filter papers and acetone evaporated using a rotary evaporator at 30 to 40°C with rotary speed of 3 to 6 rpm for 8 hours (Udo, 2011). The resulting extract was air dried in order to remove traces of solvent.

2.3 Toxicity of plant powders

Portions of 1, 2.5 and 5g of each plant powders corresponding to 5, 12.5 and 25% w/w concentration (Fatope *et al.*, 1995) were weighed and each added to a 20g of clean undamaged and uninfested cowpea seeds in 250ml plastic containers. The seeds in the controls contained no plant powders. The containers with their contents were gently shaken to ensure thorough admixture of the cowpea seeds and treatment powders. Ten pairs of a General adults *C. maculatus* were introduced to each of the containers and covered. The insects were sexed based on the observation reported by Odeyemi and Daramola (2000). Three replicates of the treatments and untreated controls were laid out in Complete Randomized Design. The adult mortality was assessed after every 24 hours for 96

hours. On day 5, all insects, both dead and alive were removed from each container and oviposition were noted before returned the seeds to their respective containers. Adult emergence (F1) was then recorded at 6 weeks. The percentage adult emergence was calculated thus:

$$\% \text{ Adult emergence} = \frac{\text{No of adults emerged}}{\text{No of eggs laid}} \times \frac{100}{1}$$

At day 42, the cowpea seeds were re-weighed and the % loss in weight was determined and recorded. The percentage seed damaged were also evaluated as follows:

$$\% \text{ seed damage} = \frac{\text{Number of seeds damaged}}{\text{Total number of seeds}} \times \frac{100}{1}$$

Weevil Perforation Index (WPI) of the weevil to cowpea seeds were calculated using the methods of Fatope *et al.*, (1995); Adedire and Ajayi (1996) respectively. The weevil perforation is define as follows:

$$\text{WPI} = \frac{\% \text{ treated cowpea seeds perforated}}{\% \text{ control cowpea seeds perforated}} \times \frac{100}{1}$$

2.4 Toxicity of plants extracts

The toxic effect of plants oils on adult *C. maculatus* was accomplished using 250ml plastic containers containing 20g of cowpea seeds with concentration of 0.5, 1 and 2% w/v plant extracts in acetone. The oils were thoroughly mixed with the aid of a glass rod and agitated for 5-10 mins to ensure uniform coating. The containers were left open for 30 mins so as to allow traces of acetone to evaporate off. Ten copulating pairs of *C. maculatus* were introduced into the containers and mortality was observed daily for 4 days. Cowpea seeds that were solvent treated served as the control experiment (Arannilewa *et al.*, 2006). Three replicates of the treatments and untreated controls were laid out in Complete Randomized Design. Adult insects were considered dead where no response was observed after probing them with forceps.

The effect of extracts on adult emergence was recorded after 42 days. At the end of 42 days, the extent of weight loss as a result of weevil development, damaged to seeds and weevil perforation index (WPI) were calculated as described under toxicity of plant powders.

2.5 Statistical analysis

Data were subjected to analysis of variance and where significant differences existed, treatment means were separated using the New Duncan's Multiple Range Test (Zar, 1984).

3. Results

3.1 Toxicity of plants powders to *Callosobruchus maculatus*

Table 1 compares the effectiveness of two plants powders on mortality of cowpea bruchid. All the plants powders at different concentration had above 60% mortality when compared with untreated which had no mortality after 72 hours of post treatment. Cashew seed powders evoked 63.3%, 80% and 100% mortality of adult bruchid at rates of 1/20g, 2.5/20g and 5/20g of cowpea seeds after 72 hours of post treatment respectively. There was no significant different ($P>0.05$) in mortality of adult bruchid among the treatment with *A. occidentale* powder evoking 100% mortality irrespective of concentration after 96 hours of post treatment. In similar vein, *A. sativum* caused 60.7%, 73.3% and 100% mortality of *C. maculatus* at rates of 1/20g, 2.5/20g and 5/20g of seeds at 72hours of post treatment. Garlic powder was able to produced 100% mortality of bruchid at rates of 2.5/20g and 5/20g of cowpea seeds apart from cowpea seeds treated at rate of 1g that evoked 83.3% mortality of bruchid after 4 days of exposure.

3.2 Effect of plants powders on oviposition and adult emergence of *C. maculatus*

All the plants powders significantly reduced the number of eggs laid by *C. maculatus* (Table 2). The *A. occidentale* powders at all concentration completely prevented the emergence of adult *C. maculatus* when compared with the control that recorded 90 adult emergence. Seeds treated with *A. sativum* had no adult emergence apart from seeds treated at rate 1g that recorded 15 adult emergence.

3.3 Toxicity of plants Extracts to *Callosobruchus maculatus*

Significant mortality of adult *C. maculatus* ($P<0.05$) were observed at all concentration of the extract after 48 hours of post treatment with *A. occidentale* at 1.5ml per 20g of cowpea seeds evoking 100% adult mortality after

24 hours of post treatment (Table 3). All extracts of the two plants at different concentrations were able to evoked 100% mortality of adult bruchid apart from the extract of *A. sativum* at 0.5ml per 20g of cowpea seeds that produced 80% adult mortality of *C. maculatus* after 72 hours of post treatment.

3.4 Effect of plants Extracts on oviposition and adult emergence of *C. maculatus*

All the plant extracts significantly reduced the number of eggs laid by cowpea bruchid compared to untreated cowpea seeds that had 84 eggs (Table 4). The percentage adult emergence in the untreated was significantly different ($P>0.05$) from all the treated cowpea seeds that recorded no adult emergence. The extract completely inhibited the F1 development of *C. maculatus*.

3.5 Damage assessment of cowpea seeds treated with plant powders and extracts

Cowpea seeds treated with plants powders showed no significant different ($P.0.05$) in the reduction of damaged caused by *C. maculatus* (Table 5). However, the weevil perforation index (WPI) of 5.2 recorded on cowpea seeds treated with powder of *A. sativum* treated at rate 1g was significantly different ($P>0.05$) from weevil perforation index of the untreated cowpea seeds. In similar vein in Table 6, there was neither seed damage nor weight loss recorded in the treated cowpea seeds at all concentration. The weevil perforation index (WPI) was zero for all the tested plants extracts compared to untreated cowpea seeds.

4. Discussion

Cashew, *A. occidentale* seed powder and oil completely killed adult *C. maculatus* on stored cowpea seeds. This insecticidal effect was in agreement with Dungum *et al.* (2005), Oparaeke and Bunmi (2006) and Adedire *et al.* (2011). The mode of action of this plant powder oil could be as a result of the powder and oil coating the treated cowpea seeds which prevented contact between the seeds and the bruchid resulting into starvation. They also blocked the spiracles which may lead to suffocation as suggested by (Hall and Harman, 1991; Adedire *et al.*, 2011). The insecticidal activity of *A. occidentale* oil extract could be linked to the presence of anacardic acid and cardinal (Rehm and Espig, 1991). Others include quercetin and kaempferol glycosides (Oliver-Bever, 1986).

Garlic powder and oil may have been very toxic to *C. maculatus* as a result of the strong choky odours it produce which may disrupt normal respiratory activity of insect, thereby leading into asphyxiation and subsequent death (Adedire and Ajayi, 1996). This result agrees with the finding of Bhatnager-Thomas and Pal (1974) who reported the effectiveness of garlic oil against *Musca dominica* and *Trogoderma granarium*. Nasseh (1990) reported the insecticidal activities of *A. sativum* for the control of *Epilachna verivestis*. Arannilewa *et al.* (2006) reported 85% adult mortality of *Sitophilus zeamais* and 9 emerged adult maize weevil on treated maize grains with methanol extract of *A. sativum*.

The reduced oviposition and no adult emergence observed in all the treated seeds with oils extract could be as a result of high adult mortality of *C. maculatus*. The oil inhibits locomotion which affect mating activities an effect that had been reported by many authors (Okonkwo and Okoye, 1996; Adedire, 2002, Akinkurolere *et al.*, 2006, Oni and Ileke, 2008; Adedire *et al.*, 2011; Ileke and Oni, 2011). The few eggs that were laid are unable to stick to the surface of the seeds as a result of oil presence also prevents progeny production. The lower F1 adult bruchid in cowpea seeds treated with *A. sativum* powder at rate of 1g may be due to concentration used. The ovicidal and larvicidal of the plants powders and oils may be due to the present of lipophilic compound (Richards, 1978). Don-Pedro (1990) reported that eggs mortality may be as a result of physical properties of the oils resulting into blockage of respiratory pores (spiracle) while the larvicidal properties of the plants oils could be as a result of both physical and chemical toxicity of oils (Ivbijaro, 1984).

The plants powders and oils completely protected the seeds from been damaged by *C. maculatus*. The protectant ability of these plants powders and oils were highly remarkable. This may be as a result of ovicidal and larvicidal properties of the tested plant that completely killed few eggs that were laid and also prevent the few once that hatched into larva from going into pupa stage.

5. Conclusion

The result obtained from this study confirmed that powders and oils of *A. occidentale* seed and *A. sativum* can be used as biopesticides against *C. maculatus* especially when weevil perforation index is taken into consideration.

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Table 1. Effect of plants powders on percentage mortality of *Callosobruchus maculatus* adults

Plant powder	g/20g cowpea	Mean % mortality \pm S.E. at 24h to 96h Post treatment			
		24	48	72	96
<i>A. occidentale</i>	1.0	20.0 \pm 0.0b	46.7 \pm 0.3b	63.3 \pm 0.0b	100.0 \pm 0.0c
	2.5	33.3 \pm 0.0c	53.3 \pm 0.0b	80.0 \pm 0.0c	100.0 \pm 0.0c
	5.0	53.3 \pm 0.0d	73.3 \pm 0.0c	100.0 \pm 0.0d	100.0 \pm 0.0c
<i>A. sativum</i>	1.0	13.3 \pm 0.0b	46.7 \pm 0.3b	60.0 \pm 0.0b	83.3 \pm 0.0b
	2.5	30.0 \pm 0.0c	50.0 \pm 0.0b	73.3 \pm 0.0c	100.0 \pm 0.0c
	5.0	50.0 \pm 0.0d	70.0 \pm 0.0c	100.0 \pm 0.0d	100.0 \pm 0.0c
Control	0.0	0.0 \pm 0.0a	0.0 \pm 0.0a	0.0 \pm 0.0a	0.0 \pm 0.0a

Each value is a mean of \pm standard error of three replicates. Means within the same column followed by the same letter(s) are not significantly different at ($P>0.05$) from each other using New Duncan Multiple Range Test.

Table 2. Effect of Plants powders on oviposition and adult emergence of *Callosobruchus maculatus*

Plant powders	g/20g cowpea	No of Eggs	%Adults Emergence
<i>A. occidentale</i>	1.0	10.0 \pm 0.0a	0.0 \pm 0.0a
	2.5	10.3 \pm 0.0a	0.0 \pm 0.0a
	5.0	10.0 \pm 0.0a	0.0 \pm 0.0a
<i>A. sativum</i>	1.0	26.7 \pm 0.3b	14.8 \pm 0.1b
	2.5	10.0 \pm 0.0a	0.0 \pm 0.0a
	5.0	10.3 \pm 0.0a	0.0 \pm 0.0a
Control	0.0	86.7 \pm 0.3c	89.7 \pm 0.3c

Each value is a mean of \pm standard error of three replicates. Means within the same column followed by the same letter(s) are not significantly different at ($P>0.05$) from each other using New Duncan Multiple Range Test.

Table 3. Effect of plants extract on percentage mortality of *Callosobruchus maculatus* adults

Plant powder	Conc. ml/20g	Mean % mortality \pm S.E. at 24h to 96h Post treatment			
		24	48	72	96
<i>A. occidentale</i>	0.5	46.7 \pm 0.3bc	76.7 \pm 0.3bc	100.0 \pm 0.0b	100.0 \pm 0.0b
	1.0	60.0 \pm 0.0d	80.0 \pm 0.0c	100.0 \pm 0.0b	100.0 \pm 0.0b
	1.5	100.0 \pm 0.0e	100.0 \pm 0.0b	100.0 \pm 0.0b	100.0 \pm 0.0b
<i>A. sativum</i>	0.5	30.0 \pm 0.0b	66.7 \pm 0.3b	100.0 \pm 0.0b	100.0 \pm 0.0b
	1.0	53.3 \pm 0.0c	70.0 \pm 0.0bc	100.0 \pm 0.0b	100.0 \pm 0.0b
	1.5	70.0 \pm 0.0d	100.0 \pm 0.0d	100.0 \pm 0.0b	100.0 \pm 0.0b
Control	0.0	0.0 \pm 0.0a	0.0 \pm 0.0a	0.0 \pm 0.0a	0.0 \pm 0.0a

Each value is a mean of \pm standard error of three replicates. Means within the same column followed by the same letter(s) are not significantly different at ($P>0.05$) from each other using New Duncan Multiple Range Test.

Table 4. Effects of Plants extract on oviposition and adult emergence of *Callosobruchus maculatus*

Plant powders	Concentration ml/20g	No of Eggs	%Adults Emergence
<i>A. occidentale</i>	0.5	10.3± 0.0a	0.0± 0.0a
	1.0	10.0± 0.0a	0.0±0.0a
	1.5	10.0± 0.0a	0.0±0.0a
<i>A. sativum</i>	0.5	20.0± 0.0a	0.0±0.0a
	1.0	14.7± 0.3a	0.0 ±0.0a
	1.5	10.3± 0.0a	0.0± 0.0a
Control	0.0	83.3± 0.0b	86.7± 0.3b

Each value is a mean of ± standard error of three replicates. Means within the same column followed by the same letter(s) are not significantly different at (P>0.05) from each other using New Duncan Multiple Range Test.

Table 5. Protectantability of the plants powders on cowpea seeds

Plant part powders	g/20g cowpea	Mean total no of seeds	Mean no of damaged seeds	Mean % seed damaged	% weight loss	Weevil Perforation Index (WPI)
<i>A. occidentale</i>	1.0	93.3	0.0± 0.0a	0.0± 0.0a	0.0± 0.0a	0.0± 0.0a
	2.5	93.3	0.0± 0.0a	0.0± 0.0a	0.0± 0.0a	0.0± 0.0a
	5.0	94.7	0.0± 0.0a	0.0± 0.0a	0.0± 0.0a	0.0± 0.0a
<i>A Sativum</i>	1.0	94.3	4.0 ±0.0a	4.2± 0.4a	0.7 ±0.3a	5.2 ±0.1a
	2.5	94.7	0.0± 0.0a	0.0± 0.0a	0.0± 0.0a	0.0± 0.0a
	5.0	93.3	0.0± 0.0a	0.0± 0.0a	0.0± 0.0a	0.0± 0.0a
Control	0.0	94.3	76.7±0.3b	81.9±0.2b	84.7±0.3b	50.0±0.0b

Each value is a mean of ± standard error of three replicates. Means within the same column followed by the same letter(s) are not significantly different at (P>0.05) from each other using New Duncan Multiple Range Test.

*Weevil Perforation Index (WPI). Value lower than 50 is an index of positive protectant effect while WPI greater than 50 is an index of negative protectantability.

Table 6. Protectantability of the plants extracts on cowpea seeds

Plant part powders	Conc. ml/20g	Mean total no of seeds	Mean no of damaged seeds	Mean % seed damaged	% weight loss	Weevil Perforation Index (WPI)
<i>A.occidentale</i>	0.5	94.3	0.0± 0.0a	0.0± 0.0a	0.0± 0.0a	0.0± 0.0a
	1.0	94.7	0.0± 0.0a	0.0± 0.0a	0.0± 0.0a	0.0± 0.0a
	1.5	94.3	0.0± 0.0a	0.0± 0.0a	0.0± 0.0a	0.0± 0.0a
<i>A. Sativum</i>	0.5	94.3	0.0± 0.0a	0.0± 0.0a	0.0± 0.0a	0.0± 0.0a
	1.0	93.3	0.0± 0.0a	0.0± 0.0a	0.0± 0.0a	0.0± 0.0a
	1.5	93.7	0.0± 0.0a	0.0± 0.0a	0.0± 0.0a	0.0± 0.0a
Control	0.0	93.7	72.3±0.0b	76.6±0.4b	82.7±0.3b	50.0±0.0b

Each value is a mean of ± standard error of three replicates. Means within the same column followed by the same letter(s) are not significantly different at (P>0.05) from each other using New Duncan Multiple Range Test.

*Weevil Perforation Index (WPI). Value lower than 50 is an index of positive protectant effect while WPI greater than 50 is an index of negative protectantability.