Insecticidal Activity of *Alstonia boonei* De Wild Powder against Cowpea Bruchid, *Callosobruchus maculatus* (Fab.) [Coleoptera: Chrysomelidae] in stored Cowpea Seeds

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Abstract

The bioactivity of *Alstonia boonei* De Wild (Apocyanaceae) against *Callosobruchus maculatus* (Fab.) (Coleoptera: Chrysomelidae) in stored cowpea seeds was evaluated in the laboratory by admixing leaf, stem bark and root powders of *A. boonei* with cowpea seeds at ambient temperature of 28 ± 2^0C and 70±5% relative humidity. The powders were incorporated into 20g of cowpea seeds, *Vigna unguiculata* at 0.0% (control) 2.5%, 5.0%, 12.5% and 25.0% (w/w) concentration. The ability of the plant powders to protect cowpea seeds was assessed in terms of mortality rates after 24 - 96 hours post treatment, oviposition and adult emergence, percentage weight loss and damage after the first filial generation (F_1). All the tested plant part powders significantly ($P<0.05$) reduce the longevity of adult *C. maculatus* on treated cowpea seeds. From the study, the plant powders could be ranked in order of effectiveness thus; stem bark > leaf > root. *Alstonia boonei* can be used as biopesticide against *C. maculatus* and its incorporation into traditional storage pest management is strongly recommended.

Keywords: Insecticidal activity, Biopesticide, Weevil Perforation Index, Synthetic insecticides, *Callosobruchus maculatus, Alstonia boonei*

1. Introduction

Cowpea bruchid, *Callosobruchus maculatus* (Fab.) is a major pest of wide range of stored legume seeds especially of the cowpea, *Vigna unguiculata* (Ofuya 2001). In many countries of Western and Central Africa, cowpea is a major dietary staple. It is often the main source of protein for the families. Cowpeas are rapidly broken down by the cowpea weevil within three to five months in storage (Ajayi & Lale, 2000; Mbailao *et al*., 2006). During storage, the cowpea weevil causes heavy qualitative and quantitative losses. The damaged seeds are unsuitable for human and animal consumption and they can not be used for planting. Preservation of the quality of the seeds for the following year is one of the worrying problems of farmers. The heavy post harvest losses and quality deterioration caused by storage pests is a major problem facing agriculture in developing countries such as Nigeria (Ashamo, 2007).

The destructive activities of insects and other storage pests have been subdued by chemical control methods comprising fumigation of stored commodity with carbon disulphide, phosphine or dusting with malathion, carbaryl, pirimiphos methyl or permethrin. These chemicals have been reported to be effective against stored products pests (Ogunwolu & Idowu, 1994; Oni & Ileke, 2008; Adedire *et al*., 2011; Ileke & Oni, 2011). In the developed countries, conventional fumigation technology is currently being scrutinised for many reasons, such as ozone depletion potential of methyl bromide and carcinogenic concerns with phosphine (Adedire *et al*., 2011).

The problems with many synthetic insecticides include high mammalian toxicity, high level of persistence in the environment, health hazards, residual effects, poor knowledge of application, increasing costs of application, pest

resurgence, genetic resistance by the insect and lethal effects on non-target organisms (Ajayi & Lale, 2000; Ashamo, 2007; Akinkurolere *et al.,* 2009; Oni & Ileke, 2008; Ileke & Oni, 2011). One solution to these problems might be to totally replace synthetic chemicals with compounds, which occur naturally in plants. Most of these plant products are cheap, readily available, edible and ecologically safer means of controlling insect pest infestations of stored cereal and grains especially in the tropics (Lale, 1992; Adedire & Ajayi, 1996; Adedire & Lajide, 2003; Ashamo, 2007; Akinkurolere *et al*., 2006; 2009; Adedire *et al*., 2011; Ileke & Oni, 2011).

Alstonia boonei De Wild is a medicinal plant used extensively in West and Central Africa for the treatment of malaria, fever, intestinal helminthes, snake bite, arrow poison, impotence, toothache and oedema (Terashima, 2003; Betti, 2004; Abel & Busia, 2005; Akinmoladun *et al*., 2007; Ileke & Oni, 2011). The major phytochemicals in the plant are saponin, alkaloids, tannins and cardiac glycosides (Fasola & Egunyomi, 2005, Akinmoladun *et al.,* 2007). Despite its popularity as a medicinal plant, the insecticidal potential of *A. boonei* has not been fully investigated has been done for other medicinal plants like neem, *Azadirachta indica* and *Anacardium occidentale* (Kilonzo, 1991; Onu & Baba, 2003; Maina & Lale, 2004; Adedire *et al.,* 2011). The objective of the present study was to examine the insecticidal activities of the leaf, stem bark and root of *A. boonei* powders against the cowpea bruchid, *C. maculatus*, a major insect pest of cowpea and legumes (Lale & Ofuya, 2001).

2. Materials and Methods

2.1 Insect rearing

Newly emerged adult *C. maculatus* used for this study were obtained from already existing culture in the Postgraduate Research Laboratory of the Department of Biology, Federal University of Technology, Akure, Ondo State, Nigeria. They were subsequently reared inside 1 litre Kilner jars, on uninfested cowpea seeds *Vigna unguiculata* variety Ife brown obtained from the Agricultural Development Programme (ADP), Ado Ekiti. Ekiti State, Nigeria. The culture was placed in an insect rearing cage at ambient temperature of 28 ± 2^0C and 75 ± 5% relative humidity which were monitored with the aid of a whirling thermohygrometer.

2.2 Preparation of Alstonia boonei powders

The plant parts of *Alstonia boonei* used for this study were sourced fresh from Akola farm at Igbara-Odo Ekiti, Ekiti State, Nigeria. These plant materials were 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 $1mm^2$ perforation (Ileke & Oni, 2011). The powders were packed in plastic containers with tight lids and stored in a refrigerator at 4^0C prior to use.

2.3 Collection of cowpea seeds

Cowpea, *Vigna unguiculata* variety Ife brown obtained from newly stock cowpea seeds free of insecticides at Agricultural Development Programme (ADP), Ado Ekiti. Ekiti State, Nigeria, was used for this study. The cowpea were first cleaned and disinfested by keeping them in a freezer at -5^0C for 7 days to kill all hidden infestations. The disinfested seeds were then dried in a Gallenkamp oven (Model 250) at 40^0C for 4 hours (Jambere *et al*., 1995) before they were stored in plastic containers with tight lids disinfested by swabbing with 90% alcohol.

2.4 Insect bioassay

Leaf, stem bark and root powders were admixed with 20g of cowpea seed at 2.5, 5.0, 12.5 and 25.0% w/w concentration (Fatope *et al*., 1995) 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 day-old adults *C. maculatus* were introduced to each of the containers and covered. The insects were sexed following the reports of Odeyemi & Daramola (2000). Three replicates of the treated and untreated controls were laid out in Complete Randomized Design. The adult mortality was assessed after every 24hours for 96hours. Adults were considered dead when probed with sharp objects and there were no responses. On day 5, all insects, both dead and alive, were removed from each container and ovipositions were noted before returning the seeds to their respective containers. Progeny emergence (F_1) was then recorded at 5 weeks. The containers were sieved out and newly emerged adult bruchid were counted with an aspirator. The percentage adult emergence was calculated using the method of Odeyemi & Daramola (2000).

At week 6, the cowpea were re-weighed by using Metler weighing balance and the % loss in weight was determined using the method of Odeyemi & Daramola (2000).

After re-weighing, the numbers of damaged cowpea were evaluated by counting wholesome and bored or seed with bruchid emergent holes. Percentage of seed damaged was also computed using the method of FAO (1985).

Weevil Perforation Index (WPI) was adopted from the analysis of damage using the method of Fatope et al. (1995), Adedire & Ajayi, 1996. The weevil perforation is define as follows:

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

2.6 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 Effect of alstonia boonei powder on adult mortality of C. maculatus

The stem bark powder was able to caused 100% mortality of adult *C. maculatus* within 72 hours of application. Leaf, stem bark and root powders of *Alstonia boonei* tested at 5% level showed similar bioactivity against *Callosobruchus maculatus* after 96 hours of post treatment. All the tested plant parts powders significantly ($P<0.05$) reduce adult *C. maculatus* on treated cowpea seeds (Table 1). There was a significant difference ($P<0.05$) in mortality of adult insects amongst the treatments, with stem bark powder causing 100% mortality of adult *C. maculatus* at all tested concentrations after 72 hours of application. This was followed by leaf powder at 5.0, 12.5 and 25%w/w concentration evoking 100% mortality of *C. maculatus* after 96 hours of exposure. Root powder of *A. boonei* was the least toxic causing 100% bruchid mortality after 96 hours of post treatment at 25%w/w concentration.

3.2 Effect of alstonia boonei powder on oviposition and adult emergence of C. maculatus

All the plant parts powders effectively reduced oviposition by *C. maculatus* (Table 2). The number of eggs laid by *C. maculatus* on treated cowpea seeds was significantly ($P>0.05$) lower than number of eggs laid on untreated cowpea seeds. There was no significant difference ($P<0.05$) in the mean number of eggs laid on the treated cowpea seeds with leaf, stem bark and root powders of *A. boonei*. The percentage adult emergence in the untreated seeds was significantly different ($P>0.05$) from emergence in the treated cowpea seeds. No progeny production took place in cowpea seeds treated with stem bark powder (Table 2). However, there was adult emergence in cowpea seeds treated with leaf powder at 2.5%w/w concentration and root powder at 2.5 and 5%w/w concentration on treated cowpea seeds. These did not show any significant difference ($P<0.05$) when compared with other treated seeds.

3.3 Protectantability of alstonia boonei powder on cowpea seeds

Leaf, stem bark and root powders of *A. boonei* completely prevented infestation and damage of the treated cowpea seeds (Table 3). There was no significant difference ($P<0.05$) in seeds damage, weight loss and Weevil Perforation Index (WPI) in the treated cowpea seeds. However, the Weevil Perforation Index (WPI) of 8.7 at 2.5%w/w concentration and 11.47 and 4.3 at 2.5 and 5%w/w concentration obtained for leaf and root powders of *A. boonei* respectively were significantly different ($P>0.05$) from Weevil Perforation Index WPI of the control. The untreated cowpea seeds recorded 73.93% damage as revealed by emergent holes of the bruchid. The weight of untreated cowpea seeds was significantly ($P>0.05$) reduced compared with treated cowpea seeds.

4. Discussion

The high mortality rate of adults *C. maculatus* recorded on cowpea seeds treated with leaf, stem bark and root powders of A. *boonei* could be due to high toxic effect of the plant parts on adult *C. maculatus*. Stem bark powder of *A. boonei* prevented the emergence of *C. maculatus* adults, an effect that is in agreement with the observation of Oigiangbe *et al*. (2007) who reported that aqueous extracts of the stem bark of *A. boonei* adversely affected the longevity of *Maruca vitrata*. Our present observations also corroborate the report of Osawe *et al.* (2007) who reported that the aqueous extracts of the stem bark and leaves of *A. boonei* adversely affected the survival and growth of *Sesamia calamists.* The result of this investigation are also similar to the observations of Ileke & Oni (2011) who obtained 100% mortality of adult *Sitophilus zeamais* in wheat grains treated with *A. boonei* stem bark powder.

The lethal effect of the stem bark powder on the bruchid could be as a result of contact toxicity. Insects breathe by means of trachea that usually open at the surface of the body through spiracles (Adedire *et al*., 2011). The spiracle might have been blocked by the plant powders thereby leading to suffocation of adult bruchids. These toxic effect has been attributed by various authors to the presence of some chemical compounds of the triterpenoids and indole

alkaloid group such as alstonine, astonidine and porphine that have been identified from the stem bark of *A. boonei* (Phillipson *et al*., 1987; Anonymous, 1992; 2001).

The plant powders also prevented oviposition, adult emergence, reduction in weight loss and seeds damage by *C. maculatus* on treated cowpea seeds. These powders inhibits locomotion which affect mating activities and sexual communication as well as deterring females from laying eggs, as well as complete suppression of the developmental stages of insects which have been reported by a number of authors (Ivbijaro & Agbaje, 1986;Ofuya, 1992; Okonkwo & Okoye, 1996; Adedire 2002; Maina & Lale, 2004; Mbailao *et al*., 2006, Akinkurolere *et al*., 2006, Okosun & Adedire, 2010; Adedire *et al*., 2011; Ileke & Oni, 2011). The protectantability of the plant powders were highly remarkable.

5. Conclusion

It is evident from this study that all the plant-part powders tested have the potential of being used as biopesticides. Higher concentration of the leaf and root powders could be more effective since it is expected to contain high active components. Further research still needs to be carried out on oil extracts of *A. boonei* which will reduce the bulkiness of the powders when used for control of pests in bags or in storage bins since their potential as biopesticides against stored products insects appears great.

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Table 1. Mortality of adult *Callosobruchus maculatus* in cowpea seeds treated with *Alstonia boonei* powders

Plant part powders	Conc. w/w	Mean % mortality ± S. E. at 24h to 96h Post Treatment			
		24h	48h	72h	96h
Leaf	2.5	10.00±0.00^{a}	30.00± 0.00^{c}	57.68± 0.41^{c}	77.67± 0.32^{bc}
	5.0	27.03±0.05^{b}	45.01± 0.20^{d}	78.45 ±0.30^{d}	100.00± 0.00^{e}
	12.5	42.03 ±0.05^{de}	76. 30 ±0.06^{fg}	100.00±0.00^{f}	100.00 ±0.00^{e}
	25.0	50.06 ±0.75^{e}	82.71 ± 0.30^{gh}	100.00±0.00^{f}	100.00± 0.00^{e}
Stem bark	2.5	30.00 ±0.00^{bc}	70.26 ± 0.25^{f}	100.00± 0.00^{f}	100.00± 0.00^{e}
	5.0	38.21 ±0.58^{cd}	87.31± 0.67^{h}	100.00±0.00^{f}	100.00 ±0.00^{e}
	12.5	50.48 ±0.17^{e}	100.00±0.00^{i}	100.00±0.00^{f}	100.00± 0.00^{e}
	25.0	70.26 ± 0.25^{f}	100.00±0.00^{i}	100.00±0.00^{f}	100.00± 0.00^{e}
Root	2.5	0.00 ±0.00^{a}	20.00±0.00^{b}	45.01±0.20^{b}	67.30 ± 0.56^{b}
	5.0	10.00±0.00^{a}	30.00±0.00^{b}	60.00± 0.00^{c}	87.70 ± 0.30^{cd}
	12.5	24.64±0.30^{b}	63.33±0.01^{e}	87.70±0.30^{de}	96.67±0.32^{de}
	25.0	45.01±0.20^{de}	84.31± 0.67^{gh}	90.01±0.02^{ef}	100.00± 0.00^{e}
Control	0.00	0.00 ±0.00^{a}	0.00±0.00^{a}	0.00 ±0.00^{a}	0.00 ±0.00^{a}

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 2. Effect of *Alstonia boonei* powders on oviposition and adult emergence of *Callosobruchus maculatus*

Plant part powders	Conc. w/w	No of Eggs	% Adults Emergence
Leaf	2.5	30.33 ± 0.01^{c}	19.78 ± 0.40^{bc}
	5.0	10.33± 0. 01^{a}	0.00 ±0.00^{a}
	12.5	10.00± 0.00^{a}	0.00 ±0.00^{a}
	25.0	10.00± 0.00^{a}	0.00 ±0.00^{a}
Stem bark	2.5	10.33± 0. 01^{a}	0.00 ±0.00^{a}
	5.0	10.33± 0. 01^{a}	0.00 ±0.00^{a}
	12.5	10.00± 0.00^{a}	0.00 ±0.00^{a}
	25.0	10.00± 0.00^{a}	0.00 ±0.00^{a}
Root	2.5	40.33 ± 0.01^{c}	24.79 ±0.41^{c}
	5.0	16.67 ± 0.32^{b}	12.06 ± 0.10^{b}
	12.5	10.33± 0. 01^{a}	0.00 ±0.00^{a}
	25.0	10.33± 0. 01^{a}	0.00 ±0.00^{a}
Control	0.00	81.47± 0.20^{d}	84.09± 0.20^{d}

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 3. Protectantability of *Alstonia boonei* powders on cowpea seeds

Plant part powders	Conc. w/w	Mean total no of seeds	Mean no of damaged seeds	Mean % seed damaged	% weight loss	*Weevil Perforation Index (WPI)
Leaf	2.5	93.33	6.00 ± 0.00[a]	6.43± 0.46[a]	0.80±0.00[a]	8.70±0.33[ab]
	5.0	93.67	0.00 ± 0.00[a]	0.00 ± 0.00[a]	0.00±0.00[a]	0.00±0.00[a]
	12.5	94.33	0.00 ± 0.00[a]	0.00 ± 0.00[a]	0.00±0.00[a]	0.00±0.00[a]
	25.0	94.67	0.00 ± 0.00[a]	0.00 ± 0.00[a]	0.00±0.00[a]	0.00±0.00[a]
Stem bark	2.5	94.33	0.00 ± 0.00[a]	0.00 ± 0.00[a]	0.00±0.00[a]	0.00±0.00[a]
	5.0	94.33	0.00 ±0.00[a]	0.00 ±0.00[a]	0.00±0.00[a]	0.00±0.00[a]
	12.5	93.33	0.00 ± 0.00[a]	0.00 ± 0.00[a]	0.00±0.00[a]	0.00±0.00[a]
	25.0	94.33	0.00 ± 0.00[a]	0.00 ± 0.00[a]	0.00±0.00[a]	0.00±0.00[a]
Root	2.5	94.33	8.00 ± 0.00[a]	8.48± 0.65[a]	10.40±0.20[a]	11.47±0.70[b]
	5.0	94.33	3.00± 0.00[a]	3.18 ± 0.20[a]	5..67±0.40[a]	4.30±0.60[a]
	12.5	94.33	0.00 ± 0.00[a]	0.00 ± 0.00[a]	0.00±0.00[a]	0.00±0.00[a]
	25.0	93.33	0.00 ± 0.00[a]	0.00 ± 0.00[a]	0.00±0.00[a]	0.00 ±0.00[a]
Control	0.00	93.33	69.0 ± 0.00[b]	73.93±1.20[b]	83.47±0.70[b]	50.00±0.00[c]

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.