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Laboratory Assessment of Some Plants Latex as Biopesticide Against Cowpea Bruchid, *Callosobruchus maculatus* (Fab.) [Coleoptera: Chrysomelidae]

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Abstract

Laboratory evaluation of *Calotropis procera, Alstonia boonei, Jatropha curcas* and *Argemone mexicana* latex as biopesticide against cowpea bruchid, *Callosobruchus maculatus* (Fab.) [Coleoptera: Chrysomelidae] were evaluated at ambient temperature and relative humidity of 28±2^{o}C and 75±5% respectively. The plants latex was tested at rates of 0.5 ml, 1 ml, 1.5 ml and 2 ml / 20 g of cowpea seeds. Adult mortality and adult emergence of the insects were investigated. Results showed that at rates of 1 ml, 1.5 ml and 2 ml/ 20 g of cowpea seeds, *A. boonei* latex evoked 100% mortality of adult cowpea bruchid after 4 days of post treatment. This is followed by *C. procera* and *J. curcas* which caused 100% mortality of cowpea bruchid at rates of 1.5 ml and 2 ml / 20 g of cowpea seeds while the least effective plant *Argemone mexicana* latex. There was no adult emergence in seeds treated with *A. boonei* latex at tested concentrations and *C. procera* and *J. curcas* at rates of 1.5 ml and 2 ml / 20 g of cowpea seeds compared with untreated that had 87.75% adults emergence. The results obtained from this study revealed that *Alstonia boonei, Calotropis procera* and *J. curcas* latex were effective in controlling of *C. maculatus* and could serve as an alternative to synthetic insecticides for the protection of stored cowpeas against bruchids.

Keywords: *Calotropis procera, Alstonia boonei, Jatropha curcas, Argemone mexicana, Callosobruchus maculatus*

1. Introduction

Cowpea, *Vigna unguiculata*, is one of the most important proteinous grain legumes in Nigeria and other tropical countries, where it forms a major source of protein to combat malnutrition in young children (Akinkurolere et al., 2006; Adedire et al., 2011; Ileke et al., 2013a). In addition to the high protein content of cowpea, it also has high iron content but is low in fat (Adedire et al., 2011). One of the major problems encountered in agriculture in developing countries is post harvest losses which usually occur during storage (Adedire et al., 2011). Cowpea bruchid, *C. maculatus* has been recognized for years as the major insect pest of cowpea seeds (Ofuya, 2001; Ileke & Bulus, 2012; Ileke et al., 2013a). Insects damage of cowpea seeds start in the field just before harvest and the insects developmental stages were carried into the store where the population builds up rapidly (Ofuya, 2001; Ileke et al., 2012; Ileke et al., 2013b).

Efficient control of stored products insect pests has long been the aim of stored products entomologists throughout the world (Ileke et al., 2013b). The control of stored products insects like *C. maculatus* has centred mainly on the use of synthetic insecticides (Asawalam et al., 2007). However, the use of these chemicals is hampered by many attendant problems such as development of insect resistant strains, their toxic residues getting into food of animals and man, workers safety and high cost of procurement (Sighamony et al., 1990; Ileke & Oni, 2011). These problems have necessitated research on the use of alternative eco-friendly cheaper means of insect pests control methods amongst which are the use of powdered plant parts and their extracts (Lajide et al., 1998; Asawalam &

Adesiyan, 2001; Adedire & Lajide, 2003; Ileke & Bulus, 2012). Hiltertue, the use of botanicals as insecticides have only been concentrated on the use of their powders and oil extracts, therefore, there is still need for searching other parts or constituents of botanicals that could have greater insecticidal effect. Therefore, keeping in mind the importance of application of some environmentally sound plant based molecules as potential substitute to synthetic pesticides; this research work investigated the biopesticide potential of *Calotropis procera, Alstonia boonei, J. multifida* and *Argemone mexicana* latex against cowpea bruchid, *Callosobruchus maculatus* which is scarce in literature.

2. Materials and Methods

2.1 Insect Culture

The insects used to establish a laboratory colony of *C. maculatus* came from a batch of infested cowpea seeds, *V. unguiculata* Walp variety Ife brown collected from Agricultural Development Project, Akure, Ondo State, Nigeria. Beetles were reared subsequently by replacement of devoured and infested cowpea seeds with fresh un-infested cowpea seeds in 2-L kilner jars covered with muslin cloth to allow air circulation. Insect rearing and the experiments were carried out in the Research Laboratory, Department of Environmental Biology and Fisheries, AdekunleAjasin University, Akungba Akoko, Ondo State, Nigeria, at ambient temperature of 28±2^{o}C and 75±5% relative humidity.

2.2 Collection of Cowpea Seeds

Cowpea seeds used for this study were obtained from a newly stocked seeds free of insecticides at Agricultural Development Project (ADP), Akure, Ondo State, Nigeria. Firstly, the grains were cleaned and disinfested by keeping at -5^{o}C for 7 days to kill all hidden infestations. This is because all the life stages, particularly the eggs are very sensitive to cold (Koehler, 2003). The disinfested cowpea seeds were then placed inside a Gallenkamp oven (model 250) at 40^{o}C for 4 hours (Jambere et al., 1995) and later air dried in the laboratory to prevent mouldiness (Adedire et al., 2011) before they were stored in plastic containers with tight lids disinfected by swabbing with 90% alcohol.

2.3 Collection of Plants Latex

The stem of each *C. procera, A. boonei, J. multifida* and *A. mexicana* was cut with knife to allow the plant latex to come out into a container. Ten (10 ml) of each of the plant latex were collected in separate beaker and corked tightly to prevent evaporation and solidification. They were then labeled and kept in refrigerator to keep then fresh.

2.4 Identification and Sexing of C. maculatus Adults

The identification and sexing of *C. maculatus* were carried out in the Research Laboratory, Department of Environmental Biology and Fisheries, Adekunle Ajasin University, Akungba Akoko, Ondo State, Nigeria using Binocular Microscope based on observations of Halstead (1963), Appert (1987), Odeyemi and Daramola (2000). Male have comparative shorter abdomen and the dorsal side of the terminal segment is sharply curved downward and inward. In contrast the females have comparatively longer abdomen and the dorsal side of the terminal segment is only slightly bent downward. The female also has two dark visible spots on their elytra (Halstead, 1963; Odeyemi & Daramola, 2000; Ileke et al., 2013a).

2.5 Bioassay

0.5 ml, 1.0 ml, 1.5 ml and 2.0 ml of *C. procera, A. boonei, J. multifida* and *A. mexicana* latex were mixed separately with 20 g of un-infested cowpea seeds in 250 ml plastic containers. The latex and seeds were thoroughly mixed using a glass rod and then agitated for 5-10 min to ensure uniform coating. Control experiment was also set up without latex. Ten pairs of *C. maculatus* adults were introduced into each of the containers and covered. Four replicates of the treated and untreated controls were laid out in Complete Randomized Block Design in insect cage. Beetle mortality was observed after 4 days post treatment. The beetles were confirmed dead when there was no response to probing with sharp pin at the abdomen. The total number of eggs laid per replicate was recorded after 4 days post treatment. The experimental set up was kept inside the insect rearing cage for further 30 days for the emergence of the first filial (F_1) generation. The percentage number of adult beetle emergence was calculated according to the method described by Odeyemi and Daramola (2000).

$$\%\ \text{Adult emergence} = \frac{\text{Total number of adult emergence}}{\text{Total number of eggs laid}} \text{X100}$$

2.6 Statistical Analysis

Data were subjected to analysis of variance (ANOVA) and treatment means were separated using the New Duncan's Multiple Range Test. The ANOVA was performed with SPSS 16.0 software (SPSS, Inc., 2007).

3. Results

3.1 Effectiveness of Plant Latex as Insecticide

The effectiveness of *C. procera, A. boonei, J. multifida* and *A. mexicana* latex on the survival of cowpea bruchid, *C .maculatus* at different concentrations after 4 days of post treatment is presented in Table 2. The results revealed that in each treatment, the mortality of cowpea bruchid increased gradually with increase in concentration. Cheesewood, *A. boonei* latex caused 100% mortality of *C. maculatus* at rate 1 ml/20 g of cowpea seeds after 4 days of post treatment. The corresponding values for *C. procera, J, multifida* and *A. mexicana* were 82.5%, 75% and 42.5% mortality of adult cowpea bruchid respectively. The toxicities of these plant latex increase with an increased in concentration. At concentration of 2.0 ml/20 g of cowpea seeds, 100% mortality of adult cowpea bruchid was obtained in seeds treated with *C. procera, A. boonei* and *J. multifida* latex (Table 2). Generally, the result indicated that various plants latex significantly (P<0.05) reduced the number of cowpea bruchid, *C. maculatus*.

Table 1. Plants latex evaluated for insecticidal activities against *C. maculatus*

Scientific name	Family	Plants used	Common name
Calotropis procera	Apocynaceae	Latex	Apple of Sodom
Alstonia boonei	Apocynaceae	Latex	Cheesewood
Jatropha curcas	Euphorbiaceae	Latex	Physics nut
Argemone mexicana	Papaveraceae	Latex	Mexicana poppy

Table 2. Effect of plant latex on mortality (%) of adult *C. maculatus* after 4 days of infestation

Plant latex/Concentration	0.5 ml	1.0 ml	1.5 ml	2.0 ml
C. procera	60.00±4.08c	82.50±7.50cd	100.00±00.00c	100.00±00.00c
A. boonei	85.00±2.89d	100.00±00.00d	100.00±00.00c	100.00±00.00c
J. multifida	52.50±7.50c	75.00±2.89c	100.00±00.00c	100.00±00.00c
A. Mexicana	30.00±4.08b	42.50±7.50b	55.00±2.89b	67.50±2.50b
Control	0.00±0.00a	0.00±0.00a	0.00±0.00a	0.00±0.00a

Each value is a mean ± S. E. of four replicates. Means followed by the same letter along the column are not significantly different (P<0.05) using New Duncan's Multiple Range Test.

3.2 Effect of Plant Latex on Oviposition of C. maculatus

Table 3. Effect of plant latex on oviposition of *C. maculatus*

Plant latex/Concentration	Number of Eggs laid			
	0.5 ml	1.0 ml	1.5 ml	2.0 ml
C. procera	7.75±0.84a	7.50±1.31a	5.00±1.90ab	5.00±1.90a
A. boonei	5.75±0.84a	5.50±1.31a	2.50±1.30a	2.25±1.30a
J. multifida	15.25±2.37ab	10.00±1.90a	7.75±0.84ab	5.25±1.30a
A. mexicana	20.00±1.90b	15.75±0.84ab	15.50±1.30b	12.75±0.84a
Control	45.50±1.30d	45.50±1.30c	45.50±1.30c	45.50±1.30b

Each value is a mean ± S. E. of four replicates. Means followed by the same letter along the column are not significantly different (P<0.05) using New Duncan's Multiple Range Test.

The plants latex effectively reduced oviposition by cowpea bruchid, *C. maculatus* (Table 3). The number of eggs laid by *C. maculatus* on cowpea seeds treated with *C. procera, A. boonei* and *J. multifida* latex significantly lower than number of eggs laid by cowpea bruchid on cowpea seeds treated with *A. mexicana* latex. There was significantly different (P>0.05) in the number of eggs laid on the seeds treated with *A. mexicana* latex and control.

3.3 Effect of Plant Latex on Adult Emergence of C. maculatus

The percentage adult emergence in the untreated cowpea seeds was significantly different (P>0.05) from the emergence in the treated cowpea seeds (Table 4). There was no adult emergence of *C. maculatus* adult in seeds treated at rates 1.5 ml and 2.0 ml/20g of seeds after 4 days of post treatment.

Table 4. Effect of plant latex on percentage adult emergence of *C. maculatus*

Plant latex/Concentration	% Adult Emergence			
	0.5 ml	1.0 ml	1.5 ml	2.0 ml
C. procera	27.75±0.84bc	27.50±1.31c	0.00±0.00a	0.00±0.00a
A. boonei	0.00±0.00a	0.00±0.00a	0.00±0.00a	0.00±0.00a
J. multifida	20.00±1.90b	10.00±1.90b	0.00±0.00a	0.00±0.00a
A. mexicana	35.00±2.89c	32.75±0.84c	25.50±1.30b	17.75±0.84b
Control	87.75±0.84d	87.75±0.84d	87.75±0.84c	87.75±0.84c

Each value is a mean ± S. E. of four replicates. Means followed by the same letter along the column are not significantly different (P<0.05) using New Duncan's Multiple Range Test.

4. Discussion

Latex is a plant colourless or milky sap which contains protein, starch, alkaloids and it coagulates on exposure to air. Studies by many researchers had shown insecticidal potential of *A. boonei* stem bark on the mortality of adult *Maruca vitrata, Sesamia calamists*, *Sitophilus zeamais* and *C. maculatus* (Ogunleye, 2000; Ogunleye & Omotoso, 2011; Oigiangbe et al., 2007; Oigiangbe et al., 2007; Ileke & Oni, 2011; Ileke et al., 2012; Ileke et al., 2013b; Ojo & Ogunleye, 2013). In this study, the latex of *A. boonei* was the most toxic to adult of *C. maculatus*. The insecticidal effects of this plant latex on the beetle could be linked to the presence of some chemical compounds of the triterpenoids, indole and alkaloid group such as alstonine, astondine, and porphine that have been identified from the stem bark of *A. boonei* (Phillipson et al., 1987; Anonymous, 1992, 2001).

The latex of *J. multifida* was able to caused 100% mortality of adult cowpea bruchid at rates of 1.5 ml and 2.0 ml after 4 days of post treatment. This result is in agreement with the finding of Ogunleye (2010). Ogunleye and Omotoso (2011) reported that seed oil of *J. curcas* effects 100% mortality of adult *S. zeamais* at rates of 0.3 ml and 0.4 ml after 24 hours of application. This research work collaborates with the finding of Ogunleye and Omotoso (2011) who reported 100% mortality of adult *C. maculatus* on cowpea seeds treated with drops of *J. multifida* latex after 24 hours of application. Member of the family Euphorbiceae have been reported to have insecticidal property (Adebowale & Adedire, 2006).

The insecticidal activity of *C. procera* have been tested and reported by Vikash (2003). The plant latex at rates 1.5ml and 2.0ml evoked 100% mortality of adult *C. maculatus* after 4 days application.

A. mexicana was the least toxic among the plant latex tested causing 67.5% adult mortality of cowpea bruchid after 4 days of application. Administrator (2010) listed alkaloids like berberin, protopine, argenmouine, codeine and caspsin as metabolites present in *A. mexicana.*

All the latex significantly reduced oviposition and adult emergence of adult cowpea bruchid. The effect of the latex on oviposition could be due to respiratory impairment which probably affects the process of metabolism and consequently other systems of the body of the insects (Onolemhemhem & Oigiangbe, 1991; Adedire et al., 2011; Ileke & Bulus, 2012). The inability of the eggs to stick to the cowpea seed due to the presence of the latex which also reduced adult emergence arising from egg mortality (Adedire et al., 2011; Ileke et al., 2013). It has been reported that one of the main mechanisms of action of plant metabolites is their ability to penetrate the chorion of bruchid eggs via the micropyle and cause the death of developing embryos through asphyxiation (Don

– Pedro 1989a; b). It is evident from this study that all the tested plants latex has the potential of being used as a biopesticides.

5. Conclusion

This study has revealed the insecticidal efficacy of *C. procera, A. boonei, J. multifida* and *A. mexicana* latex as cowpea protectant against *C. maculatus* and could serve as an alternative to synthetic insecticides for the protection of stored cowpeas against cowpea bruchids.

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