ENTOMOCIDAL POTENTIALS OF BITTER LEAF (*VERNONIA AMYGDALINA*) IN THE CONTROL OF COWPEA BRUCHID,

CALLOSOBRUCHUS MACULATUS

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ABSTRACT

Powders and ashes from *Vernonia amygdalina* were evaluated for their efficacy as contact insecticides on cowpea bruchid, *Callosobruchus maculatus* in the laboratory at ambient tropical conditions of temperature and relative humidity. The powders and ashes were applied at rates 0.0 (control), 2.00g/20g of cowpea seeds. The result indicated that the plant ashes and powders tested as contact insecticides significantly ($P<0.05$) reduced the number of tested insects. However, stem bark ash and powder was the most toxic to *C. maculatus* that evoked 100% mortality of adult cowpea bruchid after 72 h of exposure. There was no progeny development of the bruchid in samples treated with *V. amygdalina*. The survival of the bruchid from eggs to adults when treated with the plant part powders showed that there was significantly ($P<0.05$) more % progeny development in the control compared to others. This study showed that all the tested plant parts powders and ashes were toxic to cowpea bruchid and the powders can be mixed with cowpea seeds to prevent hatching of the eggs thereby aiding their control.

Keywords: Entomocidal, *Callosobrochus maculatus*, *Vernonia amygdalina,* cowpea seed

INTRODUCTION

Cowpea, *Vigna unguiculata* is an important food crop in tropical countries especially in West Africa where it is a cheap source of dietary protein (Adedire, 2002). The dry seed consists of about 25% protein and 67% carbohydrate. It is also a good source of calcium, iron, vitamins and carotene. Initial infestation of cowpea seeds occurs in the field just before harvest and the insects are carried into the store where population builds up rapidly (Adedire, 2002).*Callosobruchus maculatus* has become a major storage insect pest of the seeds. Some people relish vegetable dishes of young cowpea leaves, immature pods, or immature seeds (Alabi *et al*., 2006). The cowpea bruchid is a cosmopolitan insect, with its initial infestation starting in the field just before harvest and the insects are carried into the store where the population increases rapidly (Ofuya, 2001; Ileke *et al*., 2012).The magnitude of infestation between *C. maculatus* and human beings for this important crop necessitates its control to avoid food shortage and promote self-sufficiency.The general use of insecticides to protect stored grains has been enhanced by the public suspicion of any chemical of a persistent nature, whether or not evidence is available of any adverse effects (Adedire, 2002). Limited information is available on the use of mixture of plant powders against the control of *C. maculatus* this work is aimed at determining the potentials/efficiency of the leaf, root and stem bark ash and powders of *Vernonia amygdalina* against *C. maculatus*.The objectives of this research are to investigate the insecticidal potentials of *V. amygdalina*, against cowpea bruchid, *C. maculatus*, the effectiveness of *V. amygdalina* stem bark, leaf, and root ash and powders as contact and fumigant entomocide in the management of *C. maculatus*andalsoinvestigate the fecundity of *C. maculatus* treated with *V. amygdalina* ash and powder at rate 2g/20g of cowpea seeds.

MATERIALS AND METHODS

Insect culture

The insects used to establish a laboratory colony of *C. maculatus* came from a batch of infested cowpea seeds, *Vigna unguiculata* (L.) Walp variety Ife brown collected from Agricultural Development Project, Akure, Ondo State, Nigeria. The cowpea were first cleaned and disinfested by keeping them in a freezer at -5^{0}C for 10 days. The frozen seeds were allowed to thaw and placed inside a Gallenkamp oven (Model 250) at 40^{0}C for 4 hours before they were stored in plastic containers with tight lids.

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 at ambient temperature of $28+2^{o}$C and 75+5% relative humidity.

Identification and sexing of adult *Callosobruchus maculatus*

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 using Binocular Microscope based on observations of Halstead (1998), Appert (1997), 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 (Odeyemi and Daramola, 2000).

Plant collection

The plant evaluated in this work is *V. amygdalina* .They were obtained in fresh form, free of insecticides from Akungba community, Ondo State, Nigeria and authenticated by the Plant Science and Technology Department of Adekunle Ajasin University, Akungba Akoko, Ondo State. This plant material was rinsed in clean water to remove sand and other impurities, cut into smaller pieces before air dried in a well-ventilated laboratory and ground into very fine powder using an electric blender. The powders were further sieved to pass through $1mm^{2}$ perforations. The powders were packed in plastic containers with tight lids and stored in a refrigerator at 4°C prior to use.

Collection of Cowpea Seeds

Cowpea seeds used for this study were obtained from a newly stocked seeds free of insecticides at Agricultural Development Program (ADP), Akure, Ondo State, Nigeria. Firstly, the seeds were cleaned and disinfested by keeping at -5°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°C for 4 h (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.

Effect of contact toxicity of plants powders on adult mortality, oviposition and progeny development of *Callosobruchusmaculatus*

Fine powders of *V. amygdalina*were mixed with cowpea seeds at the rate of 2.00g of cowpea seeds in 250ml plastic containers. Ten pairs of 2 – 3 days old adult's *C. maculatus* were introduced to each of the containers and covered. Four replicates of the treated and untreated controls were laid out in Complete Randomized Design. The adult mortality was assessed after every 24 h for 96 h. Adults were considered dead when probed with sharp objects and there were no responses. At the end of day 4, all insects, both dead and alive, were removed from each container and oviposition were counted and recorded before returning the seeds to their respective containers. Percentage adult mortality was corrected using Abbott (1998) formula thus:

$$P_T = \frac{P_o - P_c}{100 - P_c} \times \frac{100}{1}$$

Where P_T = corrected mortality (%)

P_O = observed mortality (%)

P_C = control mortality (%)

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 containers were sieved out and newly emerged adult cowpea bruchid were counted and recorded. The percentage adult emergence was calculated using the method of Odeyemi and Daramola (2000).

$$\% \text{ Adult emergence} = \frac{\text{Total number of adult emergence}}{\text{Total number of eggs laid}} \times \frac{100}{1}$$

Percentage weight loss of the cowpea seeds was determined by re-weighing after 35 days and the % loss in weight was determined as follows:

$$\% \text{ Weight loss} = \frac{\text{Change in weight}}{\text{Initial weight}} \times \frac{100}{1}$$

After re-weighing, the numbers of damaged cowpea seeds were evaluated by counting wholesome seeds and seeds with bruchid emergent holes. Percentage seed damaged was calculated as follows:

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

Beetle Perforation Index (BPI) used by Fatope*et al.* (1995) was adopted for the analysis of damage. Beetle perforation index (BPI) was defined as follows:

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

BPI value exceeding 50 was regarded as enhancement of infestation by the weevil or negative protectability of the extract tested.

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 20.0 software (SPSS, Inc. 2007).

RESULTS

Toxicity of *Vernonia amygdalina* ash and powder to *Callosobrochus maculatus*

The effectiveness of the ash and powders of the leaf, stem bark, and root of *V. amygdalina* on the survival of cowpea bruchid, *C. maculatus* is presented in Table 1. There were no significant differences on the mortality of cowpea bruchids treated with various plant powder parts. However, stem bark ash powder was the most toxic to *C. maculatus* that evoked 100% mortality of adult cowpea bruchid after 72 h of exposure. The result indicated that various plant powders tested as contact insecticides significantly (P<0.05) reduced the number of tested insects. Generally, *V. amygdalina* stem bark ash and powders were more toxic than the other tested plant powder parts (leaf and root).

Table 1: Percentage mortality of adult *C. maculatus* treated with *V. amygdalina* ash and powders at rate 2g/20g of cowpea seed

V. amygdalina ash and powders	Mean % Mortality ± SE on Days			
	1	2	3	4
Ash (L)	25.00 ± 2.89^{c}	40.00 ± 4.08^{cd}	72.50 ± 7.50^{cd}	85.00 ± 4.89^{c}
Ash (S)	40.00 ± 4.08^{d}	75.00 ± 3.89^{e}	100.00 ± 0.00^{e}	100.00 ± 0.00^{d}
Ash (R)	20.00 ± 4.08^{bc}	32.50 ± 7.50^{bc}	65.00 ± 2.89^{c}	80.00 ± 4.08^{bc}
Powder (L)	15.00 ± 2.89^{bc}	35.00 ± 2.89^{bc}	60.00 ± 4.08^{bc}	87.00 ± 2.50^{cd}
Powder (S)	25.00 ± 2.89^{c}	50.00 ± 5.79^{d}	85.00 ± 4.89^{d}	100.00 ± 0.00^{d}
Powder (R)	10.00 ± 2.01^{b}	22.50 ± 3.50^{b}	50.00 ± 5.79^{b}	65.00 ± 2.50^{b}
Control	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 ± standard error of means (S.E.M) of four replicate means within column followed by the same letters (s) are not significantly different at (P> 0.05) using New Duncan's Multiple Range Test.

Keys: L – Leaf, S¬ – Stem bark, R – Root

Fecundity of *C. maculatus* treated with *Vernonia amygdalina*

Table 2 presented the oviposition and % progeny development of *C. maculatus* after been exposed to various plant powders as contact insecticide at 2g/20g of cowpea seeds. Progeny development was significantly suppressed by various plant powders with the stem bark ash and powder completely inhibiting the emergence of *C. maculatus* (100% efficiency).

Table 2: Fecundity of *C. maculatus* treated with *V. amygdalina* ash and powder at rate 2g/20g of cowpea seeds.

V. amygdalina	Oviposition	% number of progeny development
Ash (L)	6.25 ± 1.30^{ab}	9.25 ± 1.30^{a}
Ash (S)	2.25 ± 1.30^{a}	0.00 ± 0.00^{a}
Ash (R)	12.00 ± 1.90^{b}	8.33 ± 1.64^{b}
Powder (L)	7.75 ± 0.84^{ab}	12.90 ± 1.21^{b}
Powder (S)	3.00 ± 1.90^{a}	0.00 ± 0.00^{a}
Powder (R)	15.75 ± 0.84^{b}	19.05 ± 2.03^{b}
Untreated	50.00 ± 5.79^{c}	82.50 ± 7.50^{c}

Each value is a mean ± standard error of means (S.E.M) of four replicate means within column followed by the same letters (s) are not significantly different at (P> 0.05) using New Duncan's Multiple Range Test.

Keys: L – Leaf, S¬ – Stembark, R – Root

DISCUSSION

Results reported in this research shows that ash and powders of *V. amygdalina* parts have insecticidal effects on cowpea bruchid, *C. maculatus*. The stembark ash and powders applied as contact insecticides were very toxic to *C. maculatus* causing 100% mortality of cowpea bruchid at rate of 2g/20g of cowpea seeds within 4 days of application. The observed mortality and low progeny may be due to the thickening and hardening properties of the ash powders of *V. amygdalina* on the body of adult *C. maculatus* which may hinder mobility, and the choky effects of this powder also disrupt mating activities, sexual communication and inhibit locomotion hence, reducing oviposition and eradicating progeny development, an effect that has being reported by many researchers (Ofuya, 1992; Adedire, 2002; Maina and Lale, 2004; Adedire *et al.,*2011; Ileke *et al.,*2012). This is in agreement with the finding of Musa *et al.,* (2009) who reported the efficacy of *V. amygdalina* in the management of *C. maculatus*. Adedire and Lagide (2008) reported the effectives of *V. amygdalina* in the control of *S. zeamais* causing 100% mortality.

The plants also prevented oviposition and adult emergence of bruchid. The effect of the plant ash and powder on oviposition could be due to respiratory impairment which probably affects the process of metabolism and consequently other systems of the body of the bruchid (Osisiogu and Agbakwuru 1978; Onolemhemhem and Oigiangbe 1991; Adedire *et al*., 2011). Plant product has been reported to inhibit locomotion (Adedire *et al*., 2011); hence, the beetles were unable to move freely thereby affecting mating activities and sexual communication (Ileke*et al*., 2012). The inability of the eggs to stick to the cowpea seed due to the presence of the ached, powder and latex also reduced adult emergence arising from egg mortality (Adedire *et al*., 2011). It has been reported that one of the main mechanisms of action of plant extracts is their ability to penetrate the chorion of bruchid eggs via the micropyle and cause the death of developing embryos through asphyxiation (Don-Pedro 1996a, 1996b). The protectant ability of the extracts was highly remarkable. It is evident from this study that all the extracts tested have the potential of being used as biopesticides.

CONCLUSION AND RECOMMENDATION

Bitter leaf (*Vernonia amygdalina*) plant is medicinal, readily available, safe, eco-friendly, biodegradable and has not been reported to be toxic to man, this study has revealed its insecticidal potentials against stored cowpea and it is therefore recommended as an entomocide and could also serve as an alternative to synthetic insecticides for the protection of stored cowpea seeds against cowpea bruchid, *C.maculatus*.

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