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ENTOMOTOXICANT POTENTIAL OF BITTER LEAF, VERNONIA AMYGDALINA POWDER IN THE CONTROL OF COWPEA BRUCHID, CALLOSOBRUCHUS MACULATUS (COLEOPTERA: CHRYSOMELIDAE) INFESTING STORED COWPEA SEEDS

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Abstract: Powders from *Vernonia amygdalina* were evaluated for their efficacy as contact insecticides on cowpea bruchid, *Callosobruchus maculatus* in the laboratory at temperature of 30±2°C and 75±5% relative humidity. The powders were applied at rate 2g/20g of cowpea seeds. The result showed that the plant powders tested as contact insecticides significantly (P<0.05) reduced the number of adults bruchid. However, leaf of *V. amygdalina* was the most toxic to *C. maculatus* that evoked 100% mortality of adult cowpea bruchid after 72 hours 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 treated with the plant part powders showed that there was significantly (P<0.05) more % progeny development in the control compared to treated ones. This study showed that all the tested plant parts powders were toxic to cowpea bruchid and the powders can be mixed with cowpea seeds to prevent hatching of the eggs thereby helping in their management. From the study, the order of effectiveness of the plant powders could be ranked thus; Leaf>stem bark>root. Bitter leaf can be used as entomotoxicant against *C. maculatus* and its incorporation into traditional storage pest management is strongly recommended in developing countries.
Keyword: Beetle Perforation Index, *Callosobruchus maculates,* Entomotoxicant, *Vernonia amygdalina.*
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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 and Ajayi, 2003; Adedire *et al*., 2011). The dry seed consists of about 25% protein and 67% carbohydrate. It is also a good source of calcium, iron, vitamins and carotene (Adedire *et al*., 2011). 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 (Ajayi *et al*., 2000). *Callosobruchus maculatus* has caused enormous weight loss, reduced viability and reduced commercial value of cowpea seeds (Adedire and Akinneye, 2004). It has been reported that both quantitative and qualitative losses arising from physical, chemical and biological factors (e.g. fungi, rodents, birds and insects) occur during storage of grains (Adedire and Akinneye, 2004). The magnitude of infestation by *C. maculatus* for this important crop necessitates its control to avoid food shortage and promote self-sufficiency. To protect the cowpea seeds from insect infestation, some farmers and traders use synthetic insecticides, which are applied as sprays or in powder form to reduce quality loss. In Nigeria, the abuse and misuse of these chemical pesticides have several repercussions one of which is acute and chronic poisoning in man (Akunne and Okonkwo, 2006); others include sudden deaths, blindness, skin irritation and pest resurgence in the ecosystem (Omoloye, 2008; Akunne *et al*., 2013). Furthermore, the development of resistant strains, killing of non target species, pollution of part of the ecosystem, toxic residue, worker's unsafety and increasing costs are recorded as environmental repercussion of abuse and misuse of pesticides (Akunne and Okonkwo, 2006; Ofuya *et al*., 2008; Akunne *et al*., 2013). Plant materials that are safe, to the environment, users and consumers' alike, inexpensive, repellents and antifeedants need to be exploited as suitable alternatives to the expensive, toxic and environmentally unsafe synthetic insecticides (Isman, 2006; Akunne *et al*., 2013). Although several workers have demonstrated the possible application of powder or extracts from plant materials to *C. maculatus* (Maina and Lale, 1995). Moreso, researches have shown that botanicals have been extensively used on agricultural pests and to very limited extent on insect pests of stored products (Ufele *et al*., 2013). There have also been some degrees of success and achievements in the use of powders of some medicinal plants against insect pests of stored products (Brisibe *et al.*, 201; Akunne *et al*., 2013). *Vernonia amygdalina*, a member of the Asteraceae family, is a small shrub that grows in the tropical Africa with petiolate leaf of about 6 mm diameter and elliptic shape. It is commonly called "bitter leaf" because of its bitter taste. The bitterness can, however, be abated by boiling or by soaking the leaves in several changes of water. The bitter taste is due to anti-nutritional factors such as alkaloids, saponins, tannins, and glycoside (Juliar *et al*., 2005). The plant has being used traditionally to treat sexually transmitted diseases such as gonorrhea and malaria in rift valley and western parts of Kenya (Erasto *et al.,* 2007) and cancer cells (Farombi, 2004). The aqueous extract of this plant have been found to have cell growth inhibitory effects in prostate cancer cell line (Adebayo *et al*., 2014). The plant has antihelmintic, antitumorigenic, hypoglycaemic and hypolipidaemic activity and both the leaves and the roots are used traditionally in phytomedicine to treat fever, kidney heart disease and stomach discomfort (Farombi, E.O. and Owoeye, 2011). Many studies have shown that *V. amygdalina* extracts may strengthen the immune system through many cytokines (including NFκB, pro inflammatory molecule) regulation (Igile *et al*., 1994). The specific objective of this research is to investigate the effectiveness of *V. amygdalina* stem bark, leaf and root powders as contact entomocide in the management of *C. maculatus.*

EXPERIMENTAL

Insect culture

The initial culture of *C. maculatus* was obtained from infested cowpea seeds from the Environmental Biology Research Laboratory, Adekunle Ajasin University, Akungba Akoko, Ondo State, Nigeria. Eighty pairs of *C. maculatus*, sexed according to the report of Halstead (1963), Appert (1987), Odeyemi and Daramola (2000) were introduced into two litre plane glass kilner jar containing 300g of *Vigna unguiculata* variety Ife brown. Insect rearing and the experiments were carried out at ambient temperature of 30±2^{o}C and 75±5% relative humidity.

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^{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 and later air dried in the laboratory to prevent mouldiness (Adedire *et al*., 2011) before they were stored in plastic containers with tight lids.

Plant Collection

The plant evaluated in this work is *V. amygdalina* (leaf, stem and seed). They were obtained in fresh form, free of insecticides from Ipinsa community, Akure, Ondo State, Nigeria and authenticated by the Plant Science and Technology Department of Adekunle Ajasin University, Akungba Akoko, Ondo State. This plant material were 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^{o}C prior to use.

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 (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 (Ileke, 2014). 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 and Daramola, 2000).

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

Fine powders of *V. amygdalina* were admixed with cowpea seeds at the rate 2.00g/20g 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 hours for 96 hours. 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:

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

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 beetle 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 16.0 software (SPSS, 2007).

RESULTS AND DISCUSSION

Toxicity of *Vernonia amygdalina* powder to *Callosobrochus maculatus*
The effectiveness of leaf,stem bark and root powders 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, leaf powder was the most toxic to *C. maculatus* that evoked 100% mortality of adult cowpea bruchid after 72 hours 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* leaf powder were more toxic than the other tested plant powder parts (stem bark and root).

Fecundity of *C. maculatus* treated with *Vernonia amygdalina* powders
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 leaf completely inhibiting the emergence of *C. maculatus* (100% efficiency).

Protectabilty of *V. amygdalina* powders on cowpea seeds
Vernonia amygdalina stem bark powder completely prevented infestation and damage of the treated cowpea seeds (Table 3). There was neither seed damage nor weight loss recorded in the treated cowpea seeds. Beetle Perforation Index (BPI) was zero for *V. amygdalina* leaf powder except in seeds treated with *V. amygdalina* stem bark and root powders that recorded 2.19 and 3.19% for seed damage respectively. However, the BPI of 3.30 and 5.04 were recorded on seeds treated with *V. amygdalina* stem bark and root powders respectively. In the untreated cowpea seeds, 43.42% damage occurred as revealed by emergent holes of the bruchids. As a result of the feeding activity of *C. maculatus* larvae on the cowpea seeds, the weight of the untreated cowpea seeds was significantly ($P< 0.05$) reduced compared with the treated seeds with *V. amygdalina* powders.

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

V. amygdalina powders	Mean % Mortality + SE on Days			
	1	2	3	4
Powder (S)	15.00 ± 2.89^{bc}	35.00 ± 2.89^{bc}	60.00 ± 4.08^{bc}	87.00 ± 2.50^{cd}
Powder (L)	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 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

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

V. amygdalina	Ovi-position	% number of progeny development
Powder (S)	7.75 ± 0.84^{ab}	12.90 ± 1.21^{b}
Powder (L)	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 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

Table 3: Protectability of *V. amygdalina* powder on cowpea seeds

V. amygdalina	**Mean total number of seeds**	**Mean number of damaged seeds**	**Mean % of seeds damaged**	**% weight loss**	**Beetle perforation index (BPI)**
Powder (S)	91.50	2.00 ± 0.03[a]	2.19 ± 0.05[a]	3.04 ± 0.07[a]	5.04 ± 1.31[b]
Powder (L)	94.50	0.00±0.00[a]	0.00±0.00[a]	0.00±0.00[a]	0.00±0.00[a]
Powder (R)	94.00	3.00 ± 0.07[a]	3.19 ± 0.07[a]	4.85 ± 1.01[a]	3.30 ± 1.42[b]
Untreated	95.00	41.25±4.11[b]	43.42±4.21[b]	75.65±3.26[b]	50.00±0.00[c]

Each value is a mean ± standard error 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

Effect of *V. amygdalina* powder on viability of stored cowpea seeds

The percentage of cowpea seeds that germinated after treatment with powder of *V. amygdalina* parts is presented in Table 4. At the end of seven-day germination period, all the treated seeds recorded high germinability. The untreated cowpea seeds and seeds treated with *V. amygdalina* leaf, stem bark powders and control had the highest percentage germination of 100%. The least percentage germination was recorded in *V. amygdalina* leaf powder which had 95% viability. However, this value was not significantly different from the germination observed in other treatments. Phytochemicals derived from plant sources can act as larvicides, insect growth regulators, repellents and ovipositor attractants, and these different activities have been observed by many researchers (Venketachalam and Jebasan; Ishii *et al*., 2010; Ntonifor *et al*., 2011; Asmanizar *et al*., 2012). Plants are considered rich sources of bioactive chemicals and may be an alternative source of insect control agents so as to ensure food security in developing countries such as Nigeria. Results reported in this research shows that powders of *V. amygdalina* parts have insecticidal effects on cowpea bruchid, *C. maculatus.* The leaf powder 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. This agrees with the report of Moses and Dorathy (2011) who reported that bitter leaf gave the best protection against cowpea weevil when compared with garlic and ginger. Musa *et al.* (2009) also reported on efficacy of Mixed Leaf Powders of *V. amygdalin* and *Ocimum gratissimum* against *C. maculatus.* Akunne *et al*. (2013) reported on efficacy of mixed application of leaf powders of *V. amygdalina* and *Azadirachta indica* against adult *C. maculatus.* The observed mortality and lower adult emergence could result from death of immature stages as a result of treatment, an effect that has been 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 Lajide (2008) reported the effectives of *V. amygdalina* in the control of *S. zeamais* causing 100% mortality. The insecticidal effects of this plant powders on the beetle could be linked to the presence of some chemical compounds like sesquiterpene lactones containing vernodalin, vernodalol and 11, 13-dihydrovernodalin, these have insecticidal properties which act as an insect feeding deterrent (Pascual *et al*., 2001). The plants also prevented oviposition and adult emergence of bruchid. The effect of the plant powder on oviposition could be due to respiratory impairment which probably affects the process of metabolism of the bruchid (Osisiogu and Agbakwuru 1978; Onolemhemhem and Oigiangbe 1991; Adedire *et al*., 2011; Ileke *et al*., 2012). Plant product has been reported to inhibit locomotion (Adedire *et al*., 2011); hence, the beetles were unable to move freely thereby affecting mating activities (Ileke et al. 2012). Reduction in progeny development may be due to early mortality and partial or complete retardation of embryonic development (Dike and Mbah, 1992). There was no marked difference between the percentage germination in treated cowpea seeds compared with the untreated. This shows that powders of *V. amygdalina* parts have no adverse effect on germination. It had been reported that seeds treated with powders and extracts did not loose their viability (Das, 2002; Onu and Aliyu, 1995; Keita *et al*., 2001). Bitter leaf, *V. amygdalina* plant is medicinal, readily available, safe, eco-friendly and has not been reported to be toxic to man, this study has revealed its insecticidal potentials against stored cowpea bruchid and it is therefore recommended as an entomotoxicant against *C. maculatus.*

Table 4: Effects of *V. amygdalina* powder on viability of stored cowpea seeds

V. amygdalina	**% Viability**
Powder (S)	100.00 ± 0.00[a]
Powder (L)	100.00 ± 0.00[a]
Powder (R)	95.00 ± 2.89[a]
Untreated	100.00 ± 0.00[a]

Each value is a mean ± standard error 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.

Phytochemicals derived from plant sources can act as larvicides, insect growth regulators, repellents and ovipositor attractants, and these different activities have been observed by many researchers (Venketachalam and Jebasan; Ishii *et al*., 2010; Ntonifor *et al*., 2011; Asmanizar *et al*., 2012). Plants are considered rich sources of bioactive chemicals and may be an alternative source of insect control agents so as to ensure food security in developing countries such as Nigeria. Results reported in this research shows that powders of *V. amygdalina* parts have insecticidal effects on cowpea bruchid, *C. maculatus.* The leaf powder 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. This agrees with the report of Moses and Dorathy (2011) who reported that bitter leaf gave the best protection against cowpea weevil when compared with garlic and ginger. Musa *et al.* (2009) also reported on efficacy of Mixed Leaf Powders of *V. amygdalin* and *Ocimum gratissimum* against *C. maculatus.* Akunne *et al*. (2013) reported on efficacy of mixed application of leaf powders of *V. amygdalina* and *Azadirachta indica* against adult *C. maculatus.* The observed mortality and lower adult emergence could result from death of immature stages as a result of treatment, an effect that has been 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 Lajide (2008) reported the effectives of *V. amygdalina* in the control of *S. zeamais* causing 100% mortality. The insecticidal effects of this plant powders on the beetle could be linked to the presence of some chemical compounds like sesquiterpene lactones containing vernodalin, vernodalol and 11, 13-dihydrovernodalin, these have insecticidal properties which act as an insect feeding deterrent (Pascual *et al*., 2001). The plants also prevented ovi-position and adult emergence of bruchid. The effect of the plant powder on ovi-position could be due to respiratory impairment which probably affects the process of metabolism of the bruchid (Osisiogu and Agbakwuru 1978; Onolemhemhem and Oigiangbe 1991; Adedire *et al*., 2011Ileke *et al*., 2012). Plant product has been reported to inhibit locomotion (Adedire *et al*., 2011); hence, the beetles were unable to move freely thereby affecting mating activities (Ileke et al. 2012). Reduction in progeny development may be due to early mortality and partial or complete retardation of embryonic development (Dike and Mbah, 1992). There was no marked difference between the percentage germination in treated cowpea seeds compared with the untreated. This shows that powders of *V. amygdalina* parts have no adverse effect on germination. It had been reported that seeds treated with powders and

extracts did not lose their viability (Das, 2002; Onu and Aliyu, 1995; Keita *et al*., 2001).

CONCLUSION

Bitter leaf, *V. amygdalina* plant is medicinal, readily available, safe, eco-friendly and has not been reported to be toxic to man, this study has revealed its insecticidal potentials against stored cowpea bruchid and it is therefore recommended as an entomotoxicant against *C. maculatus.* However, further research is required as to know the effect of this plant on mammals when used at high concentration.

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