



Evaluation of the Efficacy of Ethanolic leaf extract of *Vernonia amygdalina* on the oviposition, egg hatchability and adult emergence of *Callosobruchus maculatus*(f.) on treated Cowpea seed

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Abstract

The efficacy of *Vernonia amygdalina* ethanolic leaf extract against the oviposition, egg hatchability and adult emergence of *Callosobruchus maculatus* (F.) on cowpea treated seed was evaluated at the New Biology Laboratory, Department of Science Laboratory Technology, Kano State Polytechnic. Leaf extract from *V. amygdalina* was obtained by Soxhlet extraction method. *C. maculatus* were obtained along with infested cowpea grains from Dawanau market in Kano State of Nigeria. The insects were reared in the laboratory and four different concentrations of the leaf extract (1.0, 1.5, 2.0, and 2.5g) were separately mixed with twenty gram (20g) of cowpea in separate Petri-dishes which correspond to (5.0, 7.5, 10, and 12.5 w/w %) respectively. Control treatment was also set along. Five (5) adult pairs of newly emerged *C. maculatus* were introduced into each Petri dish. Oviposition and egg hatchability of the female insect was significantly reduced on seed treated with higher treatment level (10.0 and 12.5 % w/w) of the extract at ($p < 0.05$), in comparison with the control treatments. *C. maculatus* adult emergence from seed with varying dose of *V. amygdalina* was significantly reduced ($p < 0.05$) compared to other treatments. *V. amygdalina* plant leaf extract recorded low oviposition (3.3 ± 0.10), low eggs hatchability (0.33 ± 0.19) and lower F1 progeny adult emergence (0.00 ± 0.00), when compared with untreated control treatment ($P < 0.05$). Therefore the plant has shown a strong effectiveness similar to that of synthetic insecticide in the preservation of cowpea seeds against *C. maculatus* during storage. It was recommended that government should encourage more planting of these plants that possess insecticidal properties.

Keywords: Adult emergence, *Callosobruchus maculatus*, leaf extract, oviposition and *Vernonia amygdalina*.

INTRODUCTION

Cowpea (*Vigna unguiculata* (L.) walp) is one of the major food (legumes) cultivated commercially in most tropics and sub-tropics (Basher *et al*; 2002). It is truly a multinational crop, providing food for man and livestock and serving as a valuable and dependable revenue-generating commodity for farmers and traders (Singh, 2002; Langyinto *et al*; 2003). It is also a valuable component of farming systems in areas where soil fertility is limiting. This is because cowpea has a high rate of nitrogen fixation (Elawad and Hall, 1987) and forms effective symbiosis with mycorrhizae (Kwapala and Hall, 1985). It accounts for about 60% of human protein intake in Nigeria (Oparaeke *et al*; 1998). The seeds and leaves are very rich in protein (24-33%) and are used in the preparation of various dishes for food and feed (Bressani, 1985). *Callosobruchus maculatus* is responsible for a great deal of damage to the seeds making it unfit for human consumption. Insect pests constitute a major factor militating against food availability and security (Ofuya

and Adedire, 2004). Cowpea bruchid (*C. maculatus*) (Fab) is a major pest of wide range of stored legume seeds especially of the cowpea *V. unguiculata* (Ofuya, 2001). Cowpea are rapidly broken down by the cowpea beetle within three to five months in storage (Ajayi and Lale, 2000; Mbailao *et al*, 2006). During storage, the cowpea beetle (*C. maculatus*) causes heavy qualitative and quantitative losses. The damaged seeds are unsuitable for human and animal consumption and cannot be used for planting. There is need to control these insect pests due to their destructive activities so as to maintain the quality of the products. The common control measures were the use of synthetic insecticides due to their swift action in eradicating the pests. Their use is being discouraged due to associated human and environmental problems, such as insect pests resistance to insecticides, environmental pollution, high cost of purchase, non-availability as well as being toxic to man and livestock (Akob and Ewete, 2007).

These drawbacks have necessitated the need for sustainable alternatives measures such as plants with natural insecticidal potency, that are easily biodegradable, environmentally friendly and safe to both producers and consumers (Ewete, *et al.*, 1996). Based on this, the present study was conducted to evaluate the efficacy of the leaf extracts of *V. amygdalina* on *C. maculatus* on treated cowpea seed. 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 and Owoaye, 2011).

MATERIALS AND METHODS

Study area

The study was conducted at New Biology Laboratory, School of Technology, Kano State Polytechnic, between the periods of March to August 2016. Latitude 11°05'24" N and Longitude 8° 32'67" E.

Collection and identification of Plant materials

Fresh leaves of *V. amygdalina* plant were collected at DanMalikitown, in Kumbotso Local government, of Kano State. These were identified and authenticated at the herbarium section, Plant Biology Department, Bayero University, Kano, Nigeria. Using identification taxonomy guide.

Processing of plant materials

Plant materials were air-dried under a room temperature for about 2 weeks following the procedure described by (Adesina, 2012). The dried leaves were pounded into powder using mortar and pestle as described by (Epidi *et al.*, 2009). The powdered particles were sieved using 0.01mm mesh size to obtain fine particles. The sieved powdered particles were kept in a glass bottle until required for extraction.

SOXHLET EXTRACTION

Solid plant materials were extracted using Soxhlet extraction method as described by (Chemat *et al.*, 2011). The filtrates were evaporated using rotary evaporator, after complete evaporation, the final crude extracts from the plant was then weighted, and preserved in sealed bottles in a refrigerator until required for bioassay.

COLLECTION OF COWPEA SEEDS

Clean cowpea seeds (*V. unguiculata* (IT93K-452-1)) were obtained from International Institute for Tropical Agriculture (IITA), Kano State, Nigeria.

PROCESSING OF COWPEA SEEDS

Obtained seeds were placed in plastic bags and kept in the freezer overnight to eliminate any possible beetle infestation coming from the field (Marcileyne *et al.*, 2004). The seeds were removed from the freezer and kept at room temperature and relative humidity for some hours to equilibrate and the moisture content of the seeds was determined before the experiment (Jacka and Asante, 2001).

COLLECTION OF TEST INSECTS AND IDENTIFICATION

A small population of cowpea beetles (*C. maculatus*) along with naturally infested cowpea seeds were obtained at Dawanau Market in Dawakin Tofa local Government area of Kano state, Nigeria. The insects were identified and authenticated at Crop protection Department, faculty of Agriculture, Bayero University Kano, Nigeria prior to rearing using identification taxonomy guide.

INSECT REARING

Insect rearing was done according to the method described by (Tapondju *et al.*, 2002). Insects were reared and bred under laboratory conditions at ambient temperature of $28 \pm 2^\circ\text{C}$ and relative humidity $75 \pm 5\%$ on the seeds of cowpea (*V. unguiculata*). Initially, 60 pairs of 1-2 day old adults were placed in a plastic bucket containing 6kg of cowpea seeds, and allowed to mate and oviposit for about 7 days (Tapondju *et al.*, 2002). Then parent stocks were removed and cowpea seeds containing eggs were covered with pieces of cloth fastened with rubber band to facilitate proper aeration and prevent the contamination and escape of beetles (Tapondju *et al.*, 2002). The subsequent progenies of the beetles were used for the experiments.

Bioassay

Bioassay were conducted based on the method described by (Talukder and Howse, 1994). Four different diluted concentrations of extract from the leaf of *V. amygdalina* (1.0, 1.5, 2.0, and 2.5g) were designed after a trial experiment. These were separately mixed with twenty grams (20g) of cowpea in separate Petri dishes which correspond to 5.0, 7.5, 10.0, and 12.5% W/W respectively. Cowpea seeds which were mixed with different concentrations of the extract were shaken properly to ensure proper coating of the seeds with the extract. The seeds were then air-dried for one hour to evaporate the solvent (Talukder and Howse, 1994).

Cypermethrin dust (positive control) was set up as standard chemical insecticides. Control treatment was also set along (which has neither extract nor Cypermethrin dust). Five pairs of the beetle *C. maculatus* that freshly emerged from the culture were released into each treatment. These were covered with a muslin cloth to facilitate proper aeration and prevent entry and exit of insects. Each treatment were replicated three times and arranged in a completely randomized design (CRD) and left on the laboratory bench for daily observation (Oparaeke, 1996). Mortality of the insect was observed after 24 hours interval for a period of 96 hours after treatment. The number of eggs laid was counted separately for each treatment on the 14th day after the introduction of beetles to seeds; this was used to calculate the percentage of egg hatching and percentage adult emergence respectively according to Abdullahi *et al.* (2011). All the eggs laid in different Petri dishes were examined and the viable eggs were identified. Viable eggs were recognized by their morphological aspects (Marcileyne *et al.*, 2004), since they become opaque as a function of their residue discharged by the larvae during penetration. The % adult emergence was also determined on 35 days after the introduction of beetles to the seeds.

Statistical analysis

One way analysis of variance (ANOVA) was used to analyse the data and least significant difference (LSD) was conducted to find out where differences in means exist using OpenStat Statistical Software (Version 08. 12. 14).

RESULTS AND DISCUSSION

The results showed that *V. amygdalina* leaf extract at all level of concentrations including cypermethrin treated seeds significantly ($P < 0.05$) reduced the oviposition and egg viability of *C. maculatus* in comparison with the untreated control treatments (Table 1). The reduction of the oviposition and egg viability by the extracts was found to be higher on seed treated with higher doses of the extract (10.0 % w/w and 12.5% w/w) and lower on seeds treated with lower doses of the extract (5.0% w/w and 7.5 % w/w) and the differences were found to be significant ($P < 0.05$). The result also showed that as the concentration increases

the number of eggs laid and egg viability also decreases. Therefore the result indicated the possibility of having a strong oviposition deterrent and ovicidal effect in the extract. The finding in this study was in accordance with the finding of Abdullahi, (2011) who tested the extract of *Balanite aegyptiaca* as oviposition deterrent against *C. maculatus* and reported that the seeds treated with extract acted as highest ovipositional deterrents and ovicidal effects. Elhag, (2000) tested the extracts from nine plant materials as oviposition deterrents against *C. maculatus* and found that pulse treated with *Rhazya stricta* leaves, neem seeds, *Heliotropium bacciferum* aerial parts and citrus peels acted as highest ovipositional deterrents. The ovicidal effect of the extract on the bruchid may also be explained in terms of asphyxiation by blocking a channel which is probably the major route of gas exchange between a thin area of the chorion and the outside (Credland, 1992). Jacob and Thomas, (2015) tested the ovicidal effect of *Secamone afzelii* leaf extract against *C. maculatus*, and reported that the maximum reduction in egg hatching was noticed on seeds treated with high doses.

Result from adult emergence showed that the adult emergence were highly reduced in all the concentrations level including the positive control (Table 2). The differences observed was found to be significant ($P < 0.05$) in comparison with the untreated control. The reduction in adult emergences was found to be higher on seed treated with higher doses of the extract (10.0 and 12.5% w/w). The reduction may be due to the decrease in the hatching rate of the eggs and also the number of eggs laid. This was in agreement with the finding of Ketohet *al.* (2006) who reported that Cymbopogon oil vapour treatment for 24 hours could be satisfactory for controlling the eggs hatchability of *C. maculatus*. Ibrahim and Aliyu, (2014) reported that there was no progeny emergence of *C. maculatus* two months after storage using bitter leaf powder at the rate of 3.0g. Adebowale and Adedire, (2006) also reported that the cowpea seeds treated with *Jatropha curcas* seed oil, reduced the number of eggs laid by *C. maculatus* and prevented the adult emergence.

Table 1: Mean Number of Oviposition and Eggs Viability of Cowpea Seeds Treated With Different Plant Leaf Extract.

Treatment	Concentration (W/W%)	Weight of cowpea	No of insect used	Mean no. of eggs laid	Mean no. of egg hatched	% eggs hatching
<i>V. amygdalina</i>	1.0 (5.0)	20	10	46.7±1.11 ^f	25.0±0.14 ^e	53.53
	1.5 (7.5)	20	10	40.0±0.37 ^e	18.3±0.10 ^c	45.75
	2.0 (10.0)	20	10	9.0±0.05 ^b	3.7±0.05 ^a	41.11
	2.5 (12.5)	20	10	3.3±0.10 ^a	0.33±0.19 ^a	10.00
Control (-ve)		20	10	124±0.28 ⁱ	108±0.54 ^h	87.09
Control (+ve) cypermethrin	1.0 (5.0)	20	10	0.00±0.00 ^a	0.00±0.00 ^a	0.00
L.S.D (0.05)				4.69	5.13	

Mean ± SE with the same letter are not significantly different from each other by (LSD P < 0.05)

Table 2: Mean Number of Adult Emergence of *C. maculatus* from Cowpea Seeds Treated With Different Plant Leaf Extract.

Treatment	Concentration (w/w %)	Weight of cowpea	No of insect used	Mean no. of eggs laid	Mean no. of adult emerged	%adult emergence
<i>V. amygdalina</i>	1.0 (5.0)	20	10	46.7±0.11 ^f	15.7±0.13 ^c	33.61
	1.5 (7.5)	20	10	40.0±0.37 ^e	13.0±0.50 ^c	32.5
	2.0 (10.0)	20	10	9.0±0.05 ^b	1.00±0.31 ^a	11.11
	2.5 (12.5)	20	10	3.3±0.10 ^a	0.00±0.00 ^a	0.00
Control (-ve)		20	10	124±0.28 ⁱ	106.7 ±0.48 ^h	86.04
Control (+ve) cypermethrin	1.0 (5.0)	20	10	0.00±0.00 ^a	0.00 ±0.00 ^a	0.00
L.S.D (0.05)				4.69	5.19	

Mean± (SE) with the same letter are not significantly different from each other by (LSD P < 0.05)

Conclusion

V. amygdalina plant leaf extract recorded low oviposition (3.3±0.10), low eggs hatchability (0.33±0.19) and lower F1 progeny adult emergence (0.00±0.00). Therefore the plant has shown a strong effectiveness similar to that of synthetic insecticide in the preservation of cowpea seeds against *C. maculatus* during storage.

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Recommendations

1. Government and non-governmental organization should encourage more planting of this plants that possess insecticidal activity.
2. Further studies should be carried out to identify and isolate the bioactive constituents responsible for this insecticidal activity.

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