Original Article

EFFICACY OF MIXED AND SINGLE APPLICATION OF LEAF POWDERS AND EXTRACTS OF *VERNONIA AMYGDALINA* (BITTER LEAF) AND *AZADIRACHTA INDICA* (NEEM) ON THE COWPEA BRUCHID, *CALLOSOBRUCHUS MACULATUS*

Onekutu Amana, *Iboyi Mark Ojonugwa and Nwannunu Ifeoma Ude

Department of Zoology, Joseph Sarwuan Tarka University, P.M.B. 2373, Benue State, Nigeria

ABSTRACT

The efficacy of Vernonia amygdalina and Azadirachta indica leaf powder and solvent extract against aspects of the biology of Callosobruchus maculatus (F.) on cowpea seed was evaluated under ambient temperature at the Biological Sciences Laboratory, Joseph Sarwuan Tarka University, Makurdi. Plant leaf powders were obtained by grinding the plant leaves using electric grinder and sieving with appropriate mesh size while leaves extracts were obtained by soaking 200g each of the powdered plant materials separately in 1 liter of methanol, n-hexane and ethyl acetate, filtered and the residue evaporated at 40˚C. The stock was used to prepare the treatments used for the study. Adult Callosobruchus maculatus collected from infested cowpea seeds at market storage site in Makurdi were used to setup an insect culture. Newly emerged bruchids were exposed to different concentrations. The highest adult mortality was recorded in seeds treated with 1g (9.3±0.7) bitter leaf powder ($p<0.05$). Similarly, least oviposition count was in cowpea seeds treated with 1.5g bitter leaf powder (3.3±0.07) while there was no record of adult bruchid emergence in seeds treated with 2g of bitter leaf powder (0.0±0.00) ($p<0.05$). When combined, the treatment ratio B75.N25 and B50.N50 significantly deterred oviposition in the bruchid while the ratio B50:N50 and an application rate of 1g to the cowpea seeds completely deterred adult emergence in the treated seeds ($p<0.05$). However, the n-hexane bitter leaf extract proved to be the most effective against the adult C. maculatus at the rate of 0.5%, recording a mean mortality of 4.00±0.0 ($p<0.05$). It was also observed that seeds treated with equal ratios of plant powder (1:1) recorded the least mean weight loss, 6 .2 ± 0.92 ($p<0.05$).

KEYWORD: **Mixed, Single, Vernonia Amygdalina, Azadirachta Indica, Extract, Callosobruchus maculatus**

Open Access
Correspondence;

Email:
*iboyi.mark@uam.edu.ng,
aonekutu63@gmail.com

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INTRODUCTION

Cowpea, (*Vigna unguiculata* (L) Walpers, Fabaceae), is an important edible legume crop in many parts of the world especially in the tropical and subtropical regions. It is used as human food due to its high protein content (Diouf, 2011) and also as livestock feed to make silage and hay. Africa was reported to account for about 70% of total world production of cowpea (IITA, 2007). All parts of the plant used as food are nutritious providing protein and vitamins, immature pods and peas are used as vegetables while several snacks and main dishes are prepared from the grains (Islam *et al*., 2006). Insect pests constitute the most visible and important constraint to cowpea production, infesting mature pods and accounting for post-harvest reduction of grains (Musa, 2012). It is infested by the bean bruchid, *Callosobruchus maculatus* (F.) between harvest and storage, leading to quantitative and qualitative losses of grains. The larvae feed within the grains and consume the endosperm. The adults leave neat circular exit holes in the grains after emergence (Musa and Adeyeye, 2015). Although, the crop is destroyed by bruchids in storage thereby causing reduction in the market values, nutrients and as a stock source, the bruchids prefer dried cowpea seeds but will attack other beans and peas in storage.

Synthetic insecticides have proved very effective in the control of the bruchid because of their speedy action and easy application (Mullai and Jebanesan, 2007) but they are toxic and adversely affect the environment by contaminating soil, water and air. Its application also leads to residual problems. Other problems associated with the use of synthetic insecticides for controlling stored product insects pests include their residual effect on grains, development of resistance in insect populations and effects on non-target organisms (Iram *et al*., 2013).

For these reasons, there is a steady increase in the use of plant products as an easier and safer means of protecting small-scale stored products against insect infestation. According to Dougond *et al*. (2019) homemade botanical insecticides are widely used by farmers in low income countries; their use is often driven by the limited availability or cost of commercial pesticides. Kouninki *et al*. (2007) pointed out that synthetic chemical pesticides cannot be used in association with flour or sources of provender because of their direct consumption by humans and animals. Hence, eco-friendly pest control strategies are very much essential to protect food grain in a safe way. Plant materials with insecticidal properties are considered to be useful for post-harvest practices and particularly relevant for small scale farmers for storage of their commodities. The efficacy of these plant products is attributed to the presence of secondary metabolites which are responsible for diverse insecticidal properties (Fotso *et al*., 2019). Furthermore, botanical pesticides are promising in that they are effective, environment – friendly, easily biodegradable, and also inexpensive (Dharmagadda *et al.,* 2005).

This work is aimed at determining the efficacy of mixed and single application of leaf powders and extracts of *Vernonia amygdalina* (bitter leaf) and *Azadirachta indica* (neem) on the cowpea bruchid, *Callosobruchus maculatus.*

MATERIAL AND METHODS

Establishment of Insect culture

The study was conducted in the Biological Sciences Laboratory, Joseph Sarwuan Tarka University Makurdi. Adults *Callosobruchus maculatus* were collected from infested cowpea seeds at market

storage site in Makurdi. An insect culture was established using clean, uninfested and untreated cowpea seeds which were air dried under the sun for 3 days and infested with *C. maculatus* adult. It was maintained under laboratory condition throughout the study period (Akunne *et al*., 2013).

Collection and Processing of Plant Materials

Preparation of powdered material

Fresh leaves of neem and bitter leaf collected from the field were washed thoroughly with tap water to remove dirt, and dried under shade till the weight was constant to prevent denaturation of chemical substances (Akunne *et al*., 2013). After which they were powdered using electric blender and sieved to obtain fine powders. The powders were stored separately in polythene bags and used to prepare different concentrations by weight of the treatments used.

Extraction of plant material

Extracts of neem and bitter leaf were prepared by soaking 200g each of the powders separately in 1 liter of methanol, n-hexane and ethyl acetate after which they were well agitated for proper mixing and kept at room temperature for 48 hours. Extracts were filtered after 48 hours using a muslin cloth and the filtrates concentrated at 40˚C using water bath to obtain the stock. Different concentrations were prepared by mixing 0.25, 0.5, 0.75 and 1.0g each of the respective stock materials prepared separately in 10 ml of methanol, ethylacetate and n-hexane in clean labeled test tubes, stopped with a plug. The test tubes were shaken vigorously to enable proper mixing and allowed to stay for 24 hours after which the contents were filtered off using Whatman no 42 (125mm) filter paper.

Effect of plant materials on the biology of Bruchids

Effect of plant powders on adult mortality

Effect of powdered plant material on the mortality of the adult bruchids was evaluated at four different concentrations. Each of the powders (bitter leaf and neem) was applied at the rate of 0, 1, 1.5 and 2 g per 10g of clean uninfested cowpea seeds in separate plastic containers (Akunne *et al*., 2013). The mixtures were shaken thoroughly to ensure proper mixing and 5 adult pairs of healthy male and female bruchids (1-2 days old) introduced into each set up and left for 15 days. Each of the treatment was replicated four times.

Insecticidal activity of plant powders was recorded after 5, 10 and 15 days of post exposure (Obadofin and Fatoba, 2014). Number of dead insects were counted and removed from the container with each check.

Effect of powdered plant material on oviposition and adult emergence

Effect of plant materials on oviposition of adult bruchid was evaluated at four different concentrations for adult mortality. A setup similar to the previous was established with 5 replicates and left for 15days. The number of eggs laid were counted and recorded for six consecutive days. After the sixth day, both dead and live bruchids were taken out while the cowpea seeds were left undisturbed for adult bruchids to emerge. The emerged adults were then counted and recorded for six consecutive days (after 21 days) (Onekutu *et al*., 2017).

Effects of powdered mixture on the different developmental stages of the bruchid

To evaluate the effects of the plant mixtures on the biology of the bruchids, similar set ups as explained above for adult

mortality, oviposition and adult emergence were made. The plant materials were then combined in different ratios of 0:0, 100:100, 75:25, 25:75 and 50:50 in the form, bitter leaf: neem and were applied at a rate of 1g, 1.5g and 2.0g respectively per 10g seeds. The various data were noted and recorded ingly as in the single powder application explained above.

Evaluation of insecticidal activity of plants extract

Insecticidal activity of the plants extracts were tested by topical application. Four set ups were made containing 10 healthy insects of separate sexes selected randomly and methanol extracts applied directly to insect body (topically) by using fine brush at different concentrations and kept in a test tube (Obadofin and Fatoba, 2014). The test tubes were plugged with cotton wool. This process was repeated for the ethyl acetate and the n-hexane extracts. Five replicates were made for each treatment. The number of dead insects were counted and recorded continuously for 96 hours with 24 hour interval between each count.

Effect of plant extracts on the quality of cowpea seeds

For each of the set ups used, 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:

$$Percentage\ seed\ damage = \frac{Number\ of seeds\ damaged}{Total\ number of seeds} X\ 100$$

(Ileke, 2015)

Statistical Analysis

The data collected from the study was entered in Microsoft Excel 2010 and exported into SPSS version 20.0 for analysis using ANOVA at 5% level of significance. Least Significant Difference (LSD)

RESULTS

Table 1 shows the mean mortality of adult *Callosobruchus maculatus* exposed to Neem and Bitter leaf powder at different concentrations. Highest mortality of adult *C. maculatus* was recorded with bruchids exposed to bitter leaf powder at a concentration of 1g (9.3±0.7). On the other hand, highest adult mortality recorded on neem powder was observed on 1g and 1.5g application rate (7.0±0.00) ($p<0.05$). LSD indicated that there was a significant difference ($p<0.05$) in the mortality recorded from the different concentrations of plant materials with the exception of the control and the 1.5g treatment concentration.

The least oviposition count was recorded on the cowpea seeds treated with 1.5g of bitter leaf powder (3.3±0.07). Similar result was also obtained when the cowpea seeds were treated with 1.5g neem powder (10.3±0.41). Even though, the oviposition count recorded using neem powder was higher compared to bitter leaf powder. ($p<0.05$). Oviposition count was significantly higher in the control experiment (Table 2). However, post hoc test indicated a significant difference ($p<0.05$) in the oviposition count in the different treatment concentrations from the plant materials used. On the other hand, least adult emergence was observed in cowpea seeds treated with 2g of bitter leaf powder 0.0±0.00 and seeds treated with 1g of neem powder 1.8±0.01 ($p<0.05$). Similarly, post hoc test indicated a significant difference

($p<0.05$) in the emergence of the adult bruchids in the different treatment concentrations from the plant materials used even though, this was not the case with the control and the 1g treatment concentration in both groups.

Mixed ratio of Neem and Bitter leaf powder had significant effect on the mortality, oviposition and adult emergence of *C. maculatus* exposed to different percentages of the plant materials as shown in Table 3. Highest adult mortality was recorded using 100% bitter leaf powder at 1.5g, 9.33±0.07. None of the mixtures recorded any similar results. However, when combined at the ratio B75.N25 and B50.N50 and applied at the rate of 2.0g, the plant materials were able to deter oviposition in the bruchids at the rate 4.8 ±0.28 and 4.8 ±0.31 respectively even though, the least oviposition count (4.2 ±0.04) was recorded with sole application of bitter leaf powder (B100) applied at the same rate. Furthermore, though sole application of the powdered materials at the rate of 2.0g resulted in no adult emergence from both seeds treated with bitter leaf and neem powder, it was observed that powdered mixture at the ratio B50:N50 and an application rate of 1g as well as the sole application of the neem powder and an application rate of 1.5g to the cowpea seeds also completely deterred adult emergence in the treated seeds ($p<0.05$). LSD indicated that apart from the control, the Bitter leaf powder applied at a concentration of 1g and the B50:N50 applied at the concentration of 2.0g, the mean mortality, oviposition and adult emergence count across the other groups showed various degree of variability ($p<0.05$).

Table 4 shows the effects of extracts of both leafs on mortality of adult *C. maculatus.* From the result obtained, it was observed that though each of the extracts had an effect on the adult *C. maculatus*. N-hexane bitter leaf extract proved to be the most effective against the adult *C. maculatus* when applied at the rate of 0.5%, recording a mean mortality of 4.00±0.0 ($p<0.05$). Apart from the ethyl acetate extracts applied at the rate of 0.5%, all the other treatment concentrations were significantly ($p<0.05$) different from each other. The LSD revealed that apart from the ethyl acetate extracts applied at the rate of 0.5% all the other treatments differed significantly from each other ($p<0.05$).

On the other hand, mean weight loss of the cowpea seeds (Table 5) was significantly different across the test plants ($p<0.05$). Control recorded the highest mean weight loss, 1.0 ± 1.11. However, seeds treated with equal ratios of plant material (1:1) recorded the least mean weight loss, 6.2 ± 0.92. Similarly, post hoc test indicated that no significant difference ($p>0.05$) occurred within cowpea seeds treated with bitter leaf and neem powder however, seeds treated with mixed ratio of plants material (1:1) significantly differed ($p<0.05$) in their weights from the seeds that received no treatment at all (control).

Table 1: Mean mortality of *Callosobruchus maculatus* exposed to Neem and Bitter leaf powder

Concentration (g)	**Mean mortality ± Standard error**	
	Bitter leaf	**Neem**
0.0	1.0±0*	1.0±0*
1.0	$9.3±0.07^{a}$	$7.0±0.00^{b}$
1.5	$7.5±0.03^{b}$	$7.0±0.00^{b}$
2.0	$7.3±0.07^{b}$	$6.0±0.00^{c}$

Means with different subscripts are statistically significant at $p<0.05$

*=Means control

Table 2: Mean number of ova laid by *Callosobruchus maculatus* exposed to Neem and Bitter leaf powder

Concentration (g)	Oviposition ± Standard error		Adult emergence ± Standard error	
	Bitter leaf	**Neem**	**Bitter leaf**	**Neem**
0.0	9.0±0.0*	9.0±0.0*	8.7±0.1*	8.7±0.1*
1.0	6.8 ± 0.02^{a}	11.5 ± 0.06^{ba}	2.75 ± 0.01^{a}	1.8 ± 0.01^{a}
1.5	3.3 ± 0.07^{b}	10.3 ± 0.41^{cb}	1.8 ± 0.02^{b}	2.5 ± 0.04^{c}
2.0	4.8 ± 0.02^{bc}	12.3 ± 0.07^{dc}	0.0±0.00	5.5 ± 0.12^{d}

Means with different subscripts are statistically significant at $p<0.05$

Table 3: Mean mortality, oviposition and adult emergence of *Callosobruchus maculatus* exposed to mixed ratio of Neem and Bitter leaf powder

Ratio (%)	Mortality	Oviposition	Adult emergence
B0:N0	**1.0±0***	**8.7±0.1***	**6.0±0***
1g			
B100	5.5 ± 0.04^{a}	7.3 ± 0.37^{a}	1.6 ± 0.02^{a}
N100	8.33 ± 0.07^{b}	6.4 ± 0.18^{b}	1.6 ± 0.02^{a}
B75:N25	4.75 ± 0.23^{a}	6.8 ± 0.02^{a}	1.0 ± 0.00^{b}
B25:N75	4.5 ± 0.40^{a}	6.0 ± 0.0^{b}	1.0 ± 0.00^{b}
B50:N50	2.5 ± 0.32^{c}	5.5 ± 0.05^{c}	0.0±0.00
1.5g			
B100	9.33 ± 0.07^{a}	6.2 ± 0.80^{a}	1.0 ± 0.00^{a}
N100	9.00 ± 0.00^{a}	5.8 ± 0.02^{a}	0.0±0.00
B75:N25	3.00 ± 0.00^{b}	5.5 ± 0.05^{a}	0.8±0.00
B25.N75	5.5 ± 0.04^{c}	5.0 ± 0.00^{b}	1.0 ± 0.00^{a}
B50.N50	4.8 ± 0.02^{c}	5.8 ± 0.20^{a}	1.6 ± 0.00^{b}
2.0g			
B100	9.0 ± 0.00^{a}	4.2 ± 0.04^{a}	0 .0±0.00
N100	9.0 ± 0.00^{a}	5.0 ± 0.00^{b}	0.0±0.00
B75.N25	4.5 ± 0.03^{b}	4.8 ± 0.28^{b}	1.0 ± 0.00^{a}
B25:N75	5.0 ± 0.00^{b}	3.6 ± 0.04^{c}	1.0 ± 0.00^{a}
B50.N50	5.5 ± 0.05^{b}	4.8 ± 0.31^{b}	1.8 ± 0.21^{b}

Means with different subscripts are statistically significant at $p<0.05$

Table 4: Mean mortality of *Callosobruchus maculatus* exposed to organic solvent extracts of Neem and Bitter

Concentration (%)	Extracts					
	Methanol		Ethyl acetate		n-hexane	
	Bitter leaf	Neem	Bitter leaf	Neem	Bitter leaf	Neem
0.25	2.66 ± 0.2^{a}	2.0 ± 0.0^{ba}	3.0 ± 0.0^{a}	2.6 ± 0.0^{b}	2.0 ± 0.0^{a}	3.0 ± 0.0^{ba}
0.5	2.00 ± 0.0^{b}	3.0 ± 0.0^{ab}	3.6 ± 0.0^{a}	3.3 ± 0.3^{a}	4.00 ± 0.0^{b}	3.0 ± 0.0^{ba}
0.75	2.66 ± 0.2^{a}	2.3 ± 0.0^{bc}	3.3 ± 0.0^{a}	1.3 ± 0.7^{c}	3.33 ± 0.7^{c}	3.3 ± 0.4^{ca}
1.0	3.00 ± 0.0^{a}	1.60 ± 0.0^{ba}	3.3 ± 0.0^{a}	1.60 ± 0.2^{c}	1.30 ± 0.3^{a}	3.3 ± 0.7^{cb}

Means with different subscripts are statistically significant at $p<0.05$

Table 5: Mean weight loss of infested cowpea seeds treated with neem and bitter leaf Extract

Treatment	Mean weight loss± Standard error
Control	10 ± 1.11^{a}
Bitter leaf	6.4 ± 0.92^{b}
Neem	$7.2 \pm 1.00^{a,b}$
Mixed ratio (1:1)	6.2 ± 0.92^{b}

* Means ± SE within the column with different subscripts are significantly different ($p < 0.05$).

DISCUSSION

The results of this experiment showed that the mortality of the adult bruchids, oviposition, emergence and weight loss of cowpea seeds treated with the different plant materials differed significantly with treatment application. Irrespective of the concentration of the powders used, there was noticeably high mortality of the bruchids observed from both plant powders. Both powders are toxic to the bruchid and other insects of stored products (Akunne *et al*., 2014: Nta *et al.,* 2017).

The findings revealed that 1g of bitter leaf powder had a significant effect on the mortality of the adult *C. maculatus* than the other concentrations used. This contrast with Kabeh and Jalingo (2007) who reported that the mortality of bruchids in seeds treated with bitter leaf was dose- and time-dependent due to the presence of hydrocyanic acid and oxalic acid. Abdulrasak and Samuel (2015), had reported that bitter leaf powder has been known to have insecticidal potential and feeding deterrent effects on *C. maculatus* in stored cowpea. This result however does not conform to the findings of Nyamandi and Maphosa (2013), who reported neem plants as biopesticides for stored food protection. Though high mortality of bruchids was recorded using bitter leaf powder, the mortality was not as in Ileke (2015) who recorded 100% adult mortality of the bruchids in seeds treated with bitter leaf powder. Dougoud *et al.* (2019) opined that the efficacy of botanicals varied among trials due to variation in active ingredient, content or concentration in plant materials, variation in preparation method or conditions and the way they were tested.

Oviposition was observed to be greatly reduced when the seeds were treated with bitter leaf powder compared to neem powder. The reduction in the oviposition of *C. maculatus* observed in this study might be due to deterrence effect of the plant materials to the bruchids with the level of mortality, oviposition's expected to be low. Rajapakse (2006), had reported that the mechanical effects of large quantities of powders themselves could have an effect on oviposition. It could also be that the effect of the plant powder on oviposition was due to respiratory impairment on the process of metabolism of the bruchid (Adedire *et al*., 2011; Ileke *et al*., 2012; Nta *et al*., 2017).

The complete deterrence of emergence could be attributed to a reduction in oviposition, resulting in fewer eggs been laid. It could also mean that the plant material has an effect on the bruchid ova thereby reducing its viability. This will result in reduction in the damage caused by the bruchids hence offering a better storage treatment than when left untreated. Abdulrasak and Samuel (2015) reported that reduction rates in fecundity either reduced or inhibited progeny emergence of *C. maculatus.* In a report by Abdulrasak and Samuel (2015) and Ileke (2015) in an earlier work, reported zero adult emergence in seeds treated with bitter leaf powder. The deterrence effect on adult emergence observed may also be due to early mortality and partial or complete inhibition of embryonic development (Dike and Mbah, 1992).

Mixed application of bitter leaf and neem powders had no significant effect on mortality of adult *C. maculatus* but deterred adult emergence when compared with sole application.

Akunne *et al*. (2013) and Mundi *et al*. (2012) also reported that mixed application of both plant materials were not as effective as sole application.

The extracts of neem and bitter leaf showed a very high repellant activity in this study as most of the bruchids were seen trying to escape from the container as soon as the extracts were introduced. This implies that the neem and bitter leaf extracts could be used as insect repellents. Mortality rate was higher amongst the cowpea bruchids exposed to bitter leaf extracted with n–hexane than those of other solvents while the ethyl acetate extracts of neem gave the highest mortality as compared to the methanol and n–hexane extracts of neem. Nnamonu and Anyam (2014) reported 100% mortality of *Callosobruchus maculatus* using n-hexane as well as ethyl acetate extracts of *A. lepidophyllus*. Adedire and Lajide (2008) also reported 100% mortality of maize weevil, *Sitophilus zeamais* treated with *V. amygladina*. However, Moses and Dorathy (2011) reported that *V. amygladina* leaf gave the best protection against cowpea weevil when compared with other plants (garlic and ginger). The effect of the plant (*V. amygladina*) extract on the bruchid may be linked to the presence of compounds like sesquiterpene lactones containing vernodalin, vernodalol and 11, 13-dihydrovernodalin which has been shown to act as an insect feeding deterrent (Pascual *et al*., 2001).

The weight loss recorded from the cowpea seeds treated with different concentration of the plant materials revealed that cowpea seeds treated with mixture of the plant materials recorded the least mean weight loss. This contrast with Ileke (2015), who reported that *Vernonia amygdalina* stem bark powder completely prevented infestation and damage of the treated cowpea seeds thereby resulting in neither seed damage nor weight loss recorded in the treated cowpea seeds. Similarly, Bamphitlhi *et al*. (2015) and Musa and Adeyeye (2015), in separate works reported that bitter leaf and aqueous extracts of *Nicotiana tabacun* L. respectively reduce weight loss in cowpea seeds infested with bruchids.

Worthy of note in this study is the fact that even though neem and bitter leaf powders showed a strong effect on cowpea bruchids when applied to the seeds, it did not eliminate the bruchids completely but drastically reduced them to significantly low levels as compared to unprotected seeds. This implies that both neem and bitter leaf powders do not offer total exclusion of cowpea bruchids rather; they lower the bruchids numbers and effects on the cowpea seeds.

CONCLUSION

Based on the findings of this study, it can be concluded that though bitter leaf powder were effective in controlling the bruchids, mixed powders of neem and bitter leaf and their extracts, might also serve as alternatives to synthetic insecticides for rural farmers, hence reducing the cost of production and storage of cowpea as the synthetic insecticides are relatively costly and not sustainable in the long run due to environmental contamination. The use of these plant products would be cost effective and sustainable, mainly due to the fact that neem and bitter leaf are easily grown. And have little or no harmful effects on non-target organisms.

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