



Bio-insecticidal and Grain Protectant Activities of Mixed Powder of *Ocimum gratissimum* and *Vernonia amygdalina* against *Callosobruchus maculatus* (Coleoptera: Bruchidae) in Calabar, Nigeria

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Authors' contributions

The work was carried out in collaboration between all authors. Author AIN designed the study, performed the statistical analysis, wrote the protocol and wrote the first draft of the manuscript. Authors BEA and NSU managed the analyses of the study. Author BEA managed the literature searches. All authors read and approved the final manuscript.

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ABSTRACT

The bio-insecticidal and grain protectant activities of mixed powder of *Ocimum gratissimum* and *Vernonia amygdalina* upon *Callosobruchus maculatus* was carried out in Calabar, Nigeria during the dry season (August to November) of 2015. The mortality rate of cowpea weevil treated *V. amygdalina*: *O. gratissimum* at 100: 0 concentrations were 17.0%, 27.0%, and 47.0% for a period of 24hrs, 48hrs and 72hrs, respectively. Concentrations 0:100 gave a percentage mortality of 47.0%, 67.0% and 73.0% at 24hrs, 48hrs and 72hrs, respectively. 80: 20 and 20: 80 concentrations gave 33.3% and 47.0% at 24hrs and 48hrs, but 80:20 gave 47.0% while 20:80 gave 57.0% mortality rate at 72hrs. 50: 50 concentrations gave 73.0%, 77.0%, and 80.0% mortality rate at 24hrs, 48hrs and

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72hrs, respectively. The 0: 0 concentrations gave 0.0%, 3.3%, and 7.0% mortality rate at 24hrs, 48hrs and 72hrs, respectively. The effect on the oviposition of cowpea weevil revealed that the mean value of eggs laid on seeds were 5.0, 2.3, 3.3, 2.8, 1.0 and 7.0 for 100:0; 0:100; 80:20; 20:80; 50:50 and 0:0 concentrations, respectively. The effects of mixture of *V. amygdalina*: *O. gratissimum* powder on the emergence of cowpea weevil gave a mean value of adult weevil emergence as 5.3, 3.0, 5.0, 3.3, 1.3 and 8.0 for 100:0, 0:100, 80:20, 20:80, 50:50 and 0:0 concentrations respectively. Mixed powder of *V. amygdalina*: *O. gratissimum* with 50:50% concentrations conferred the highest bio-insecticidal potency against *C. maculatus* during the experimental investigation. This was evident from the fact that treatment with *V. amygdalina*: *O. gratissimum* (50:50%) concentration attained 80.0% mortality at 72hrs of exposure, which was followed by *V. amygdalina*: *O. gratissimum* (0: 100%) concentration with 73.0% mortality.

Keywords: Bio-insecticidal; oviposition; *Vernonia amygdalina*; *Ocimum gratissimum*; ovicidal; *Callosobruchus maculatus*.

1. INTRODUCTION

Cowpea is a legume crop that can be grown prosperously in extreme environment like high temperature, low rain and impoverished soil with little input [1,2]. Cowpea (plant) can be utilized at all stage of growth as vegetable crops, the delicate green leaves are useful food source in Africa and are prepared as a pot herb-like spinach [1,3]. The dry and mature seed are good for boiling and canning and food for livestock [4,5]. The nutrient content of mature cowpea include: carbohydrate 63.6%, Protein 24.8%, fibre 6.3%, fat 1.9%, thiamine 0.00074%, Riboflavin 0.00042% and Niacin 0.00281% they are rich in lysine, tryptophan and amino acid [6]. Cowpea (*V. unguiculata*) is an important diet protein in Africa where *Callosobruchus maculatus* has become a major storage pest of the seed. Cowpea is prone to pest infestation, during pod, dry seed stage in the field and in storage [7]. Infestation was often less serious problem because farmers' cultivated traditional varieties which though low yield were generally more resistant to attack by pest [1,5]. However, the introduction of high yielding pulse varieties has results in high storage losses since they are usually more susceptible to pest damage, the pesticide properties of many plants have been known for a long time and natural pesticide based on plant extracts such as Rotenone, Nicotine and Pyrethrum have been routinely used in pest control during the earlier half of the century [1,4,7]. After the World War II, plant extract based pesticide loss their importance with the production of synthetic organic chemicals, which were concentrated product with a high knock down effect on pest organism [1,7]. The use of these organo-chlorine and organo-phosphorus compound however has led to the unhealthy effect on environment and human beings [1,5]. Bio-pesticides of plant origin have

been found to have wide spectrum insecticidal properties with reduced persistence and toxicity in relation to organo-chlorine and organo-phosphorus compound [1,7,8]. They are easily available, can be produced within farmer's vicinity providing a more sustainable approach to pest [1,8,9].

Pesticide plants are utilized in two main ways: for the active compound is isolated, identified and chemically synthesized. If feasible, these compounds are synthesized and marketed by the industries producing them. The second method which is suitable for farmers in developing countries involves the use of plant substances or products which are obtained and used directly. These practices are labour intensive, but are often economically sound and do not require sophisticated technology [5,10,11].

According to Nisbet et al. [12], ground plant parts and their extracts suppress fecundity and fertility of many insects. Researchers have reported that plant parts or extracts mixed with grains reduces insect oviposition, egg hatchability, post embryonic development and progeny production. According to Boeke et al. [13], plant derivatives also reduce the survival rates of larvae, pupal and adult emergence. Plant extracts, powders and essential oil from different bioactive plants were reported as repellent against different economically important stored products insects [14].

Some plant substances employed as protectant on stored cowpea grain attacked by *Callosobruchus maculatus* include *Vernonia Amygdalina* (Bitter leaf) and *Ocimum gratissimum* (scent leaf). Botanically, they can be used as plant substance (either powder or extract) to control pest in stored products and in the field. The leaves composite include lactone, oxalic

acid, hydrocyanic acid (HCN) is reported to be active principal extractable non-quantitatively by different solvent, while the hydrocyanic acid is very volatile [14,15].

Ocimum gratissimum (scent leaf) have a wide use throughout West Africa as febrifuge, anti-malaria and anti-convulsant. The crushed leaf juice is of great help in the treatment of convulsion; stomach pain and catarrh, oil from the leaves has been found to possess anti-septic, anti-bacterial and anti-fungal activities [16]. It is used as spices in terms of the flavour, it is a source of income, it may also be used as plant substance (either powder or extracts) to control pest of stored product [17].

2. MATERIALS AND METHODS

2.1 Collection of Plant Materials

Fresh mature leaves of *Vernonia amygdalina* and *Ocimum gratissimum* were collected from the botanical garden in University of Calabar in Calabar Municipality, Nigeria (5°45'N and 8°30'E). The collected plants were taken in sterile bags to the herbarium unit in Botany department where their identities were confirmed.

2.2 Preparation of Plants Powders

The collected test plants leaves were separately air-dried on a well-ventilated laboratory bench in Zoology and Environmental Biology department at temperature of 30±2°C and relative humidity of 76±5% for 14 days. The crisp dried plant materials were separately milled in an electric miller (super-master model SMB 2977, Japan), and the obtained powders were separately sieved through a 0.5 mm mesh net to have fine homogenous powder. The leaf powders of the test plants (*V. amygdalina* and *O. gratissimum*) were compounded in the ratios of 100:0, 0:100, 80:20, 20:80 and 50:50 respectively. Each compounded powder which was stored in labelled air-tight container and kept at ambient laboratory condition ns was used for assessment within 48 hours.

2.3 Collection and Preparation of Cowpea Seeds

The black eyed variety of cowpea seeds were purchased from a local market (Ika Ika Oqua market) in Calabar, Cross River state, Nigeria. The seeds were taken to the laboratory where they were carefully inspected with hand lens and

infested seeds (seeds having one or more feeding holes) were removed. The un-infested seeds were sterilized by storing in deep freezer (Thermocool model 750) at temperature of minus 15°C for 48 hours. Twelve (12) hours before application of treatment, the sterilized seeds were removed and air-dried on laboratory bench for acclimatization.

2.4 Experimental Procedure

Fifty (50) grams of sterile cowpea seeds were weighed separately into eighteen (18) plastic transparent containers (15 cm in diameter). The set-up was treated in a completely randomized designed with 5% (w/w) of the different combinations of *V. amygdalina* and *O. gratissimum*. An untreated 50 grams of cowpea seeds was used as control.

Four pairs of freshly emerged (age = 0-24hr) adult *C. maculatu* (4 males and 4 females) were taken from a cultured stock and introduced into each container, covered with 0.53 mesh net to enhance aeration and exclude escape of insects. The *Vernonia amygdalina* and *Ocimum gratissimum* powder of varying proportion were then introduced. Each treatment was replicated thrice.

2.5 Data Collection

Adult mortality count was carried out at 24, 48, 72 and 96 hours after treatment. At each count, the number of dead weevils was noted, the dead weevils were carefully removed and discarded while the live ones were allowed to continue the experiment. Insects were confirmed dead if it did not move when torch with entomological pin. At 60 days post treatment, all insects (dead and alive) were removed from the experimental containers using a pair of forceps.

From the data collected, percentage of mortality, were calculated using the formulae

$$\% \text{ mortality} = \frac{\text{number of dead weevils} \times 100}{\text{Total number of weevils}} \quad 1$$

2.6 Anti-oviposition Test

At seven (7) days post treatments, all weevils (dead and alive) were removed from each experimental set-up/ container. Twenty seeds were randomly selected and taken from each experimental unit and examined using hand lens, number of eggs laid as they were counted. This count data gave the anti-oviposition index of the respective treatment.

Table 1. Treatment of *Vernonia amygdalina* and *Ocimum gratissimum*

Plant leaf	Treatments
Va ₁₀₀ : Og ₀	<i>Vernonia amygdalina</i> 100g + <i>Ocimum gratissimum</i> 0g
Va ₀ : Og ₁₀₀	<i>V. amygdalina</i> 0g + <i>O. gratissimum</i> 100g
Va ₈₀ : Og ₂₀	<i>V. amygdalina</i> 80g + <i>O. gratissimum</i> 20g
Va ₂₀ : Og ₈₀	<i>V. amygdalina</i> 20g + <i>O. gratissimum</i> 80g
Va ₅₀ : Og ₅₀	<i>V. amygdalina</i> 50g + <i>O. gratissimum</i> 50g
Va ₀ : Og ₀	Control

2.7 Adult Emergence Test

Thirty one (31) days after completing the mortality test the number of adult *C. maculatus* emerging from each treatment group were counted. This count data which was taken for seven days gave the reproductive potential inhibition/ adult cumulative emergence index.

2.8 Data Analysis

All data collected from respective study parameter were subjected to one way analysis of variance, means were separated using LSD (least significant difference) and conclusion drawn at 95% confidence limit.

3. RESULTS

At 96 hours post-treatment, the highest cumulative percentage of mortality (85%) of *C. maculatus* was obtained in compounded *V. amygdalina* and *O. gratissimum* in the ratio 50:50. This was closely followed 76% obtained from *V. amygdalina*: *O. gratissimum* in the ratio 0:100. These two treatments had similar effect and significantly different ($P < 0.5$) mortality than all other treatment groups. Va20g + Og 80g

treatment gave 62% cumulative percentage mortality while the control had the least (7.00%). The above information is presented in Table 2.

3.1 Anti Oviposition

At seven days after treatment the highest number of eggs laid were recorded in the untreated control (8.3) and this was significantly different ($P < 0.05$) from the number laid in all other treatment groups. The least number of eggs (1.20) were observed in mixed Va 50: Og 50 treatment group. *V. amygdalina* and *O. gratissimum* treatments in the combination ratios of 20:80 and 0:100 had similar effects of (2.8 and 2.3, respectively on oviposition). This was followed by comparable mean egg oviposition of 4.0 and 3.6 in *V. amygdalina* and *O. gratissimum* Va80:Og20 and Va100: Og 0 combination, respectively. The above information is as presented in Table 3.

3.2 Adult Emergence / Reproduction Inhibition

The different combinations of test plant leaf powders (50:50, 80:20, 20:80, 100:0 and 0:100), showed significant effect on reproduction and

Table 2. The effect of *V. amygdalina* and/or *O. gratissimum* powder(s) on mortality of *Callosobruchus maculatus* adult

Treatment Leaf powders	Combination (g)	% Mortality			
		24 hrs	48 hrs	72 hrs	96 hrs
<i>V. amygdalina</i> : <i>O. gratissimum</i>	Vam 100g ±Ogr 0g	17.0±2.0	27.0±3.6	47.0±4.4	51.0±1.7
<i>V. amygdalina</i> : <i>O. gratissimum</i>	Vam 0g ±Ogr 100g	42.0±3.2	64.0±1.0	73.0±2.0	76.0±2.0
<i>V. amygdalina</i> : <i>O. gratissimum</i>	Vam 80g ±Ogr 20g	31.3±0.6	47.0±4.4	48.0±1.0	50.0±1.0
<i>V. amygdalina</i> : <i>O. gratissimum</i>	Vam 20g ±Ogr 80g	34.0±1.0	47.0±4.4	57.0±2.0	62.0±1.0
<i>V. amygdalina</i> : <i>O. gratissimum</i>	Vam 50g ±Ogr 50g	75.0±2.6	77.0±1.0	80.0±1.2	86.0±4.0
Control (<i>V. amygdalina</i> : <i>O. gratissimum</i>)	Vam 0g ± Ogr 0g	0.0±0.0	3.3±0.6	7.0±0.6	8.0±2.0

Note: Vam = *Vernonia amygdalina*; Ogr = *Ocimum gratissimum*; g = gram; % = Percentage; hrs = Hours

adult emergence when compared with the control. The highest percentage inhibition of reproduction and the lowest number percentage of emerged adults were recorded in Va50:Og50 treatment i.e. the treatment with equal proportion of test plant powders while the control showed the least reproductive inhibition capacity but highest percentage of emerged adult (78.50). Va80:Og20 and Va20:Og80 respectively gave 45.75 and 40.25% of emerged adults. The percentage of adult emerging from Va100:Og0 and Va0 and Og100 treatment group was 39.00% and 54.25% respectively (Table 4).

Table 3. Effect of *V. amygdalina* and *O. gratissimum* combination on oviposition rate of adult *C. maculatus*

Treatment	Number of seeds	Mean number of eggs laid
Va100:Og0	20	4.00±1.00
Va0:Og100	20	2.30±0.57
Vago:Og20	20	3.60±0.57
Va20:Og80	20	2.80±0.57
Va50:Og50	20	1.20±0.57
Va0:Og0	20	8.30±0.57

Table 4. Effect of mixed leaf powder of *V. amygdalina* and *O. gratissimum* treatment on adult *C. maculatus* emergence

Treatment	Adult emergence %
Vag100:Og0	54.25±0.57
Va0:Og100	39.00±2.65
Va80:Og20	45.75±0.57
Va20:Og80	40.25±0.57
Va50:Og50	35.50±0.57
Va0:Og0	78.50±0.57

4. DISCUSSION

Table 2 presents the result of *V. amygdalina* and/or *O. gratissimum* powders on mortality rate of *Callosobruchus maculatus* adult at different duration. Va50:Og50 gave 86% - the highest percentage mortality at 96 hours duration followed by Va0:Og100 which gave 76% mortality at 96 hours duration. This means that the longer the duration of powder application, the greater the effect on the insect. The findings from this research is in agreement with an earlier research carried out by Musa et al. [5]. The significantly higher mortality obtained in 50:50 and 0:100 combinations of *V. amygdalina* and *O.*

gratissimum was probably due to the pungent odour of *O. gratissimum*. This confirms reports by Kouninki et al. [18] that leaves of aromatic plants are toxic to beetles and weevils attacking, storage production. Secondly the combinations of two aromatic plant in equal proportion may have increased the insecticidal potency thus resulting in higher mortality [19] reduced oviposition and inhibition of development and subsequent adult emergence Emeazor et al. [20].

Table 3 presents the result of effects of *V. amygdalina* and *O. gratissimum* combination on oviposition rate of adult *C. maculatus*. Va50:Og50 combination gave the best result with an average number of eggs laid to be 1.20. The said treatment (Va50:Og50) reduced the average number of laid eggs from 8.30 to 1.20, which is a nice result. Significantly reduction in oviposition, observed in the various combination of test plant when compared with control also indicate that the pungent odour may have affected the behavior of *C. maculatus* through the smell sensilla present in their antennal. This odour probably created a vapour barrier preventing the weevils from contacting the cowpea and ovipositing on them [1,5,7,21].

From the Table 4, it can be seen that Va50:Og50 gave the least percentage adult emergence. This concentration gave an excellent result as compare to the control treatment which gave 78.50% of adult emergence. Results obtained from the biological assays indicated that leaf powders of *V. amygdalina*, *O. gratissimum* and the different combinations of the leaf powders of plants had varying insecticidal potency against *C. maculatus*. When compared with the control, all plant material were effective in increasing insect mortality and reducing oviposition, development and adult emergence thus reducing the overall population of *C. maculatus* at 31 days after treatment.

The combinations of the plant powders may have also increased toxicity to the immatured stages and adult of *C. maculatus* [19,22]. The reduced oviposition, suppression of development and subsequent reduction in adult emergence confirms the reports of [9,23]. Lale [24] said that powders of medicinal plants when applied alone or in combination are effective in controlling insect pest of stored agricultural produces. The powders may have blocked the spiracles and other respiratory structures of the insects thereby suffocating and death.

5. CONCLUSION

The results showed that 50:50 and 0:100 ratio compounded *V. amygdalina* and *O. gratissimum* had 85% and 76% effect on the insects. The two treatments had similar effect with a significant difference at $P < 0.5$ mortality. The highest percentage inhibition of reproduction/adult emergence was recorded at Va50:Og50 treatment. When compared with the control, all plant material were effective in increasing insect mortality and reducing oviposition, development and adult emergence thus reducing the overall population of *C. maculatus* at 31 days after treatment. The potency of the combined concentrations of *V. amygdalina*: *O. gratissimum* (50:50%) of these plant substances was due to the odour they produce, which may have exerted a toxic effect by disrupting normal respiratory activities of the cowpea weevil, thereby resulting in asphyxiation and subsequent dead; it may also have acted as contact poison on the weevil. It equally may have ovicidal and larvicidal properties. It is recommended that similar studies be conducted using separately other parts of the test plant like the stem and bark of *V. amygdalina* and *O. gratissimum* to further evaluate their bio-insecticidal potency against stored grain pests.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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