

Phytochemical analysis and pesticidal effects of *Ocimum gratissimum* leaf oil extract in the management of *Callosobruchus maculatus* infesting *Vigna unguiculata*.

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Abstract

The study aimed at evaluating the phytochemicals present in the leaves of *Ocimum gratissimum*, *V. amygdalina*, *P. guineense*, *G. latifolium* and *A. indica* harvested from farms at Anaku in Ayamelum LGA of Anambra State and subsequently test their potency in causing mortality and reducing loss and damage due to *C. maculatus* infestation. Phytochemical analysis of the plant materials, leaf oil extraction and pesticidal studies were carried out in the Laboratory. Five serially diluted concentrations (1.25 % v/v, 2.5 % v/v, 5.0 % v/v, 10 % v/v and 20 % v/v) of the leaves oils extracted with N-Hexane were used. The experiment also has two control treatments (C1 – acetone only and C2 – no treatment). The data collected on insect mortality, seed damage and weight loss were analyzed using Analysis of Variance (ANOVA) separated using the Student–Newman-Keuls (SNK) test. The highest corrected mortality, 100.0% was recorded at concentrations ≥ 5.0 % v/v. The least corrected mortality, 66.7% was recorded at 1.25% v/v concentration. Bean seeds treated with a 20.0% concentration of the oil extract recorded the least percentage of damaged seed and weight loss with a Mean \pm SE value of 8.3 \pm 0.8 while Bean seeds treated with a 1.25% concentration of the oil extract recorded the highest percentage of damaged seeds and weight loss with Mean \pm SE value of 22.2 \pm 1.9. The five plants showed a similar level of performance in the management of *C. maculatus* with *O. gratissimum* having the best overall performance. Their pesticidal activity is related to their phytochemical constituents and their bitterness.

Keywords: Phytochemical; Pesticidal; *Callosobruchus maculatus*; Student–Newman-Keuls test

1. Introduction

Callosobruchus maculatus (F), Cowpea Bruchid (Coleoptera: Bruchidae), is a principal pest of grain in temperate and tropical regions of the world [1]. Its attack starts in the field if *Vigna unguiculata* is left unharvested and soon spread to the store after harvesting and storage. *Vigna unguiculata* var. white (iron beans) is one of the most severely damaged legume seeds [2]. Since the adults do not feed, damage done to *V. unguiculata* has been attributed to the larvae of *C. maculatus*. The larvae bore and penetrate undamaged grains and reduce into powdery form. This results in an estimated 90% loss both in quality and quantity of the seeds in storage [3]. There is equally a reduction in the degree of usefulness and fitness of the seeds either for planting or human consumption [4]. Uncontrolled attacks of the weevils to stored bean products and subsequent weight loss will invariably lead to hunger in society as the original weight and quality of the legumes before infestation that gives maximum satisfaction when consumed has been drastically reduced by the Bruchids [2].

Attempts to control *C. maculatus* in the recent past have relied heavily on the use of synthetic pesticides and have brought a lot of problems such as the development of resistance, unacceptable levels of pesticide residues, hiking cost

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of production, environmental toxicity, poisoning of farmers and consumers of produce infested by the pests [1]. Repeated application of pesticides leaves residues that may rise beyond the tolerance limits, thereby rendering the grains unsuitable for human and animal consumption.

However, current research efforts on chemical product development are being focused more on ecologically tolerable control measures including the use of inert materials, plant powder, oils and extracts [5]. There is also increased awareness that plants possess chemicals which naturally protect them from pests and pathogens. The tropical region is well endowed with a wide variety of plants with defensive chemicals and quite a number of them have been used traditionally in protecting *V. unguiculata* against Bruchid attack. Interestingly, botanicals have been used for centuries and were widely used in the United States until the 1940s and 50s when synthetic pesticides were introduced. The synthetics quickly became popular because they did not break down as quickly as possible but insecticides that last longer can potentially leave residues in the soil, water supply and on food [6].

On the other hand, botanicals degrade rapidly and therefore are considered safer for the environment than common synthetic chemicals. They may have to be applied more frequently than synthetic products because of their low durability. By weighing merits and demerits, science has recognized the need to reduce pesticide usage to achieve a cleaner environment, but this would certainly reduce agricultural production [7]. Thus, the conflict between the goals of reduced pesticide usage and the production of sufficient food and legumes for the ever-increasing human population provides a strong impetus for the development of cost-effective and ecologically friendly alternatives.

Traditionally, plant materials are sometimes mixed with legumes or seeds in storage for reducing Bruchid attacks [7]. Several findings have shown that coating legume seeds with oils extracted from plants are effective in Bruchid damage control [8]. Plant oils are toxic to young larvae, adults and eggs of *C. maculatus* [9]. The mode of action is partially attributed to interference with normal respiration resulting in suffocation. Plant oils may also contain insecticidal or repellent compounds, including fatty acids and other compounds after application [10]. Most of the plants used have been subjected to empirical verification for effectiveness against *C. maculatus*. Neem kernel powder and oily extracts protect cowpea seeds from *C. maculatus* damage [11]. Leaves powders from *Ocimum gratissimum* also exhibit insecticidal action on the adults and eggs of *C. maculatus*. The use of *Ocimum* plant in form of extractor oils is more beneficial for the control of stored pests which are known to have developed resistance to most forms of synthetic pesticide. Plant extracts from *Annona senegalensis* root bark have been proven to be more effective than the powder in the control of *C. maculatus* [12].

Plants parts have considerable potential as biopesticides in controlling storage insect pests. The pest control potentials remained largely untapped due to continued dependence on broad-spectrum synthetic chemical insecticides by farmers. Against this background, a laboratory investigation was conducted to evaluate the phytochemicals present in *Ocimum gratissimum* and subsequently test its potency in causing mortality and reducing loss and damage due to *C. maculatus* infestation.

2. Material and methods

2.1. Study design

The study was carried out with the plant leaves harvested from farms at Anaku in Ayamelum LGA of Anambra State. The Insects, *C. maculatus* studied were obtained from infested *V. unguiculata* (beans) purchased from Eke Awka market. Phytochemical analysis of the plant materials was done at Springboard Research Laboratories, Udoka Housing Estate, Awka Anambra State. Leaf oil extraction and pesticidal studies were carried out in the Laboratory unit of the Parasitology and Entomology Department, Nnamdi Azikiwe University Awka.

The study was an experimental work involving the treatment of *V. unguiculata* infested with *C. maculatus*, using five serially diluted concentrations of *Ocimum gratissimum* essential oils extracted with N-Hexane. The experiment on the pesticidal effect of the essential oil extracts was set up in a Completely Randomized Design (CRD). Five different concentrations of the essential oil extracts: 1.25 % v/v, 2.5 % v/v, 5.0 % v/v, 10 % v/v and 20 % v/v were prepared through serial dilutions of the raw extracts. Two replicates were used for each leaf oil concentration. The experiment also has two control treatments (C1 – acetone only and C2 – no treatment).

2.2. Culture of *C. maculatus*

The insect pest culture (*C. maculatus*) was acquired from infested beans from a warehouse in Eke-Awka market and reared in the laboratory in ex-potiskum variety (preferred substrate) of bean seeds from the same market. The

transparent plastic container used for the rearing was covered with fine mesh nets to prevent the escape or entrance of insects. The rearing containers were allowed to stand for two weeks at a temperature of 30°C and relative humidity of 80% r.h to yield enough adults of the insects before being used for the experiment.

2.3. Experimental Bean Seeds

Ife Bpc bean seeds from the Institute of Agricultural Research and Training, Moore Plantation, Ibadan were used for this study. Mature, wholesome seeds from the sample devoid of debris and emergence holes were heat sterilized at 40°C for 1 hour before use to clear any existing infestation. Subsequently, 20g of beans were weighed into labelled 60cm³ plastic containers, each container was covered with ventilated screw cap, and sealed with a net to prevent the entry of insects but allowed ventilation of the samples.

2.4. Collection, Identification and Preparation of Plant Materials (Leaves)

The plant materials (leaves) (*Ocimum grattissimum*, *V. amygdalina*, *P. guineense*, *G. latifolium* and *A. indica*) were plucked from standing plants in Coscharis farms in Anaku, Ayamelum LGA of Anambra State. The plant materials (leaves) were taken to a Botanist, Dr C.G Ukpaka of Chukwuemeka Odumegwu Ojukwu University for identification and authentication. Matured leaves *O. grattissimum* were plucked, and rinsed with water to remove dust. Subsequently, they were allowed to air-dry under shade for seven days to ensure that volatile active principles are retained in the dried sample. The dried leaves were pulverized into fine powders with an electric blender and then sieved through a 0.5mm mesh before the powders were collected into a clean container.

2.5. Phytochemical Analysis of the plants

Qualitative and Quantitative phytochemical analysis was carried out on the crude powders of *Ocimum grattissimum* at the Spring Board Laboratory situated at Road 3, House 1, Udoka Housing Estate, Awka, Anambra state. The screening was done to detect the presence or absence of some Phytochemicals (alkaloids, flavonoids, tannins, saponins, cardiac glycosides, and cyanogenic glycosides) in the plant leaf powders. The phytochemical screening was done using methods such as Wagner's reagent test, Ferric chloride test, Lead acetate test and Acid test as described by [13].

2.6. Extraction of essential oils from the plant leaves

The extractions of the pulverized samples (30g) for *Ocimum grattissimum* were done with a Soxhlet extractor for three hours using N-hexane as extracting solvent. The pulverized fine powder (30g) of each of the five leaves were wrapped in filter paper and placed inside the thimble of the Soxhlet apparatus (one after the other has been completely extracted). The bottom end of the soxhlet was greased with a jelly (Vaseline) to prevent friction and possible cracking before fixing it into the round bottom flask containing 250ml of N-hexane (extracting solvent). The apparatus was set up using a retort stand. Cold water was allowed to flow in and out through the inlet and outlet compartment of the Soxhlet condenser connected to the tap by a hose. After many refluxes, the set-up was allowed for 4 hours until the solvent in the mixture becomes cleaner. The leaf oil extracts of the five plants were removed and poured into a beaker. The procedure was repeated severally for each plant leave powder until a reasonable quantity of each extract was obtained.

2.7. Preparation of the treatment levels with the essential oils of the plants.

Serial dilutions of hexanoic extracts of the leaf essential oil were prepared in acetone. The extracts were considered as 100% essential oil concentration which was serially diluted in acetone in a 20ml syringe to give varying concentrations of 1.25%, 2.5%, 5%, 10% and 20% respectively, equivalent to 0.0125g/ml, 0.025g/ml, 0.05g/ml, 0.1g/ml and 0.2g/ml respectively, similar to Ali *et al.* (2004).

2.8. Evaluation of pesticidal effects of the leaf essential oil to determine the lethal dose

Twenty grams of disinfested bean seeds were weighed into 60cm³ plastic containers covered with a cap that had its centre carved open and sealed with a net to allow for ventilation and prevention of entrance of insects. The concentrations of the plant essential oil were: 1.25%, 2.5%, 5%, 10% and 20% diluted with Acetone using a 20ml syringe and control without treatment according to [14] while each treatment was replicated two times including the control. The dose of each concentration applied was 0.5ml. Thereafter, the acetone was allowed to evaporate completely. Subsequently, ten unsexed *C. maculatus* were introduced into each plastic container using a mouth-operated aspirator. Each container was covered with its lid to prevent the escape of the insects. These were allowed to stand on the laboratory bench in a Completely Randomized Design (CRD). Adult mortality count was carried out at twenty-four hours intervals for seven days after treatment (DAT) and insects were certified dead after probing with a pin at the abdomen and there was no response. Thereafter, all insects (dead or alive) after seven days of exposure to the treatments were

removed from the containers. Adult mortality was determined as the number of dead insects divided by a total number of insects multiplied by 100.

$$\text{Mortality} = \frac{\text{Number of dead insect}}{\text{Number of insect introduced}} \times \frac{100}{1}$$

2.9. Assessment of Damage and weight loss due to *Callosobruchus maculatus* infestation

Subsequently, after all the dead insects were removed from the containers, the samples were observed daily from the 25th day of infestation to the time of F₁ adult emergence. Thereafter, damaged and undamaged grains including exuviae and frass were sorted and separated by handpicking and sieving into separate containers. Weight loss was determined by sieving the contents of the plastic containers with a sieve of mesh size 0.25mm and reweighed. The fractions passing through the sieve are classified as insect frass (dust). Also, a damage assessment was carried out on the infested cowpea seeds by sorting the cereals damaged and undamaged categories.

The percentage grain damage was calculated using the formula:

$$\% \text{ damage} = \frac{A \times 100}{B}$$

Where:

A: number of damaged grains

B: total number of grains.

The percentage of grain weight loss was measured as the percentage of the difference between the initial weight of the grain and their final weight as described by [15].

$$\text{PGWL} = A - (B + C) \times 100$$

Where:

PGWL = percentage grain weight loss

A = the initial weight of the sample

B = weight of damaged grain

C = weight of undamaged grain

2.10. Data summary and Statistical Analysis

Correction of mortality data was done by using Abbot Formula (1925): $P_c = (P_o - P / 100 - P \times 100)$; where P_c is the corrected mortality, P_o is observed mortality and P is control mortality (if mortality in the control is up to 5%). Probit analysis to determine LC_{90} was carried out according to Finney, 1971. The data collected on insect number *C. maculatus* mortality, seed damage and weight loss were transformed into percentages and subsequently analyzed, analysis of Variance (ANOVA) at a 5% level of significance. Treatments with a significant difference were compared and separated at a 0.05% level of significance using the Student–Newman-Keuls (SNK) test at $P < 0.05$. The statistical package used was SPSS version 25.0.

3. Results

3.1. Phytochemical compositions of the plant materials used

A quantitative analysis of the phytochemicals present in the plant materials is shown in Table 1. The following phytochemicals: Sparteine, Sapogenin, Lectin, Kaempferol, Anthocyanin, Cardiac glycoside, Phytate, Flavonones, Catechin, Cyanogenic glycoside, Flavone, Proanthocyanidin, Resveratol, Oxalate, Naringenin, Steroid, Ephedrine and Ribalinidine present in the plant materials used for the study were detected and quantified. Proanthocyanin is absent in *Ocimum gratissimum*.

A qualitative analysis of the phytochemicals present in the plant materials is shown in Table 2. The following phytochemicals: Alkaloids, Flavonoids, saponins, Resins and Terpenoids were detected in all the plant materials used for the study. Cardiac glycoside is absent in *Ocimum gratissimum*.

Table 1 Quantitative phytochemical compositions of the *Ocimum gratissimum* plant used

Phytochemical Component	Quantity present ($\mu\text{g/ml}$)
Epihedrine	77.3574
Steroid	6.0626
Sparteine	0.1522
Sapogenin	0.0049
Lectin	15.5063
Kaempferol	60.4508
Tannin	19.0643
Anthocyanin	2.1752
Cardiac glycoside	19.6790
Phytate	2.7071
Flavonones	14.9159
Catechin	0.0301
Cyanogenic glycoside	8.9247
Flavone	9.7417
Proanthocyanidin	0.1897
Resveratrol	0.4703
Oxalate	2.6301
Ribalinidine	5.5794
Narigenin	4.8507
Proanthocyanin	-

Table 2 Qualitative phytochemical compositions of the plant materials used

Phytochemical Component	Presence
Alkaloids	+
Flavonoid	+
Tanin	-
Phlobo tanin	+
Saponin	+
Cardiac glycoside	-
Resins	+
Terpenoids	+

N/B: Plus sign (+) means present; Negative sign (-) means absent.

3.2. Mortality response of *C. maculatus*

Table 3 shows the mortality experience of *C. maculatus* exposed to leaf oil extract of *Ocimum gratissimum* after 7 days of exposure. All the experimental plates treated with various concentrations of *Ocimum gratissimum* leaf essential oil

recorded a mortality count greater than 40.0% as observed in untreated experimental plates (Control 2). The highest corrected mortality, 100.0% was recorded at concentrations ≥ 5.0 % v/v. The least corrected mortality, 66.7% was recorded at 1.25% v/v concentration. The difference in the mortality record observed among the different concentrations of the leaf essential oil used was statistically significant ($p = 0.000$).

Table 3 Mortality experience of *Callosobruchus maculatus* exposed to the essential oil of *Ocimum gratissimum* leaves after 7 days of exposure

Concentration (% v/v)	Mortality count				% Mortality	
	R1 (n =10)	R2 (n=10)	Total	Mean \pm SE	Observed	Corrected
1.25	7	8	15	7.5 \pm 0.5	75.0 %	66.7 %
2.5	9	7	16	8.0 \pm 1.0	80.0 %	73.3 %
5.0	10	10	20	10.0 \pm 0.0	100.0 %	100.0 %
10.0	10	10	20	10.0 \pm 0.0	100.0 %	100.0 %
20.0	10	10	20	10.0 \pm 0.0	100.0 %	100.0 %
Control 1 (Acetone)	3	2	5	2.5 \pm 0.5	25.0 %	-
Control 2 (No treatment)	4	4	8	4.0 \pm 1.0	40.0 %	-

Figure 1 shows the regression line of a probit of mortality plotted against the log of concentration for the calculation of LC_{90} (Concentration of the leaf essential oil that can kill 90.0 % of the pest population; where LC means lethal concentration). LC_{90} of 2.18 % v/v was calculated from the regression equation, $y = 3.213x + 5.1963$ from LC- P graph for *Ocimum gratissimum* leaf essential oil.

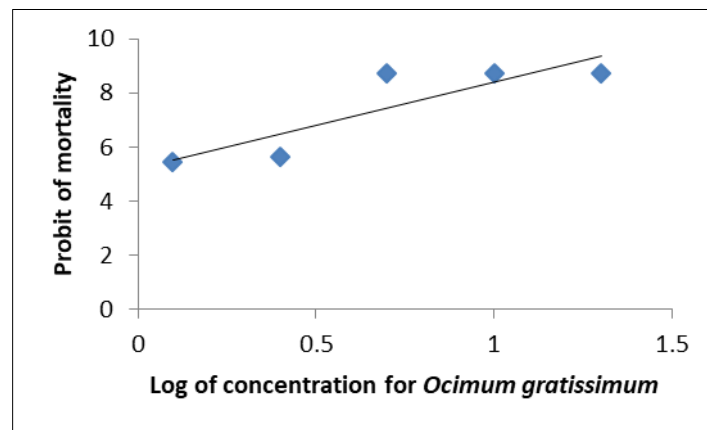


Figure 1 LC-P graph for *Ocimum gratissimum*

3.3. Seed damage and weight loss assessment

The percentage of seed damage of the experimental plates exposed to *Ocimum gratissimum* leaf oil extract is shown in Table 8. Bean seeds treated with a 20.0% concentration of the oil extract recorded the least percentage of damaged seeds, with a mean \pm se value of 8.3 \pm 0.8 while Bean seeds treated with a 1.25% concentration of the oil extract recorded the highest percentage of damaged seeds, with a mean \pm se value of 22.2 \pm 1.9. This difference in the mean percentage of the damaged bean seeds after exposure to different concentrations of *Ocimum gratissimum* leaf oil extract is statistically significant ($p = 0.007$).

The percentage weight loss of the experimental plates exposed to *Ocimum gratissimum* leaf oil extract is shown in Table 9. Bean seeds treated with a 20.0% concentration of the oil extract recorded the least percentage weight loss, with a mean \pm se value of 2.5 \pm 0.9 while Bean seeds treated with a 1.25% concentration of the oil extract recorded the highest

percentage weight loss, with a mean±se value of 11.4±0.5. This difference in the mean percentage weight loss after exposure to different concentrations of *Ocimum gratissimum* leaf oil extract is statistically significant ($p = 0.001$).

Table 4 Mean percentage seed damage after exposure to different concentrations of essential oil from the five plants

Concentration (%)	Seed damage	Weight loss
	mean ± se	mean ± se
0 (Control)	25.9±2.9 ^a	13.5±1.6 ^a
1.25	22.2±1.9 ^a	11.4±0.5 ^a
2.5	18.8±2.2 ^{a,b}	6.9±0.3 ^b
5.0	18.7±1.1 ^{a,b}	6.5±0.7 ^b
10.0	11.3±2.7 ^{b,c}	4.8±0.3 ^{b,c}
20.0	8.3±0.8 ^c	2.5±0.9 ^c

N/B: Percentage values of damaged seeds with different alphabets under the same column show significantly different effects among the different concentrations used.

4. Discussion

The study has shown that leaf oil extracts of the *O. gratissimum* screened have some pesticidal properties. This is evident in the fact that the plants extracts when used at different concentrations recorded mortality of $\geq 40.0\%$ for *C. maculatus* control. This baseline mortality value was obtained when no treatment was applied. These plants are biodegradable and edible. Thus their use in preserving bean seeds from damage due to *C. maculatus* may not lead to environmental pollution and pesticide poisoning. This is contrary to the problems associated with the use of synthetic pesticides such as unacceptable levels of pesticide residues, hiking cost of production, environmental toxicity, and poisoning of farmers and consumers of produce infested by the pests [1].

O. gratissimum leaf oil extract contain most phytochemicals that are listed: Sparteine, Sapogenin, Lectin, Kaempferol, Anthocyanin, Cardiac glycoside, Phytate, Flavonones, Catechin, Cyanogenic glycoside, Flavone, Proanthocyanidin, Resveratol, Oxalate, Naringenin, Ephedrine, Ribalinidine, Steroid, Tanin, Proanthocyanin, Alkaloids, Flavonoid, Saponin, Resins, Terpenoid, and Cardiac glycoside. Some of these phytochemicals have been reported to be used by plants as morphological and biochemical tools for defense against herbivory [16]. It then means that the leaf oil extracts of the plants may show contact and/or systemic mechanisms of action.

With *O. gratissimum* essential oil, different concentrations used (1.25%, 2.5%, 5.0%, 10.0% and 20.0%) performed significantly better than the experimental set-up without treatment. It shows that this plant has high potential for pesticidal activities which could be attributed to its phytochemical constituent. *O. gratissimum* contain all the phytochemicals listed above with the exception of Proanthocyanin, Tanin and Cardiac Glycoside. Nevertheless, concentrations $> 1.25\%$ recorded similar level of mortality in the control of *C. maculatus*. With *V. amygdalina*, *P. guineense*, and *A. indica* essential oil, different concentrations used (1.25%, 2.5%, 5.0%, 10.0% and 20.0%) performed significantly better than the experimental set-up without treatment. All the concentrations used recorded similar levels of mortality in the control of *C. maculatus*. The phytochemical components of these plants are equally similar but different in Epihedrine, Steroid, Ribalinidine and Proanthocyanin constituents. The performance of *G. latifolium* essential oil was not significantly better than that of the experimental set up without treatment. It may simply have occurred by chance since *G. latifolium* also contain similar phytochemical constituents like other plants.

The bitterness of the plant materials studied may also have contributed to the mortality records observed in this study. It may have led to reduced feeding and possible starvation of *C. maculatus*. In comparing the lethal concentrations of the leaf oil extracts for achieving 90% mortality of *C. maculatus*, LC₉₀ value of 2.18% for *O. gratissimum* essential oil was the least followed by 3.73% for *V. amygdalina*, 17.6% for *G. latifolium*, 19.05% for *P. guineense* and 192.6% for *A. indica*. This ranks the toxicity levels of the plants against *C. maculatus* as follows: *O. gratissimum* $>$ *V. amygdalina* $>$ *G. latifolium* $>$ *P. guineense* $>$ *A. indica*. In other words, the concentration of *O. gratissimum* required to kill 90% of *C. maculatus* is the smallest, and this makes it the most effective when compared to other plants studied.

After weeks of treatment, adult emergence was observed in all the plots including those used as control. It shows that these plant extracts have low residual effects and do not offer protection for a long time. This then resulted in seed

damage which reduces its attractiveness, usefulness and fitness for human consumption. This has earlier been reported by [4] and [17] who also included change in texture and unfitness for planting as part of the damages caused.

Assessment of the seed damages showed that when treatments were done using 20.0% v/v of the essential oils, the least level of seed damage was recorded. On the other hand, when treatments were done using 1.25 % v/v of the essential oils, the highest level of seed damage was recorded. At higher concentrations, the amount of bitter taste seemed high and will take a longer time to reduce than when the concentration is low. The damage in terms of seed holes observed ranged from 8.3% to 23.8% for less than two months of assessment done in this study. It, therefore, means that when left for a longer time, the percentage of seed damage may increase. This is evident in the report of [4] who estimated that in Nigeria alone, the damage in terms of holed seeds can increase to 99% after 6 months of storage. For long-term storage, the plant materials and/or their concentrations used in the present study may not offer significant protection against *C. maculatus*. It implies that the plant materials when used at concentrations as low as 20.0% and with that level of bitterness in the trader's store may cause more than 30 % damage as reported by [11].

Adult *C. maculatus* introduced in the experiment may have mated and laid eggs before they died. The eggs might have developed into larvae which then penetrated the seed thereby causing seed damage. The performances of the different leaf oil extracts in preventing seed damage were similar at the same level of concentration. For the groups treated with *O. gratissimum* leaf oil extract, the percentage number of damaged seeds was significantly reduced with 20.0% v/v concentration. Treatment with 10.0% v/v concentration however yielded a similar level of percentage seed damage though with some fluctuation that resulted in effects as low as treatment with 5.0% v/v and 2.5% v/v. The least effective treatment was with a 1.25% v/v concentration which performed at the same level as the untreated group. This is reflected in the level of mortality caused by *C. maculatus*; the higher the mortality record, the lower the chance of adult emergence and resultant reduced damage. For the groups treated with *V. amygdalina* leaf oil extract, all the concentrations used and even plots without treatment showed a similar level of effectiveness in reducing seed damage. Reproduction of an unequal number of surviving *C. maculatus* in the experimental plates treated with *V. amygdalina* may have occurred at different rates to have caused seed damage.

For the groups treated with *P. guineense* essential oil, all the concentrations used performed better in reducing seed damage than when no treatment is applied, even though treatments with concentrations $\leq 2.5\%$ v/v tend to fluctuate. The effectiveness of 5.0% v/v and 10.0% v/v concentrations in reducing seed damage were similar to the performance of 20.0% v/v concentration which has the highest performance, even though fluctuations also occurred. All the groups treated with different concentrations of *A. indica* leaf oil extract performed similarly but differently from the untreated group.

For the groups treated with *G. latifolium* essential oil, all the concentrations used and even plots without treatment showed a similar level of effectiveness in reducing seed damage.

The seed damage caused by *C. maculatus* resulted in weight loss in all the experimental units. Weight loss, just like seed damage was recorded in all the experimental plots. This resulted in a loss both in quality and quantity of seeds in storage [3]. Assessment of weight loss showed that when treatments were done using 20.0% v/v of the essential oil, the least level of weight loss was recorded. On the other hand, when treatments were done using 1.25% v/v of the leaf oil extracts, the highest levels of weight loss were recorded. The overall weight loss recorded in this study ranged from 2.2% to 11.4%. The observed difference here could be due to the difference in the study duration. While the present study was monitored for less than two months, the latter was done for eight months.

The performances of the different essential oils in preventing weight loss were similar at the same levels of concentration, except at 5.0% v/v and 10.0% v/v concentrations. These exceptions could be ignored since the pattern does not extend to treatment with a 20.0% v/v concentration. With *O. gratissimum* essential oil, different concentrations used except 1.25%, a 2.5% v/v performed significantly better in reducing weight loss of bean seeds than the experimental units without treatment. Performances of 2.5% v/v and 5.0% v/v in reducing weight loss were significantly better than that of 1.25% v/v, similar to 10.0% v/v concentration, but lower than that of 20.0% v/v concentration. With *V. amygdalina* leaf oil extracts, different concentrations used performed significantly better in reducing weight loss of bean seeds than the experimental units without treatment. The concentrations of 10.0% v/v and 20.0% v/v had the similar and highest level of performance. The 1.25% v/v concentration showed the least level of performance. Then 2.5% v/v and 5.0% v/v concentrations showed varying levels of performance up to the level similar to the performance of 1.25% v/v and 10.0% v/v respectively.

With *P. guineense* essential oil, different concentrations used performed significantly better in reducing weight loss of bean seeds than the experimental units without treatment. The 20.0% v/v concentration performed best in reducing

weight loss. This is followed by 10.0% v/v concentration, varying performances of 5.0% v/v and 2.5% v/v, and then 1.25% v/v concentration with the least level of performance. With *A. indica* essential oil, different concentrations used performed significantly better in reducing weight loss of bean seeds than the experimental units without treatment. The 20.0% v/v concentration performed best in reducing weight loss whereas 1.25% v/v performed the least. Other concentrations 2.5% v/v, 5.0% v/v and 10.0% v/v concentrations had a similar performance that showed the same kind of variation between the highest and the least performing concentrations. With *G. latifolium* leaf oil extracts, different concentrations used except for 1.25%, performed significantly better in reducing weight loss of bean seeds than the experimental units without treatment. The concentrations of 10.0 % v/v and 20.0 % v/v had the similar and highest level of performance.

5. Conclusion

The five plants showed a similar level of performance in the management of *C. maculatus*. The highest concentration, 20.0% v/v used for each plant performed best while the least concentration, 1.25% v/v used performed least with *O. gratissimum* having the best overall performance. Their pesticidal activity is related to their phytochemical constituents and their bitterness. With some surviving adults and eggs laid, this study recorded damage in quality and loss of the bean seeds treated with the plant extracts due to *C. maculatus* infestation. Nevertheless, the plant materials are edible and do not have a toxic effect when consumed by humans in preserved beans. Its biodegradable nature also helps in reducing environmental toxicity due to reduced residual effects. However, it is recommended that a higher concentration of the plant material be evaluated.

Compliance with ethical standards

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Disclosure of conflict of interest

The author declared that there was no conflict of interest during the cause of this study and producing and submitting this manuscript for publication.

References

- [1] Ofuya TI and Lale NES. Pest of Stored Cereals and Pulses in Nigeria: Biology, Ecology and Control. Dave Collins Publications, Akure, Nigeria, 2001; 174p.
- [2] Onyido AE, Zeibe CC, Okonkwo NJ, Ezugbo-Nwobi IK, Egbuche CM, Udemezie OI and Ezeanya IC. Damaged Caused by the Bean Bruchid, *Callosobruchus maculatus* (Fabricius) on different legume seeds on sale in Awka and Onitsha Markets, Anambra State, South Eastern Nigeria. International Multidisciplinary Journal, 2011; 5(4): 116-123.
- [3] Umeozor OC. Effect of the Infection of *Callosobruchus maculatus* (Fab.) on Weight Loss of Stored Cowpea (*Vigna unguiculata* (L.) Walp). Journal of Applied Science and Environmental Management, 2005; 9(1): 169-172.
- [4] Ali SM, Mahgoub SM, Hamed MS, Derkyi NSA, Acquaaah SO and Owusu-Akyaw M. Bioactivity of some natural products against the cowpea storage weevil *Callosobruchus maculatus* L. International Journal of Biology and Chemical Science, 2010; 4: 616–623.
- [5] IITA (International Institute of Tropical Agriculture) Research Highlights. Ibadan, Nigeria, 1995; Pp 68-72.
- [6] Isman MB. Botanical insecticides: for richer, for poorer. Pest Management Sciences, 2008; 64: 8–11.
- [7] Lale NES. Stored Product Pest Entomology and Acrology in Tropical Africa. 1st Edition Mole Publication, Nigeria Limited. Maiduguri, Nigeria, 2002; Pp 53-56 and 152-160.
- [8] Adedire CO, Obembe OO, Akinkurolele RO. and Oduleye O. Response of *Callosobruchus maculatus* (Coleoptera: Chysomelidae: Bruchidae) to extracts of cashew kernels. Journal of Plant Disease and Protection, 2011; 118: 75–79.
- [9] Adeniyi SA, Orjiekwe CL, Ehiagbonare JE and Arimah BD. Preliminary phytochemical analysis and insecticidal activity of ethanolic extracts of four tropical plants (*Vernonia amygdalina*, *Sida acuta*, *Ocimum*

gratissimum and *Telfaria occidentalis*) against beans weevil (*Acanthscelides obtectus*). International Journal of Physical Sciences, 2010; 5: 753–762.

- [10] Regnault-Roger C, Vincent C and Arnason, JT. Essential oils in insect control: low-risk products in a high-stakes world. Annual Revised Entomology, 2012; 57: 405–424.
- [11] Ojo JA, Olunloyo AA, and Akanni EO. Efficacy of *Moringa oleifera* Leaf Powder against *Callosobruchus maculatus* (F.) (Coleoptera: Chrysomelidae) on Stored Cowpea (*Vigna unguiculata* L. Walp). Journal of Insect Science, 2013; 5(12):240-244.
- [12] Aku AA, Ogunwolu EO and Attah JA. Journal of plant diseases and protection, 1998; 105(5):513-519.
- [13] Trease GE and Evans WC. Pharmacognosy, (11th Edition), Balliere Tindall, London, 1996; Pp 162.
- [14] Nwankwo EN, Okonkwo NJ, Uko I, Okafor EG, Ukonze BC, Ogbonna CU, Egbuche CM, Chikezie FM and Obiechina IO. Evaluation of *Moringa oleifera* (Lam) (Moringaceae) seed oil for larval control of *Aedes aegypti* L. (Diptera: Culicidae). The International Journal of Science and Technology, 2014; 12(2): 75-81.
- [15] Golob P, Changjanaan, P, Ahmed A and Cox J. Susceptibility of *Prostephanus truncatus* (Horn) (Coleoptera: Bostrichidae and *Sitophilus* species in Tanzania. Journal of Stored Product Research, 1998; 26(4):187-198.
- [16] Sharma HC, Sujana G, Rao DM. Morphological and Chemical Components of Resistance to Pod Borer, *Helicoverpa armigera* in Wild relatives of Pigeonpea. Arthropod Plant Interact, 2009; 3: 151-161.
- [17] Food and Agriculture Organization. Regional overview of food insecurity: African food insecurity prospects brighter than ever. FAO, Accra, 2015