Tropical Journal of Applied Natural Sciences
Vol. 2 Issue 2 (2024)

Tropical Journal of Applied Natural Science
ISSN: 2449-2043

Phytochemical Composition and Repellent Activities of Selected Botanical Extracts Against Cowpea Weevil for Natural Preservation of Cowpea Seeds

Idigo, M. A[1*]., Anyaegbunam, L. C[1]., Ekesiobi, A. O[1]. and Enyinnaya, J. O[2].

[1]Department of Biological Sciences, Faculty of Natural Science, Chukwuemeka Odumegwu Ojukwu University.
[2]Department of Parasitology and Entomology, Faculty of Biological Sciences, Nnamdi Azikiwe University Awka Anambra State, Nigeria.

***Correspondence**: idigomediatrix@gmail.com

Abstract

Callosobruchus maculatus (Fab.) is a significant field-to-store pest that causes quantitative and qualitative losses in stored cowpea. Management of this pest has primarily relied on synthetic pesticides, which are associated with severe health risks, including fatalities. In view of the recent increased interest in developing biopesticides as an alternative to synthetic pesticides, this study evaluated the synergistic repellent activities of botanical extracts against *C. maculatus*. Fresh leaves of *Vernonia amygdalina*, *Ocimum grattisimum*, and *Gongronema latifolium* were harvested, washed, dried, pulverized, and soaked in distilled water (1:10) and hexane (1: 10) for 24 hours. The bioactive components of the extracts were analyzed using Gas Chromatography-Flame Ionization Detector (GC-FID). Stock solutions (200mg/ml) were prepared and reconstituted in distilled water, acetone, and Tween80 to produce lower concentrations (20, 50, 100, and 150 mg/ml). The repellency effects of these reconstituted extracts against *C. maculatus* adult were assessed using a filter paper repellency method with three replicates, three negative controls, and one standard (2.5 µl/ml Deltamethrin synthetic insecticide). The analysis identified Apigenin (31.36%) as the dominant compound in the aqueous extract of *V. amygdalina*, while Artemetin (80.94%) was predominant in its hexane extract. *O. grattisimum* aqueous extract was dominated by Sinapinic acid (72.04%), and its hexane extract by Syringic acid (56.91%). *G. latifolium* aqueous extract contained Kaempferol (47.22%) as its major compound, while Luteolin (22.15%) was dominant in its hexane extract. The repellency of all extracts increased with concentration, with 200mg/ml demonstrating the highest repellency (up to 100%) over 96hours. The aqueous extract showed repellency ranging from 16.7% to 100%, acetone reconstituted hexane extract ranged from 11.1% to 100%, and Tween80 reconstituted hexane extract ranges from 6.7% to 100%. Statistical analysis revealed that differences in repellency were not statistically significant ($p>0.05$) across most concentrations, except for 50mg/ml of Tween80 reconstituted hexane extract. In descending order of effectiveness, the repellency trend was: aqueous extract > acetone reconstituted hexane extract > Tween80 reconstituted hexane extract. This finding suggest that botanical extracts, particularly aqueous formulations, are promising natural repellents for *C. maculatus* and offer a safer alternative to conventional synthetic pesticides.

Keywords: ***Callosobruchus maculatus,* Botanical extracts, Repellency, Biopesticides, Cowpea protection**

INTRODUCTION

Callosobruchus maculatus (Fabricius) (Coleoptera: Bruchidae), also known as cowpea weevil, cowpea beetle, or bruchid, is a cosmopolitan field-to-store pest and it is ranked as the principal post-harvest pest of cowpea (*Vigna unguiculata* (L.) both in Africa and Asia (Deshpande *et al*. 2011). It causes substantial quantitative and qualitative losses manifested by seed perforation and reductions in weight, market value and germination ability of seeds (Oluwafemi, 2012). Infestation begins at a low level in the field when grain moisture is high and continues in storage, causing damage ranging from making cowpea unfit for consumption to reducing viability. Because the insect population grows rapidly in storage, large losses can be reported (Muhammad *et al*., 2020).

Once infestation is established, farmers generally resort to application of synthetic pesticides otherwise loss of the entire stored product is inevitable (Mohammed *et al*., 2013). Unfortunately, the increasing cost of synthetic pesticides, combined with farmers' declining revenue in the face of ever-depreciating national currencies, has made these insecticides too expensive for most farmers (Iya & Kwaghe, 2007). Aside from being expensive, synthetic pesticides have numerous negative side effects, the most serious of which is their impact on human health. Among the others are pesticide food contamination, environmental pollution issues, natural balance disruption, toxicity to non-target organisms, and pest resistance development (Sharma *et al*., 2019). Recent studies have shown that increasing insecticide resistance, among all other things, continues to threaten the efficacy of synthetic insecticides employed in the management of insect pests (Ffrench-Constant *et al*., 2004). In most cases, the over-reliance on a single class of insecticide, to suppress insect pest populations below economic injury level, has been singled out as a cause for the development of resistance in insects (Ffrench-Constant *et al*., 2004). This suggests the need for alternative management method that would protect the crop and also the environment. According to a growing body of research, botanicals appear to be a promising alternative to synthetic pesticides (Karani *et al*., 2017).

Many botanical products have been tested for toxicity against a variety of stored-product insect pests, including the cowpea weevil, and are believed to have shown promising results as crop protectants (Moravvej & Abbar, 2008). Some of the locally available plants with pesticidal properties include Chilli pepper (*Capsicum nigrum*), Garlic (*Allium sativum*), Scent leaf (*Ocimum gratissimum*), Bitter leaf (*Vernonia amygdalina*), Ginger (*Zingiber officinale*), Utazi leaf (*Gongronema latifolium*), Neem (*Azadirachta indica*), Moringa (*Moringa oleifera*) among others. The application of these plant materials has been proven to be a viable alternative to synthetic insecticides and they do not have serious side effects as they are biodegradable, easily available, lower in cost compared to synthetic pesticides, less toxic to human and non-targeted organisms and are compatible with different human cultures (Mpumi *et al*., 2016). The mixture of two or more botanicals could play a role in increasing the insecticide activity and reduce the cost for controlling pest. In fact, it has been demonstrated that insect pests when exposed to a mixture of plant extracts combined in specific ratios recorded more insect mortality compared to when they were exposed to the plant extracts singly (Soe *et al*., 2019). This study is carried out to determine the synergistic repellent potential of the plant extracts from three plant species at various concentrations against cowpea weevil, *C. maculatus*.

MATERIALS AND METHODS

Experimental Design

This was a longitudinal study laid out in a Completely Randomized Design (CRD) to determine the repellent effect of the aqueous and N-hexane extracts (reconstituted with acetone and Tween 80 respectively) of *Vernonia amygdalina, Gongronema latifolium*, and *Ocimum gratissimum* against *Callosobruchus maculatus*. The aqueous extract and the N-hexane extracts (acetone and tween80) of *O. gratissimum, V. amygdalina,* and *G. latifolium* were mixed in a 1:1:1 volume ratio respectively. Five different concentrations of each extracts were prepared and used as treatment. Three replicates were used for the aqueous and N-hexane concentrates. A standard / positive control (Deltamethrin synthetic insecticide) and three negative controls (C_1 – acetone, C_2 – Tween 80 and C_3 – no treatment) were equally used. Percentage repellency and protection time and of the various plant extracts were all determined in this study. The independent variables were the different concentrations of the various plant extracts, while the dependent variables were the percentage repellency and protection time.

Culturing of *Callosobruchus maculatus*

The initial stock of *Callosobruchus maculatus* was obtained from infested cowpea, purchased at the Eke-Awka market, Awka, Anambra State, Nigeria. Plastic container used for the rearing was covered with fine mesh nets to prevent escape or entrance of insects. The rearing container was allowed to stand for two weeks at temperature of 30^{o}C and relative humidity of 80% R.H under laboratory condition to yield enough adults of the insects before being used for further experiment.

Experimental Cowpea Seeds

Untreated cowpea obtained from International Institute of tropical Agricultural (IITA) Ibadan, Nigeria was used for this study. Mature, wholesome seeds from the sample devoid of debris and emergence holes were heat sterilized in the oven at 60^{o}C for 3 hours and allowed to cool for an hour (Ogbonna *et al*., 2016). Afterwards, the adult *C. maculatus* was introduced into the uninfested and sterilized cowpea, and allowed to oviposit. The newly emerged adults were transferred to a sterile grain container for use in the experiment. The cultures was kept under a temperature of 32 ± 20 ^{o}C, 70 % R.H and 12L: 12D photo regime (Ogbonna *et al*., 2016). This will ensure that the adult F_1 *C. maculatus* used as the experiment's culturing stock are of the same size and age. Subsequently, 20g of the cowpea were weighed into labeled 60cm^3 plastic container; each container was covered with ventilated screw cap, sealed with nylon netting to prevent entry of insects but allowed ventilation of the samples.

Collection, Identification and Preparation of the Plant Materials

The plants that were used for the study were *Ocimum gratissimum* (Scent leaf), *Vernonia amygdalina* (Bitter leaf), and *Gongronema latifolium* (bushbuck). All the plant leaves for the experiment were harvested from Chimzik farms in Aguleri, Anambra East LGA of Anambra State. The plants were taken to the laboratory for identification and authentication by Dr. C. G. Ukpaka, an experienced botanist from the Department of the Biological science, Chukwuemeka Odumegwu Ojukwu University.

Preparation of Aqueous and Hexane Extracts

The fresh leaves of *Ocimum gratissimum, Vernonia amygdalina,* and *Gongronema latifolium* were washed, shade-dried, and blended into a fine powder. Two hundred grams of each properly homogenized leaves were soaked in six liters of distilled water (1:10) for aqueous extract and hexane (1:10) for hexane extract respectively for twenty-four hours. These were filtered using a muslin cloth and a Whatman filter paper and then concentrated using a water bath at 50-60^{o}C. Viscous extracts were obtained and stored separately in airtight bottles in a refrigerator maintained at 2-8^{o}C until when required for further work.

Preparation of the Extracts for Bioactive Component Analysis

0.2g of each extracts were weighed and transferred in a test tube and 15ml ethanol and 10ml of 50%m/v potassium hydroxide was added. The test tube was allowed to react in a water bath at 60^{o}C for 3hours. After the reaction time, the reaction product contained in the test tube was transferred to a separatory funnel. The tube was washed successfully with 20ml of ethanol, 10ml of cold water, 10ml of hot water and 3ml of hexane, which was all transferred to the funnel. This extracts were combined and washed three times with 10ml of 10%v/v ethanol aqueous solution. The ethanol solvent was evaporated and then the extracts were later cleared to remove impurities using flosiril (magnesium silicate).

Quantification of the Bioactive Compound by GC-FID

The analysis of the bioactive compound of the extracts was conducted at the Docchy Analytical Laboratories and Environmental Services Limited, Awka, Anambra State. The analysis was performed on an Agilent 6890 Gas chromatography equipped with a flame ionization detector. A RESTEK 15 meter MXT-1 column (15m x 250um x 0.15um) was used. The injector temperature was 280^{o}C with splitless injection of 2ul of sample and a linear velocity of 30cm s^{-1}, Helium 5.0pa.s was the carrier gas with a flow rate of 40 ml min^{-1}. The oven operated initially at 200 ^{o}C, it was heated to 330^{o}C at a rate of 3^{o}C min^{-1} and was kept at this temperature for 5min. the detector operated at a temperature of 320^{o}C. The active compounds were determined by the ratio between the area and mass of internal standard and the area of the identified phytochemicals. The concentrations of the different bioactive compound were express in ppm.

Preparation of Stock Solutions for Repellency Bioassay

A stock solution of 200mg/ml of each extracts was prepared. This was done by dissolving 20g of each extracts in 100ml of the relevant solvent (i.e. for the aqueous extract, distilled water was used for the reconstitution while acetone and Tween 80 were used for the hexane extract due to its hydrophobicity), making a total of three stock solutions i.e. aqueous, n-hexane(acetone) and n-hexane(tween80). From this stock, other solutions of lower concentrations (20, 50, 100 and 150 mg/ml) were prepared by using the dilution formula: $C_1V_1 = C_2V_2$, where C_1 is the stock concentration (200mg/ml), V_1 is the unknown volume to be taken from the stock, C_2 is the concentration of the new solution to be prepared (20, 50, 100, 150 mg/ml), V_2 is the total volume (10ml). All solutions were prepared and stored in a refrigerator in airtight bottles in a refrigerator maintained at 2-8 ^{o}C until the commencement of the repellency test.

Repellence Bioassay

The method described by Ogbonna *et al*. (2016) was used for the repellence test. The base of a 9 cm diameter glass Petri-dish was lined with filter paper divided into four equal parts. In each quarter equidistant to the centre in an alternate untreated (control)-treated arrangement, 4 g of treated cowpea was placed. This was replicated three times per concentration. Deltamethrin synthetic insecticide (2.5 µl/ml) was used as the standard treatment. A total of 5 unsexed adult *C. maculatus* was introduced at the centre of the Petri-dish and covered. The setup was kept in the dark at a temperature of 32 ± 20C and 70% R.H. The number of insects present in the control and treated grains was recorded after 24 h for 5 days (Soe *et al*., 2020). Percentage repellence values were computed using the formula adopted by Bett *et al*. (2017).

$$\text{Percent Repellence (PR)} = \frac{(N_C - N_T)}{(N_C + N_T)} \times 100$$

Where N_C represents the number of insects in the control and N_T is the number of insects in the treatment slide.

Data Analysis

The data was analysed using descriptive statistics such as mean and standard deviation (SD). The analysis of variance (ANOVA) at 95% confidence interval was used to compare the means across the different treatment groups. The Tukey's post hoc test was used for pairwise comparison of the means in groups that had statistically significant difference. Statistical significance was determined at 5% probability level ($p < 0.05$). The statistical analysis was performed in R version 4.1.1 (R Core Team 2021).

RESULTS

The analysis of the aqueous extracts revealed complex mixtures of bioactive constituents. A total of 30 compounds were identified both in the aqueous extracts and N-hexane extracts of the three plant species by GC-FID (Table 1 and 2). For the various aqueous extracts, *V. amygdalina* contains the most abundant component with a total of 17 compounds as against 14 compound identified in *G. latifolium* and 11 compounds identified in *O. grattisimum.* Whereas, the bioactive screening of the N-hexane extracts shows that, *O. grattisimum* contained the most abundant component with a total of 16 compounds as against 15 compound identified in *V. amygdalina* and 11 compounds identified in *G. latifolium.*

V. amygdalina (aqueous extract) and *V. amygdalina* (N-hexane extract) contain 8 compounds in common, viz., kaempferol, artemetin, ellagic acid, vanillic, naringenin, apigenin, isorhamnetin, myricetin and daidzein. *O. grattisimum* (aqueous extract) and *O. grattisimum* (N-hexane extract) contain 4 compounds in common, viz., gallocatechin-3-gallate, syringic acid, sinapinic acid and rosemarinic acid. Whereas, *G. latifolium* (aqueous extract) and *G. latifolium* (N-hexane extract) contain 10 compounds in common, viz., kaempferol, catechin, quercetin, luteolin, myricetin, daidzein, resveratrol, epicatechin, silymarin and baicalin.

Table 1: Bioactive Constituents in the Aqueous Extracts of the three plant species

Components (ppm)	**Plant species**		
	Vernonia amygdalina	***Ocimum grattisimum***	***Gongronema latifolium***
	Conc. (%)	**Conc. (%)**	**Conc. (%)**
Kaempferol	19.55	-	47.22
Nobiletin	1.69	1.72	-
Genistein	0.73	-	-
Catechin	17.41	-	11.56
Flavone	1.64	-	-
Artemetin	0.77	-	0.20
Quercetin	0.90	-	0.30
Luteolin	22.07	-	0.59
Retusin	0.88	-	0.17
Hesperidin	0.47	-	-
Ellagic acid	0.29	-	-
Vanillic acid	0.25	0.31	-
Naringenin	0.88	-	-
Apigenin	31.36	-	-
Isorhamnetin	0.48	-	-
Myricetin	0.36	0.13	0.48
Daidzein	0.27	-	0.11
Resveratrol	-	-	0.27
Tangeretein	-	2.25	0.13
Epicatechin	-	-	26.78
Silymarin	-	-	11.82
Baicalin	-	-	0.25
Ferulic acid	-	-	0.12
Gallocatechin 3 gallate	-	0.34	-
Robinetin	-	1.34	-
Naringin	-	3.77	-
Cinnamic acid	-	4.44	-
Syringic acid	-	13.42	-
Sinapinic acid	-	72.04	-
Rosemarinic acid	-	0.25	-

Table 2: Bioactive Constituents in the N-Haxane Extracts of the three plant species

Components (ppm)	**Plant species**		
	Vernonia amygdalina	***Ocimum grattisimum***	***Gongronema latifolium***
	Conc. (%)	**Conc. (%)**	**Conc. (%)**
Kaempferol	0.81	0.04	6.09
Genistein	-	2.63	-
Catechin	-	23.33	3.60
Artemetin	80.94	-	-
Quercetin	-	1.56	11.60
Luteolin	-	0.027	22.15
Ellagic acid	0.53	-	-
Vanillic acid	3.70	-	-
Naringenin	2.22	-	-
Apigenin	0.66	2.82	-
Isorhamnetin	2.19	-	-
Myricetin	1.83	-	11.64
Daidzein	0.85	0.021	12.48
Resveratrol	-	0.08	5.47
Tangeretein	0.55	-	-
Epicatechin	1.83	0.0196	4.11
Silymarin	-	-	3.80
Baicalin	-	-	6.18
Gallocatechin 3 gallate	-	0.05	-
Gallocatechin	1.17	-	-
Naringin	0.70	-	12.87
Syringic acid	-	56.91	-
Sinapinic acid	-	7.81	-
Rosemarinic acid	-	4.63	-
Daidzin	-	0.03	-
Butein	-	0.031	-
Piperic acid	-	0.016	-
Lunamarin	0.64	-	-
Reveratrol	1.37	-	-

Repellent Activities of Extract Blends against *Callosobruchus maculatus*

The repellent activity of aqueous extract, acetone reconstituted hexane extract and tween80 reconstituted hexane extract blends of the three plant species against *C. maculatus*, were presented in Table 3.

For the aqueous extracts of the three plant species, at 24hrs after application of the different concentrations of the extracts, and the standard (2.5 µl/ml Deltamethrin), 100 % repellency was also observed at 100 mg/ml and 50 mg/ml concentration when compared with that of the standard ($p< 0.05$). At 48hrs of application, no repellency was recorded at the lowest concentration (20 mg/ml). However, complete protection (100% repellency) was achieved at 150 mg/ml. A statistically significant difference in repellency was observed compared to the standard ($p = 0.02$). At 72hours, both 6.25% and 12.5% concentrations demonstrated 100% repellency. At 96 hours, the repellency differences across concentrations were not statistically significant ($p = 0.05$), though the highest concentration (200 mg/ml) maintained 100% repellency as presented in Table 3.

For the acetone reconstituted hexane extracts, at 24 hours of exposure time, Both the highest concentration (200 mg/ml) and the standard achieved 100% repellency. The lowest repellency (33%) was recorded at 50 mg/ml and 20 mg/ml. Differences across concentrations were not statistically significant ($p > 0.05$). At 48 hours, the highest concentration (200 mg/ml) maintained 100% repellency, while the lowest repellency was recorded at 50 mg/ml. Differences across concentrations were statistically significant (F (5, 12) = [4.70], $p = 0.01$). At 72 hours, the standard achieved 100% repellency, but the lowest repellency was observed at 100 mg/ml. Differences across concentrations were not statistically significant (F (5, 12) = [0.84], $p = 0.55$). At 96 hours, while the standard achieved 100% repellency, the lowest repellency (11%) was recorded at 20 mg/ml. Differences were marginally significant (F (5, 12) = [3.14], $p = 0.05$) as presented in Table 3.

For the tween80 reconstituted hexane extracts, at 24 hours of exposure, both the highest concentration (200 mg/ml) and the standard achieved 100% repellency. Although repellency varied across concentrations, differences were not statistically significant (F (5, 12) = [2.599], $p = 0.08$). At 48 hours, the highest repellency (77.8%) was observed in the standard, while the lowest repellency occurred at 50 mg/ml. Differences were not statistically significant (F (5, 12) = [1.085], $p = 0.42$). At 72 hours, the standard maintained 100% repellency, while the lowest repellency was recorded at 50 mg/ml. Differences were not statistically significant (F (5, 12) = [2.306], $p = 0.11$). At 96 hours of exposure time, the standard achieved 100% repellency, while the lowest repellency (6.7%) occurred at 20 mg/ml. Differences across concentrations were statistically significant (F (5, 12) = [4.065], $p = 0.02$) as presented in Table 3.

Table 3: Percentage repellency of different extract blends *against Callosobruchus maculatus*

Extract Blends	Exposure Time (Hours)	Repellency Concentration (%)						*P*-value
		200 mg/ml	150 mg/ml	100 mg/ml	50 mg/ml	20 mg/ml	Standard	
Aqueous Extracts (Va + Og + Gl)	24	77.8 ± 38.5[a]	61.1 ± 34.7[a]	100.0 ± 0.0[a]	100.0 ± 0.0[a]	0.0 ± 0.0[a]	100.0 ± 0.0 [a]	<0.001
	48	83.3 ± 28.9[a]	100.0 ± 0.0[a]	77.8 ± 38.5[a]	77.8 ± 38.5[a]	0.0 ± 0.0[a]	77.8 ± 38.5[a]	0.02
	72	100.0 ± 0.0[a]	83.3 ± 28.9[a]	100.0 ± 0.0[a]	77.8 ± 38.5[a]	16.7 ± 28.9[a]	100.0 ± 0.0[a]	0.01
	96	100.0 ± 0.0[a]	100.0 ± 0.0[a]	66.7 ± 57.7[a]	64.4 ± 33.6[a]	27.8 ± 25.5[a]	100.0 ± 0.0[a]	0.05
Acetone Reconstituted Hexane Extracts (Va + Og + Gl)	24	100.0 ± 0.0[a]	77.8 ± 38.5[a]	83.3 ± 28.9[a]	33.3 ± 0.0[a]	33.3 ± 28.9[a]	100.0 ± 0.0[a]	0.08
	48	100.0 ± 0.0[a]	77.8 ± 38.5[a]	66.7 ± 28.9[a]	11.1 ± 19.2[a]	16.7 ± 28.9[a]	77.8 ± 38.5[a]	0.01
	72	66.7 ± 28.9[a]	83.3 ± 28.9[a]	53.3 ± 5.8[a]	66.7 ± 57.7[a]	56.7 ± 40.4[a]	100.0 ± 0.0[a]	0.55
	96	83.3 ± 28.9[a]	37.8 ± 20.4[a]	40 ± 52.9[a]	56.7 ± 40.4[a]	11.1 ± 19.2[a]	100 ± 0.0[a]	0.05
Tween80 Reconstituted Hexane Extracts (Va + Og + Gl)	24	100.0 ± 0.0[a]	44.4 ± 50.9[a]	46.7 ± 23.1[a]	86.7 ± 23.1[b]	70.0 ± 26.5[a]	100.0 ± 0.0[a]	0.08
	48	37.8 ± 20.4[a]	70 ± 26.5[a]	60.0 ± 40.0[a]	31.1 ± 30.1[ab]	46.7 ± 23.1[a]	77.8 ± 38.5[a]	0.42
	72	70.0 ± 26.5[a]	77.8 ± 38.5[a]	66.7 ± 28.9[a]	33.3 ± 23.1[ab]	40.0 ± 34.6[a]	100.0 ± 0.0[a]	0.11
	96	60.0 ± 40.0[a]	60.0 ± 40.0[a]	60.0 ± 40.0[a]	20.0 ± 0.0[a]	6.7 ± 11.5[a]	100.0 ± 0.0[a]	0.02

Percentage values (Mean ± S.E.) followed by the same letter(s) in the same column are not significantly different at $p < 0.05$ using Tukey's post hoc test. **Va**: *Vernonia amygdalina,* **Og**: *Ocimum gratissimum,* and **Gl**: *Gongronema latifolium*

Comparative Repellency of Extract Blends

Overall, the aqueous extract showed varying levels of repellency across different concentrations and time intervals. At higher concentrations (200 and 150 mg/ml), it exhibited relatively high repellency percentages, achieving 100% effectiveness at 72 and 96 hours (Figure 1). Lower concentrations (100, 50, and 20 mg/ml), showed variable effectiveness, with repellency significantly reduced at 20 mg/ml across all time intervals (0-27.8%). N-hexane extract in acetone generally showed high repellency at 200mg/ml, with values ranging from 66.7% to 100% across all exposure time (Figure 1). However, declined at lower concentrations and longer exposure, particularly for 20mg/ml, where repellency dropped to 11.1% by 96 hours. N-hexane extract in tween 80 demonstrated initial repellency at higher concentrations (up to 100% at 200mg/ml for 24houtrs), its effectiveness was less consistent compared to the aqueous and N-hexane extract in acetone extract blends (Figure 1). Lower concentration (50 and 20 mg/ml) showed notably lower repellency, with percentages dropping to 6.7% by 96hours at 20mg/ml.

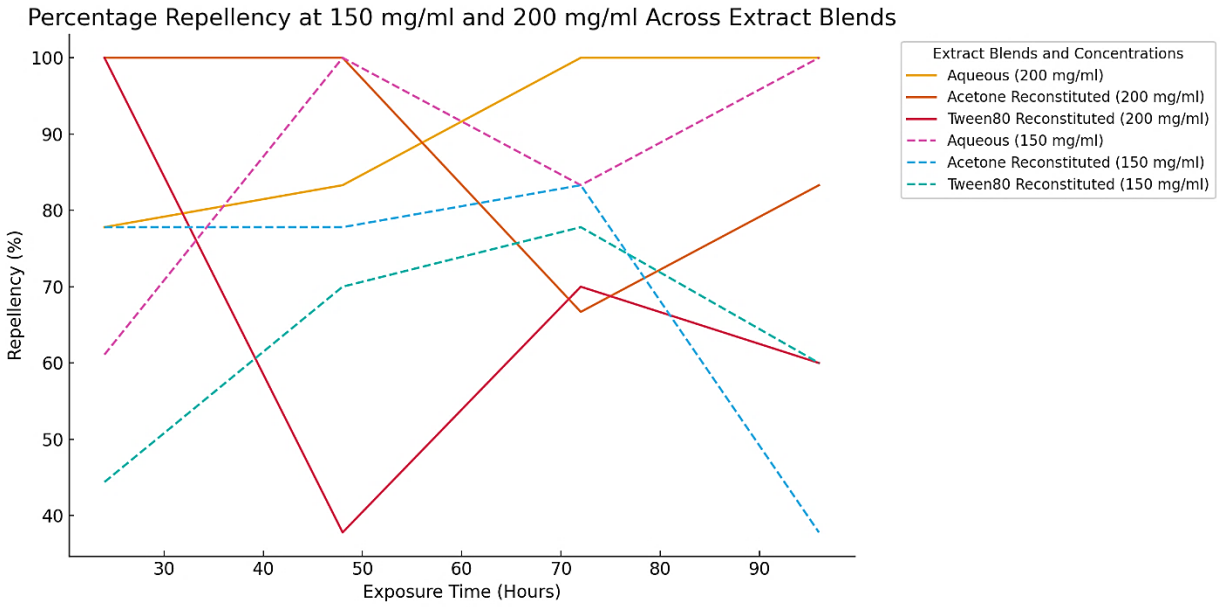

Figure 1: Graph illustrating the repellency percentages at 150mg/ml and 200mg/ml for all extract blends over different exposure time.

DISCUSSION

This work has shown the efficacy of the extracts (both aqueous and n-hexane) of *Ocimum gratissimum*, *Vernonia amygdalina*, and *Gongronema latifolium* as a plant-based repellent against *Callosobruchus maculatus*. The result from the bioactive screening showed that each plant extracts contained in abundance one of the major bioactive compounds such as flavonoids, a class of polyphenols that have been extensively studied as insecticides in crop protection because of their involvement in plant defense responses (Pereira *et al*., 2024). They can inhibit enzymatic activity and prevent the growth of larvae of different insect species (Kim, *et al*., 2000). Flavonoids are the most abundant non-nitrogenous phytochemicals and some interfere in the process of moulting and reproduction of several insects, that is, they inhibit the formation of juvenile hormone (ecdysone). It has been reported that some types of flavonoids have had an effect on agricultural pests with ovicidal effect, oviposition, fecundity, mortality, weight

reduction, and emergence of adults (Goławska *et al*., 2014; Salunke *et al*., 2005). Some of ethe flavonoids found in this study includes; quercetin, naringin, kaempferol, myricetin, daidzein, isoflavonoids, etc. Some of these phytochemicals have been reported to be used by the plants as morphological and biochemical tools for defense against herbivore (Sharma *et al*., 2009). It therefore means that the leaf extracts of the plants my show contact and/ or systemic mechanism of action. It has been reported that the natural phytochemicals from plants have a potential of being eco-friendly and can replace synthetic pesticides for insect pests control (Ashraf *et al*., 2020). The result showed that aqueous extracts of *V. amygdalina,* and *G. latifolium* contained the most abundant bioactive compounds in both plants when compared with the n-hexane counterpart. However, the reverse was observed in *O. gratissimum,* where the n-hexane extracts contained the highest amount of bioactive compounds compared to its aqueous extracts. This may be as a result of the fact that *O. gratissimum* are odoriferous and therefore only n-hexane can infiltrate into the plant tissue and increase the process of extraction (Oyebuchi & Kavaz, 2020). Moreover, the lower number of bioactive compound in the aqueous extracts of *O. gratissimum* compared to the n-hexane extracts might be due to reduced compound solubility in water as well as the extraction conditions as also pointed out by extraction (Oyebuchi & Kavaz, 2020).

All extract blends from the various extraction solvents showed repellent activity of varying degree against *C. maculatus* at different tested concentrations. In this study, *C. maculatus* responded differently to the repellent effects of the extracts in accordance with its behavioural tendency. This was also similar to the finding of Ito & Anigboro (2019) who recorded *C. maculatus* responding differently to treatments accordance with its behavioural tendency. The aqueous extract showed 100% repellency at 24 hours post-treatment, with concentrations of 50 and 100mg/ml when compared with that of the standard ($p<0.05$). It also showed total protection (100% repellency) at 48 hours post-treatment, with concentration of 150 mg/ml. It also showed 100% repellency at 200mg/ml concentration at 72 hours post-treatment. However, at 96 hours post-treatment, 100% repellency was observed at 150 and 200mg/ml concentration. Only 20 and 200mg/ml concentration showed that an increase in the exposure time increases the percentage repellency of the aqueous extracts. This may be because of the irregularity in the behavioural tendency of the tested *C. maculatus*. Also, the synergetic effect of the aqueous extracts showed good results with the concentrations ranging from ≥ 50 mg/ml used to achieve complete protection from *C. maculatus.* Since the aqueous extracts contain the most abundant compounds in both *V. amygdalina* and *G. latifolium,* the presence of these compounds could be attributed towards the higher repellent bioactivity of the synergetic effect of the aqueous extracts at concentration ≥ 50 mg/ml.

The acetone reconstituted hexane extracts also showed a high repellent activity that lasted for 48hours at a higher concentration of 200mg/ml and its repellent effect was similar to that of positive control (standard). However, at a lower concentration ranging from 50-150 mg/ml, it showed 11.1±19.2 % to 83.3±28.9 % repellency at varying hours of post-treatment. In addition, there were irregularities observed in the behavioural pattern of the tested *C. maculatus* which might have led to the poor repellent activity of the n-hexane extracts compared to the repellent effect of aqueous extracts on *C. maculatus* at concentration ≥ 50 mg/ml. Only 100mg/ml concentration showed that an increase in the exposure time decreases the percentage repellency of the acetone reconstituted hexane extracts. However, there was no statistical difference in the percentage repellency of the various concentrations of the acetone reconstituted hexane extracts on the *C. maculatus* ($p>0.05$).

On the other hand, the Tween80 reconstituted hexane extracts showed a repellent activity that lasted for less than 48hours post-treatment at a higher concentration of 200mg/ml with repellent effect similar to that of positive control (standard). For all the exposure time used, the highest percentage repellency was observed only in the standard. Therefore, a poor repellent activity was recorded in the Tween80 reconstituted hexane extracts when compared to aqueous extracts and those of the acetone reconstituted hexane extracts

The results indicated that the highest repellency of the pest across the various extracts over 96 hours was produced by the highest concentration of 200 mg/ml. These findings agreed with the study of Ito & Anigboro (2019) who reported that the powders and crude extracts of plants' types was produced by the highest treatment of 4.0g/200g. This study therefore reveals that aqueous extracts demonstrated higher repellency, particularly at higher concentrations, making them the most effective. Acetone reconstituted hexane extracts were effective but not as consistent as aqueous extract. Whereas, the Tween80 reconstituted hexane extracts appeared to be less effective overall, especially at prolonged exposure times and lower concentrations. In this study, it was also observed that there are great differences between the activities of the various plant extracts used. This can be ascribed partly to the differences in the concentration of the component used as well as the irregularity in the behavioural tendency of the experimental *C. maculatus*. However, the effectiveness and duration of repellency products depends on multiple factors as described by other researchers, such as the type of repellents (active ingredients and formulation), the mode of application, environmental factors (temperature, humidity, and wind), the attractiveness of individual people to insects, loss due to removal by perspiration and abrasion, the sensitivity of the insects to repellent and the biting density (Ahmad *et al*., 2011; Govindarajan, 2011; Singha and Chandra, 2011).

CONCLUSION

The finding obtained in this study revealed that, all the three different extract blends both had repellent activity against *C. maculatus* in the protection of cowpea (*Vigna unguiculata*). However, aqueous extracts offer a more promising alternative for eco-friendly pest management, particularly for *C. maculatus*, and warrant further exploration to optimize formulations and application strategies.

ACKNOWLEDGEMENTS

The authors thank the Department of Parasitology and Entomology, Faculty of Biosciences, Nnamdi Azikiwe University, Awka, Anambra State, Nigeria, for providing the laboratory facility needed for the repellency tests. We are also thankful to the botanist, Dr. C. G. Ukpaka, Department of Biological Sciences, Chukwuemeka Odumegwu Ojukwu University, Nigeria, for plant identification. We also appreciate the Staff and Management of the International Institute of tropical Agricultural (IITA) Ibadan, Nigeria, for providing the experimental cowpea seeds. We are also thankful to the management of Docchy Analytical Laboratories and Environmental Services Limited, Awka, and OPEC Research Consult for their technical assistance provided in bioactive analysis and data analysis respectively.

AUTHOR CONTRIBUTIONS

Idigo, M. A. conceived, designed, carried out the experiment and edited the manuscript. Enyinnaya, J. O. contributed to the interpretation of results, and edited the manuscript. Anyaegbunam, L. C. and Ekesiobi, A. O. oversaw study implementation and monitoring. Each author approved the final manuscript.

CONFLICT OF INTEREST

We declare no conflict of interest.

REFRENCES

Ahmad, N., Fazal, H., Abbasi, B. H., & Iqbal, M. (2011). In vitro larvicidal potential against *Anophelesstephensi* and antioxidative enzyme activities of *Ginkgo biloba*, *Stevia rebaudiana* and *Parthenium hysterophorous*. *Asian Pacific Journal of Tropical Medicine*, *4*(3), 169–175.

Ashraf, A., Qureshi, N. A., Shaheen, N., Iqbal, A., Fatima, H., Afzal, M., Alhewairini, S. S. and Qureshi, M. Z. (2020) Termiticidal and protozocidal potentials of eight tropical plant extracts evaluated against *Odontotermes obesus* Rambur (Blattodea; Termitidae) and *Heterotermes indicola* Wasmann (Blattodea; Rhinotermitidae). *Polish Journal of Environmental Studies*, 29, 3493–3507.

Bett, P. K., Deng, A. L., Ogendo, J. O., Kariuki, S. T., Kamatenesi-mugisha, M., Mihale, J. M., & Torto, B. (2017). Industrial Crops & Products Residual contact toxicity and repellence of Cupressus lusitanica Miller and Eucalyptus saligna Smith essential oils against major stored product insect pests. *Industrial Crops & Products*, *110*, 65-74.

Deshpande, V. K., Makanur, B., Deshpande, S. K., Adiger, S., & Salimath, P. M. (2011). Quantitative and Qualitative Losses Caused by *Callosobruchus maculatus* in Cowpea During Seed Storage. *Plant Archives*, *11*(2), 723–731.

Ffrench-Constant, R. H., Daborn, P. J., & Le Goff, G. (2004). The genetics and genomics of insecticide resistance. *Trends in Genetics*, *20*(3), 163–170.

Goławska, S., Sprawka, I., Łukasik, I., & Goławski, A. (2014). Are naringenin and quercetin useful chemicals in pest-management strategies? *Journal of Pest Science*, *87*(1), 173-180.

Govindarajan, M. (2011). Evaluation of *Andrographis paniculata* Burm. f. (Family: Acanthaceae) extracts against *Culex quinquefasciatus* (Say.) and *Aedes aegypti* (Linn.) (Diptera: Culicidae). *Asian Pacific Journal of Tropical Medicine*, *4*(3), 176–181.

Ito, E. E., & Anigboro, O. F. (2019). Evaluation of Repellent Potential of Some Botanical Products against Cowpea Weevil, *Callosobruchus maculatus* (Fabricius) (Coleoptera: Bruchidae). *Jordan Journal of Biological Sciences*, 12 (3), 267 – 273.

Iya, I. B., & Kwaghe, T. T. (2007), The Economic Effect of Spraying Pesticides on Cowpea (*Vigna unguiculata L*. Walp.) Production in Adamawa State of Nigeria. *International Journal of Agricultural Research*, *2*(7):647–650.

Karani, A., Ndakidemi, P., & Mbega, E. (2017). Botanical Pesticides in Management of Common Bean Pests: Importance and Possibilities for Adoption by Small-scale Farmers in Africa. *Journal of Applied Life Sciences International*, *12*(1), 1–10.

Kim, J. S., Kwon, C. S. & Son, K. H. (2000). Inhibition of ά-glucosidase and ά-amylase by luteolin, a flavonoid. *Bioscience, Biotechnology, and Biochemistry*, *64*, 2458–2461.

Mariana, P-T., Marcos, S-H., & Eva, A-H. (2017). Flavonoids in Agriculture. pp. 189-201. DOI: http://dx.doi.org/10.5772/intechopen.68626.

Mohammed, S. B., Ishiyaku, M. F., & Sami, R. A. (2013). Application of Cry1Ab/Ac Bt strip for screening of resistance for Maruca vitrata in cowpea. *African Journal of Biotechnology*, *12*(40), 5869–5874.

Moravvej, G., & Abbar, S. (2008). Fumigant toxicity of Citrus oils against cowpea seed beetle *Callosobruchus maculatus* (F.) (Coleoptera: Bruchidae). In *Pakistan Journal of Biological Sciences*, *11*(1), 48–54.

Mpumi, N., Mtei, K., Machunda, R., & Ndakidemi, P. A. (2016). The Toxicity, Persistence and Mode of Actions of Selected Botanical Pesticides in Africa against Insect Pests in Common Beans, P. vulgaris: A Review. *American Journal of Plant Sciences*, *07*(01), 138–151.

Muhammad, A., Yusuf, L. I., & Oaya, C. S. (2020). Insecticidal Comparison of Botanicals and Synthetic Insecticide on Cowpea Bruchid , *Callosobruchus maculatus* (Fab .) [Coleoptera : Chrysomelidae] Development on Vigna unguiculata (L .) Walp. *Journal of Entomlogy*, *17*(2), 68–73.

Ogbonna, C. U., Okonkwo, N. J., Nwankwo, E. N., Okeke, P. C., & Ebi, S. E. (2016). Bioefficacy of Zingiber officinale against *Callosobruchus maculatus* Fabricius (Coleoptera : Bruchidae) infesting cowpea. *International Journal of Entomology*, *1*(4), 19–25.

Oluwafemi, A. R. (2012). Comparative Effects of Three Plant Powders and Pirimiphos-Methyl against the Infestation of *Callosobruchus maculatus* (F .) (Coleoptera: Bruchidae) in Cowpea Seeds. *Signpost Open Access Journal of Entomological Studies*, *1*(2), 108–117.

Onyebuchi, C., & Kavaz, D. (2020). Effect of extraction temperature and solvent type of the bioactive potential of *Ocimum gratissimum* L. extracts. *Scientific reports*, *10*(1), 21760.

Pereira, V., Figueira, O., & Castilho, P.C. (2024). Flavonoids as insecticides in crop protection—A review of current research and future prospects. *Plants*, *13*, 776. https://doi.org/10.3390/ plants13060776.

Salunke, B. K., Kotkar, H. M., Mendki, P. S., Upasani, S. M., & Maheshwari, V. L. (2005). Efficacy of flavonoids in controlling *Callosobruchus chinensis* (L.) (Coleoptera: Bruchidae) a post-harvest pest of grain legumes. *Crop Protection*, *24*,888-893.

Sharma, A., Kumar, V., Shahzad, B., Tanveer, M., Sidhu, G. P. S., Handa, N., Kohli, S. K., Yadav, P., Bali, A. S., Parihar, R. D., Dar, O. I., Singh, K., Jasrotia, S., Bakshi, P., Ramakrishnan, M., Kumar, S., Bhardwaj, R., & Thukral, A. K. (2019). Worldwide pesticide usage and its impacts on ecosystem. *SN Applied Sciences*, *1*(11), 1–16.

Singha, S., & Chandra, G. (2011). Mosquito larvicidal activity of some common spices and vegetable waste on *Culex quinquefasciatus* and *Anophelesstephensi*. *Asian Pacific Journal of Tropical Medicine*, *4*(4), 288–293.

Soe, T. N., Ngampongsai, A., & Sittichaya, W. (2019). Synergistic effect of sesame oil and clove oil on toxicity against the pulse beetle, *Callosobruchus maculatus* (Fabricius) (Coleoptera : Chysomelidae). *Khon Kaen Agriculture Journal*, *47*(1), 351–356.

Soe, T. N., Ngampongsai, A., & Sittichaya, W. (2020). Bioactivity of some plant essential oils for seed treatment against pulse beetle, *Callosobruchus maculatus* (F.) (coleoptera: Bruchidae) on mung bean. *Bulgarian Journal of Agricultural Science*, *26*(1), 141–147.