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Oni MO
Department of Crop, Soil and Pest Management, Federal University of Technology, P.M.B 704 Akure, Nigeria

Olatujoye KS
Department of Crop, Soil and Pest Management, Federal University of Technology, P.M.B 704 Akure, Nigeria

Ogungbite OC
Department of Plant Science, University of Ado-Ekiti, P.M.B 5363, Ado-Ekiti, Nigeria

Ofuya TI
Department of Crop, Soil and Pest Management, Federal University of Technology, P.M.B 704 Akure, Nigeria

Correspondence
Oni MO
Department of Crop, Soil and Pest Management, Federal University of Technology, P.M.B 704 Akure, Nigeria

Potential of Crude and Fractions of Beef Steak Plant, *Acalypha wilkesiana* Muell Arg oil extract to *Callosobruchus maculatus* (Coleoptera: Bruchidae)

Oni MO, Olatujoye KS, Ogungbite OC and Ofuya TI

Abstract
The potential of crude and twelve fractions of *Acalypha wilkesiana* oil extract was tested against cowpea beetle *Callosobruchus maculatus* at 20, 30, 40 and 50 µl dosages in triplicates. The results obtained were dosage and exposure time dependent. Fraction 1 of the oil extract caused 100% insect mortality at 40 and 50 µl within 96 h of application. The fraction significantly repelled the adult beetles, inhibited oviposition, adult emergence and apparently reduced seed weight-loss. The correlation between the adult emergence and seed weight-loss were greater than that of the mortality and oviposition as reflected by their R-values. Benzyl-alcohol was found to be in abundant in fraction 1 of the oil extract. Since, fraction 1 of oil extract of *A. wilkesiana* has proven insecticidal against *C. maculatus* infestation, it can be included in the new integrated pest management strategies.

Keywords: *Callosobruchus maculatus*, cowpea, potential, *Acalypha wilkesiana,* active compounds

1. Introduction

Insect pests are the major constraints to man's development as their role in food security of many developing nations cannot be over-emphasized [4] Damage caused by insect pests to field and stored produce had been a major concern and had advanced quite an array of botanical control of pests of grains, cowpea in particular. Stored cowpea, *Vigna unguiculata,* being a major source of protein incurred a rapid degree of infestation by direct consumption of its kernel by *Callosobruchus maculatus,* hence huge economic loss of produce and products. Some of these losses are caused by insects and fungi with the speed at which they multiply being influenced by prevailing environmental conditions [21]. The bruchid. *Callosobruchus maculatus* in storage caused major loss and they are field-to store agricultural insect pests of Africa and Asia that presently range throughout the tropical and subtropical world [9]. Among the key constraints to improving food security in Africa are losses resulting from poor-post harvest management of grains estimated at 20-30% amounting to more than US$ 4 billion annually [12].

Economic losses of stored cowpea ranging from direct and indirect contamination by insect pests through feeding and reproduction thus increases grain temperature and moisture content, this promotes deterioration of the produce, thereby undermined produce returns. A nation's food security is inevitably threatened at this critical stage. In some instances, farmers are forced to sell their grains off cheaply soon after harvesting due to anticipated losses in storage and later buy food at higher prices [20]. Over the decades, a number of efforts are being implemented to help alleviate ravages caused by field pests [19] likewise post-harvest losses resulting from insects equally remain a huge challenge [35].

However, stored product pest control has relied solely on synthetic chemicals which are expensive and poses serious dangers to the flora and fauna's there-in and the environment in general. In recent decades, research efforts are focused on the use of plant-derived biopesticides such as plant powders and oils, which are relatively cheaper and ecologically more tolerable than conventional chemical insecticides [34]. In a bid to replace many organochlorine and organophosphate chemicals already banned, while the use of some is being restricted [14]. An emerging pest control tactics for stored product pests include the use of crude essential oils of botanicals that have been tested effective against *C. maculatus* [1, 18, 23, 24, 27].

A. wilkesiana is a medicinal plant, an evergreen shrub that has antimicrobial properties [25] used for treatment of gastrointestinal disorders and fungal skin infections such as *Pityriasis versicolor*, *Tinea pedis, Tinea corporis.* Leaves are used as vegetables in management of hypertension [15]. The beta carotene content, is an antioxidant that boosts immune system against cataract and damaging effects of radiation [8, 10] and its aqueous extract is used in the management of fever in infants, as well as abnormal sodium and potassium metabolism that accompanies hypertension [26]. This present study investigated the entomotoxicant ability of crude and twelve fractions of *Acalypha wilkesiana* on *C. maculatus* in stored cowpea.

2. Materials and Methods

2.1 Study Site

The survey was conducted in the Research laboratory from February to November 2016 in the Department of Crop, Soil and Pest Management, Federal University of Technology, Akure (FUTA) Nigeria located in Southwestern part of Nigeria.

2.2 Cowpea seeds and Insect Culture

Cowpea variety Ife-brown seeds were purchased from Agricultural Development Project (ADP), Akure, Nigeria. The seeds were disinfested in the freezer at -20°C until use [16]. A fresh culture of *C. maculatus* was started from a colony that had been maintained in the laboratory by placing 25 pairs of a day-old male and female sexed beetles [32], in 1L wide mouth glass jars containing 100g of cowpea seeds [3] The jars were placed in a rearing chamber maintained at 28±2°C, 70± 5% R.H [1]. Female beetles were allowed to lay eggs on the seeds for 24 hours. Insects were removed and seeds containing eggs were kept in a rearing chamber until teneral adults used for bioassay emerged [3].

2.3 Plant collection and extraction of oil from leaves

A. wilkesiana fresh leaves were collected from teaching and research farm of FUTA and were taken into the laboratory for drying. The leaves were air-dried under shade and were pulverized into fine powder with electric blender, (JTC Omniblend V, Model TM-800) and kept in air tight containers until use. About 150g of the pulverized plant was soaked in an extracting bottle containing 500ml absolute ethanol solvent for three days, the solution was decanted on the fourth day and the solvent was separated from the oil using rotary evaporator. The oil extract was then air-dried to allow escape of traces of solvent. The resulting oil extract was kept in refrigerator prior to the commencement of the bioassays.

2.4 Fractionation and characterization of the oil extract of *A. wilkesiana*

The oil extract was fractionated using column chromatography method. Silica gel 80-120 mesh (23g) was made into slurry and was eluted with ethanol and chloroform: ethanol (3:1 v:v). The fractions were identified with TLC and fractions with the same RF were pulled together [7, 37, 38]. The fractions 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11 and 12 were made after the fractions have been pulled together. The most effective fraction was characterized using GC-MS.

2.6 Bioassay

Toxicity of oil extracts

Toxicity of crude and fraction 1; 2; 3; 4; 5; 6; 7; 8; 9; 10; 11 and 12 oil extracts was investigated by exposing 10 pairs of 1-day- old adult male and female *C. maculatus* to dosages of 20, 30, 40 and 50µl of treatment series separately in each 250ml plastic containers. Two negative controls were set up, one with 1.0ml ethanol solvent (untreated control) and a positive control with 1 ml of 0.05% concentration DDVP (Dichlorvos). Each treatment was replicated three times. Data on adult mortality was recorded after 24, 48, 72 and 96 hours of application.

To determine the protectability effect of the plant extract and its fractions, 10 pairs of adult *C. maculatus* were introduced to cowpea seeds that have treated with the 20 µl dosage of the fractions and the crude oil extract in five replicates and the set up was left for four days. Both live and dead insects were removed on the fifth day and oviposition was recorded. The setup was left until adult insects emerged and seed weight loss was recorded after no emergence was observed for four consecutive days [3]. The percentage adult emergence and seed weight loss were calculated using the formulae below:

$$\%\, adult\ emergence = \frac{number\ of\ adult\ emerged}{total\ number\ of\ egg\ laid} x \frac{100}{1}$$

$$\%\, weight\ loss = \frac{initial\ weight - final\ weight}{initial\ weight} x \frac{100}{1}$$

Repellency test

The repellent effects of the most effective fraction against *C. maculatus* was assayed on Petri-dishes as described by [37]. with some minor modifications. Petri-dishes 9 cm in diameter was used to confine insects during the experiment. The fraction was applied at 20, 30, 40 and 50µl dosages and absolute ethanol was used as negative control. Filter paper 9 cm in diameter was cut into half and different dosages of the fraction were applied separately to one-half of the filter paper as uniformly as possible with a micropipette. The other half (control) was treated with 0.5ml of absolute ethanol. Both the treated half and the control half was then air-dried to evaporate the solvent completely (30seconds). A full disk was carefully remade by attaching the treated half to the negative control half with tape. Each reassembled filter paper after treatment with solid glue was placed in a Petri dish with the same oriented in one of four different randomly selected directions to avoid any insecticidal stimuli affecting the distribution of insects. Ten starved adult insects were released in the center of the central zone of each filter paper disk, and a cover was placed over the Petri dish. Three replicates were used, and the experiment was repeated three times. Counts of the insects present on each strip were made after 4, 8 and 24 h. The percentage repellency (PR) of each fraction was calculated using the formula:

$$\%\, repellency = \frac{N_c - N_t}{N_c + N_t} x \frac{100}{1}$$

Where

N_c - number of insects present in the negative control half and N_t - number of insects present in the treated half. A commercial repellent DDVP was used as positive control.

2.8 Data analysis

Data were subjected to one-way analysis of variance (ANOVA) at 0.05 significant levels, and treatment means were separated with new Duncan's Multiple Range Test version 17. Regression analysis was used to estimate the LD_{50} and LD_{95} of the oil extract using probit analysis [13]. Linear regression analysis was done to correlate the relationship between insect mortality, oviposition, adult emergence and seed weight loss.

3. Results

3.1 Effect of crude oil extract and fractions of *A. wilkesiana* on survival of *C. maculatus*

The effect of crude oil extract and fractions of *A. wilkesiana* leaves on survival of adult *C. maculatus* was presented in Table 1. The effects of the treatment were dosage and period of exposure dependent. Significant ($p < 0.05$) differences existed between the treatments at all period of application. Fraction 1 effected 100% mortality of the beetles at 40 and 50µl within 96h period of exposure and was significantly ($p < 0.05$) different from other treatments except those that recorded up to 90% mortality of the insect. Regardless of dosage and time of exposure, all treatments were significantly different from the two negative controls.

The amount of crude oil extract and fractions of *A. wilkesiana* needed to achieve 50% mortality was presented in Table 2. The regression analysis revealed that Fraction 1 of the oil extract was the most effective among the treatments as only 15.87 and 58.66µl dosages were required to achieve 50 and 95% insect mortality within 72h respectively. However, fraction 6 proved the least effective as indicated by the high amount of dosage required to cause 50 and 95% (50.84 - 78.50µl) mortality.

3.2 Effect of fractions of *A. wilkesiana* on the oviposition and adult emergence of *C. maculatus* and its ability to cause seed weight loss

The effect of the crude oil extract and fractions of *A. wilkesiana* leaves on the oviposition and adult emergence of cowpea beetle and its ability to cause weight loss of protected seeds were presented in Figure1. Fraction one recorded the lowest oviposition rate (10) of the insect and was significantly different from the others except the positive control which recorded 4.45 oviposition. Only fraction 1 and the positive control was able to prevent adult emergence and weight loss of the protected seeds.

3.3 Relationship between insect mortality and oviposition as well as between adult emergence and seed weight loss

Table 4 presents combined relationship between insect mortality at 96hr, ability of *C. maculatus* to oviposit, emerge and cause weight loss of cowpea seeds. The R values of 0.832- 0.947 that tends to 1 reflected high relationship between the adult mortality of the insect and the number of eggs laid. The R^2 values shows that only 69.3 – 89.7% of the eggs laid by the insect can be explained by the mortality of the insects. However, after the adjustment of the R^2 values, only 51.7 -83.8 % of the eggs laid can be determined by the mortality rate. The t-values of 2.240 – 7.461 that is greater than 1.98 indicates that, there was a great significant positive relationship between insect mortality and oviposition. Similarly, there was positive relationship between adult insect emergence and the weight loss of protected seeds as reflected by all their respective R values of 0.978 and below. The R^2 values showed that 95.6% and below of the seed weight loss can be determined by the emergence of the adult beetle. After the adjustment of the R^2 values, only 94.8 -95.2% of the seed weight loss was being explained by the emergence of the adult beetle. Hence the t-values of 14.232 -14.791, which is greater than 1.98 shows that the correlation between the adult emergence of the beetle and the weight loss of the cowpea was statistically significant. It was noted that the correlation between the adult emergence and seed weight loss were greater than that of adult mortality and number of eggs laid as reflected by their t-values.

3.4 Repellent activity of fraction 1 oil extract *C. maculatus*

Repellent activity of fraction 1 of oil extract of *A. wilkesiana* was presented in Table 3. The repellent activity increased with increase in the dosage of the fraction. The 50µl dose recorded the highest repellence 86.67% and was significantly different from others. However, none of the treatment was able to achieve 100% repellent within the period of observation except the positive control.

3.5 Active compounds present in fraction 1 of *A. wilkesiana* leaf oil

Table 5 presented thirteen (13) active compounds identified in *A. wilkesiana* fraction 1 leaf oil extract by GC MS analysis as listed. Moreover, Benzyl alcohol (73.438%), Octadecanoic acid (10.383%), Tetradecanoic acid, ethyl ester (3.289%) and Benzaldehyde (3.215%) appeared the most active compound of the fraction.

4. Discussion

Stored product pest is a major challenge in achieving food security in the world as a whole. In a bid to eradicate the use of synthetic chemical insecticides that has been the vogue for ages to reduce pest population to a tolerable level, the use of plant materials in various forms is gradually taking over. This practice has been suggested as one of great hopes for controlling stored product pests [2], due to several limitations associated with the use of synthetic insecticides and fumigants [17].

The result of this study demonstrated that the biotic potential of crude and twelve fractions of the medicinal plant used, have significant effect on the survival of the adult beetle. However, it was feasible that the ability of oil extracts to cause adult mortality was dosage and exposure period dependent. Dosages 40-50µl /exposure period effected increase mortality compared to lower doses and the negative controls. It was observed that the fractions obtained from the crude oil extract of the plant significantly acted differently from the crude oil extract.

At 72hr, regardless of the dosage/exposure period, fraction 1 of the oil extract treatments was more effective than other fractions and the crude extracts as reflected by its LD_{50} and the fiducial limit shown by the lethal dosage [29]. lt was feasible that the effectiveness of fraction 1 than other treatments was attributed to the fact that active components in the plant oils are more contained in this fraction than others. Benzyl alcohol observed as the most active component in fraction 1 might have disrupt or caused inability of the insects to feed on the oil coated cowpea seeds [17] which may in turn lead to starvation and also disrupt normal respiratory activity of the insect, hence lead to asphyxiation and death of the insect [4, 6, 28] In similar vein, the repellent action of fraction 1is likened to the active component, suspected to have acted as repellant in a vapour form on the olfactory receptors of the weevils. This according to [3, 11, 17, 30, 31], attributed the toxicity of essential oil to insect pests to the components of the oils. The plant oil extract used significantly prevented or reduced hatching or inability of the eggs to hatch due to breathing pore (Chorion) blockage of the eggs and thereby caused subsequent death of the larvae which might have emerged into adult [4] thereby prevented or reduced adult emergence. Relationship existed between insect mortality at 96 h, number of eggs laid, adult emerged and weight loss of the protected seeds as reflected by their regression analysis.

In the present study, it is obvious that the presence of benzyl alcohol an active component in *A wilkesiana* have proven a great biotoxic potential, against *C. maculatus*. Previous related studies have tested and reported the toxicity of crude essential oils to survival and development of *C. maculatus* [4, 29] but few studies specifically tested the effectiveness of pure oil components on the insect [3].

Table 1: Percentage mortality of adult *C. maculatus* treated with crude and fractions of *A. wilkesiana* oil extract

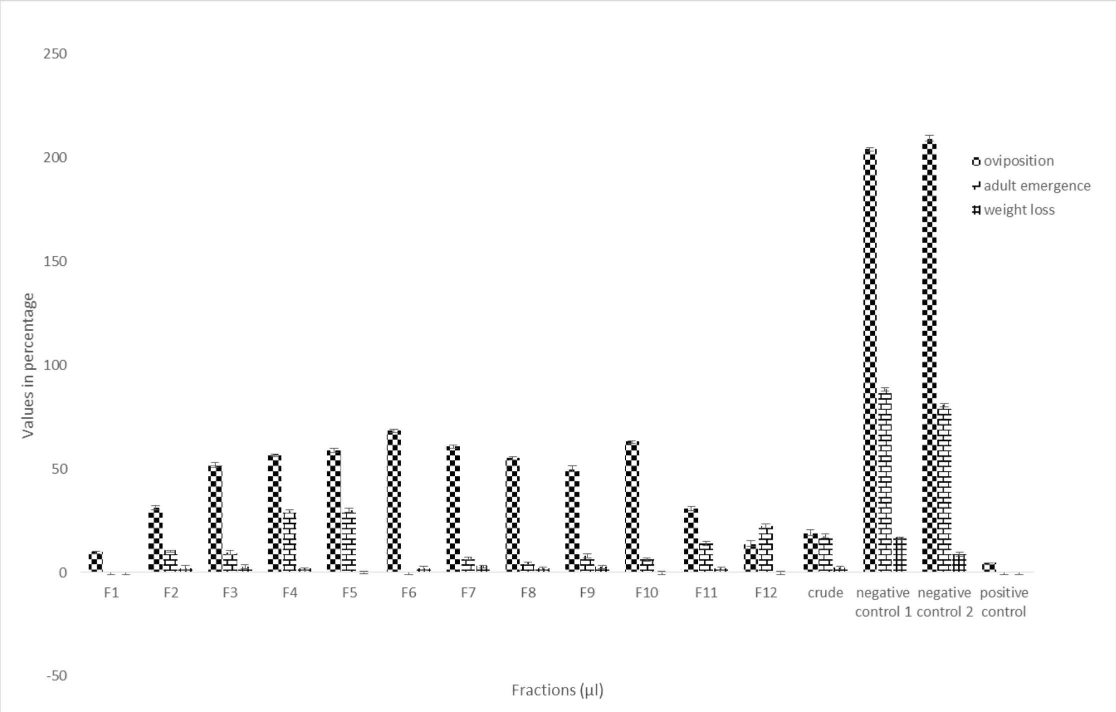
	Dosage (µl)	Percentage mortality in hours			
		24	48	72	96
Crude oil	20	23.33 ± 1.33^{b}	36.67±1.33^{b}	53.33±1.33^{b}	61.67±1.67^{b}
	30	26.67 ± 1.67^{b}	48.33±1.67^{c}	61.67±2.41^{b}	76.67±2.67^{c}
	40	41.67 ± 1.67^{c}	80.00±2.89^{d}	85.00±2.89^{c}	93.33±1.33^{d}
	50	49.00 ± 2.58^{c}	85.00±2.89^{d}	93.33±2.41^{cd}	98.33±1.67^{d}
Fraction 1	20	56.67±1.33^{b}	70.00±2.77^{b}	76.67±2.67^{b}	90.00±2.77^{b}
	30	58.33±1.67^{b}	78.00±1.67^{c}	86.67±1.33^{bc}	95.00±2.00^{b}
	40	68.33±1.67^{c}	83.33±1.33^{cd}	95.00±1.89^{cd}	100.00±0.00^{b}
	50	81.67±1.67^{d}	91.67±1.67^{de}	98.33±1.67^{d}	100.00±0.00^{b}
Fraction 2	20	25.00±2.87^{b}	55.00±2.87^{b}	63.33±1.33^{b}	68.33±2.01^{b}
	30	33.33±1.33^{bc}	58.33±2.01^{b}	70.00±2.77^{bc}	73.33±2.41^{bc}
	40	35.00±2.87^{c}	63.33±1.33^{b}	80.00±2.37^{c}	86.67±2.89^{cd}
	50	40.00±2.00^{c}	66.67±2.67^{b}	85.00±2.63^{c}	95.00±1.89^{d}
Fraction 3	20	3.33±1.33^{a}	15.00±1.87^{b}	25.00±1.10^{b}	46.67±1.33^{b}
	30	11.67±1.67^{bc}	21.67±2.41^{b}	55.00±1.05^{c}	66.67±1.33^{c}
	40	21.67±1.41^{c}	36.67±1.33^{c}	56.67±1.33^{c}	73.33±1.57^{cd}
	50	31.67±1.67^{d}	45.00±1.87^{c}	73.33±2.37^{d}	80.00±2.37^{d}
Fraction 4	20	10.00±0.00^{b}	28.33±1.67^{b}	41.67±1.67^{b}	58.33±1.41^{b}
	30	13.33±1.33^{bc}	35.00±1.89^{b}	56.67±1.33^{c}	73.33±1.67^{c}
	40	21.67±1.67^{c}	53.33±1.67^{c}	73.00±1.33^{d}	75.00±1.89^{c}
	50	31.67±1.67^{d}	56.67±1.33^{c}	78.33±1.67^{d}	86.67±1.67^{d}
Fraction 5	20	10.00±0.00^{bc}	23.33±1.33^{b}	51.67±1.41^{b}	66.67±1.67^{b}
	30	11.67±1.67^{bc}	28.33±1.67^{bc}	56.67±1.33bc	73.33±1.33^{bc}
	40	15.00±1.89^{c}	33.33±1.67^{cd}	65.00±2.00^{c}	76.67±1.33^{c}
	50	18.33±1.67^{c}	40.00±1.89^{d}	68.33±1.41^{c}	93.33±1.41^{d}
Fraction 6	20	6.67±1.33^{b}	16.67±1.33^{b}	26.67±1.33^{b}	43.33±2.67^{b}
	30	10.00±0.00^{b}	23.33±1.33^{bc}	40.00±0.00^{c}	55.00±2.89^{bc}
	40	11.67±1.67^{c}	26.67±1.33^{c}	43.33±1.33^{c}	60.00±2.77^{c}
	50	16.67±1.67^{c}	31.67±1.67^{c}	51.67±1.67^{d}	65.00±1.89^{c}
Fraction 7	20	3.33±1.33^{a}	16.67±1.33^{b}	28.33±1.67^{b}	43.33±1.33^{b}
	30	3.33±1.33^{a}	23.33±1.33^{bc}	35.00±1.89^{bc}	55.00±1.89^{c}
	40	16.67±1.33^{b}	28.33±1.67^{cd}	41.67±1.67^{cd}	56.67±1.33^{cd}
	50	21.67±1.67^{b}	35.00±1.89^{d}	45.00±1.89^{d}	65.00±1.89^{d}
Fraction 8	20	3.33±1.33^{ab}	13.33±1.33^{b}	23.33±1.33^{b}	28.33±1.67^{b}
	30	3.33±1.33^{ab}	15.00±1.89^{b}	33.33±1.33^{c}	45.00±1.89^{c}
	40	6.67±1.33^{ab}	23.33±1.33^{c}	43.33±1.67^{d}	51.67±1.67^{c}
	50	11.67±1.67^{b}	30.00±0.00^{c}	46.67±1.33^{d}	60.00±0.00^{d}
Fraction 9	20	3.33±1.33^{a}	10.00±2.00^{ab}	20.00±2.77^{b}	28.33±2.00^{b}
	30	6.67±1.33^{a}	20.00±2.17^{bc}	28.33±1.41^{bc}	48.33±1.67^{c}
	40	16.67±1.33^{b}	26.67±1.33^{cd}	38.33±1.41^{cd}	56.67±1.33^{c}
	50	30.00±0.00^{c}	36.67±1.67^{d}	48.33±1.67^{d}	71.67±1.67^{d}
Fraction 10	20	6.67±1.33^{ab}	13.33±1.67^{b}	18.33±1.67^{b}	26.67±1.33^{b}
	30	13.33±1.33^{b}	20.00±1.89^{b}	28.33±1.67^{c}	35.00±2.00^{b}
	40	23.33±1.33^{c}	28.33±1.67^{c}	33.33±1.67^{c}	48.33±1.67^{c}
	50	25.00±1.89^{c}	33.33±1.67^{c}	45.00±2.00^{d}	56.67±1.33^{c}
Fraction 11	20	16.67±1.33^{b}	35.00±2.00^{b}	50.00±0.00^{b}	71.67±1.67^{b}
	30	23.33±1.33^{bc}	41.67±1.67^{bc}	58.33±2.41^{bc}	76.67±1.33^{bc}
	40	26.67±1.33^{c}	46.67±1.67^{c}	63.33±1.33^{c}	83.33±1.33^{c}
	50	35.00±1.87^{d}	61.67±1.67^{d}	78.33±2.41^{d}	95.00±1.20^{d}
Fraction 12	20	6.67±1.33^{a}	26.67±1.33^{b}	40.00±0.00^{b}	51.67±1.67^{b}
	30	16.67±1.33^{b}	40.00±0.00^{c}	55.00±2.00^{c}	56.67±1.67^{b}
	40	26.67±1.33^{c}	43.33±1.33^{c}	58.33±1.67^{c}	65.00±2.00^{c}
	50	33.33±1.33^{c}	53.33±1.33^{d}	71.67±2.00^{d}	76.67±1.33^{d}
	NC 1	0.00±0.00^{a}	3.33±1.33^{a}	3.33±1.33^{a}	6.67±1.33^{a}
	NC 2	0.00±0.00^{a}	3.33±1.33^{a}	6.67±1.33^{a}	13.33±1.33^{a}
	PC	73.33±1.33^{d}	100.00±0.00^{e}	100.00±0.00^{e}	100.00±0.00^{e}

Each value is mean ± standard error of three replicates. Values followed by the same letters are not significantly ($P > 0.05$) different from each other using New Duncan's Multiple Range Test.*Note: Negative control (1) is treatment with neither oil nor ethanol, Negative control (2) is treatment with only ethanol and positive control is treatment with DDVP.

Table 2: Lethal concentration of the crude and fractions oil extracts required to achieve 50& 90% mortality of adult *C. maculatus* after 72 h post treatment

Treatments	Slope (±SE)	Intercept (±SE)	X^2	LD_{50} (FL@95%)	LD_{95} (FL@95%)	Significant
Crude	1.59±0.17	-0.88±0.07	32.73	45.57 (43.82-47.53)	63.76 (61.56-65.72)	0.0001
Fraction 1	1.06±0.17	0.06±0.07	19.94	15.87 (13.34-17.26)	58.66 (56.39-60.52)	0.030
Fraction 2	0.66±0.17	-0.66±0.07	14.64	58.07 (56.76-60.42)	75.31(74.73-77.18)	0.146
Fraction 3	1.58±0.18	-1.12±0.08	19.62	55.09 (53.97-58.24)	75.98 (73.91-78.49)	0.033
Fraction 4	1.33±0.17	-0.64±0.07	12.29	37.01 (35.64-38.56)	72.38 (70.86-74.86)	0.260
Fraction 5	0.76±0.17	-0.76±0.07	8.59	58.88 (57.09-60.35)	85.24 (82.34-87.93)	0.571
Fraction 6	1.05±0.17	-0.61±0.07	7.79	50.84 (49.17-51.19)	78.50 (76.77-79.95)	0.649
Fraction 7	0.76±0.67	-0.59±0.07	5.91	62.91 (60.19-64.91)	89.41 (88.31-90.19)	0.823
Fraction 8	1.11±0.17	-0.74±0.07	10.55	61.60 (59.73-63.51)	87.02 (86.73-89.03)	0.393
Fraction 9	1.32±0.17	-0.90±0.08	26.46	59.76 (57.50-61.91)	85.06 (83.90-88.34)	0.003
Fraction 10	1.23±0.18	-0.93±0.08	6.99	62.76 (60.55-64.49)	89.51 (88.14-90.76)	0.726
Fraction 11	1.02±0.16	-0.45±0.07	16.45	47.76 (45.18-49.91)	80.48 (78.66-81.51)	0.87
Fraction 12	1.10±0.17	-0.62±0.07	10.32	49.66 (48.07-50.77)	84.55 (82.95-86.13)	0.413

Note: LD: lethal dosage; SE: standard error; x^2: Chi-square; FL: Fiducial limits



Note: Oviposition is not in percentage.

Fig 1: Effects of different fractions of *A. wilkesiana* on oviposition, adult emergence adult *C. maculatus* and weight loss of protected cowpea seeds.

Table 3: Repellent activity (%) of fraction 1 of *A. wilkesiana* oil against adult *C. maculatus*

Dosages (µl)	Percentage repellent in hours		
	4	8	24
20	13.33±2.67a	20.00±0.00b	20.00±0.00b
30	26.67±2.67b	26.67±2.67b	26.67±2.67b
40	33.33±2.67bc	46.67±2.67c	73.33±2.67c
50	40.00±3.54c	66.67±2.67d	86.67±2.67cd
Negative control	6.67±2.67a	6.67±2.67a	6.67±2.67a
Positive control	80.00±3.54d	100.00±0.00e	100.00±0.00e

Each value is mean ± standard error of three replicates. Values followed by the same letters are not significantly (P > 0.05) different from each other using New Duncan's Multiple Range Test.*Note: negative control is treatment with ethanol and positive is treatment with DDVP

Table 4: Relationship between insect mortality at 96 h of application and number of eggs laid as well as between adult emergence and seed weight loss of cowpea seeds treated with crude oil extract and fractions of *A. wilkesiana* oil

Treatments	Parameters	R	R^2	AR	K±S.E	RC±S.E	RE	t-value	Sig.
Crude	Mortality	0.832	0.693	0.517	6.14±2.74	-0.11±0.09	O=6.14-0.11(M)	2.240	0.055
	Adult emergence	0.978	0.956	0.952	0.01±0.05	0.07±0.001	W=0.01+0.07(A)	14.791	0.0001
Fraction 1	Mortality	0.902	0.814	0.708	10.58±7.14	0.34±0.15	O=10.58+0.34(M)	2.309	0.011
	Adult emergence	0.968	0.937	0.931	-0.001±0.01	0.02±0.002	W=-0.001+0.02(A)	12.246	0.0001

Fraction 2	Mortality	0.845	0.713	0.549	56.52±19.91	-0.21±0.53	O=56.52-0.21(M)	2.840	0.044
	Adult emergence	8.883	0.780	0.757	0.03±0.05	0.05±0.01	W=0.03+0.05(A)	5.946	0.0001
Fraction 3	Mortality	0.964	0.930	0.889	45.04±12.96	0.55±0.34	O=45.04+0.55(M)	3.475	0.0001
	A.emergence	0.832	0.692	0.661	-0.01±0.07	0.05±0.01	W=-0.01+0.05(A)	4.739	0.001
Fraction 4	Mortality	0.965	0.932	0.893	64.91±6.50	0.04±0.13	O=64.91+0.04(M)	9.981	0.0001
	Adult emergence	0.942	0.888	0.876	-0.74±0.16	0.67±0.01	W=-0.74+0.67(A)	8.886	0.0001
Fraction 5	Mortality	0.952	0.907	0.853	81.76±12.42	-0.34±0.19	O=81.76-0.34(M)	6.581	0.001
	Adult emergence	0.955	0.911	0.902	4.05±1.28	0.63±0.06	W=4.05+0.63(A)	10.130	0.0001
Fraction 6	Mortality	0.879	0.773	0.643	103.29±15.95	0.04±0.47	O=103.29+15.95(M)	6.478	0.021
	Adult emergence	0.940	0.884	0.872	0.00±0.01	0.17±0.02	W=0.00+0.17(A)	8.727	0.0001
Fraction 7	Mortality	0.943	0.889	0.825	94.03±12.15	-0.34±0.38	O=94.03-0.34(M)	7.738	0.002
	Adult emergence	0.994	0.988	0.987	-0.06±0.06	0.18±0.01	W=-0.06+0.18(A)	28.668	0.0001
Fraction 8	Mortality	0.974	0.949	0.919	80.99±4.24	-0.83±0.22	O=80.99-0.83(M)	-3.798	0.0001
	Adult emergence	0.959	0.919	0.911	-0.01±0.01	0.05±0.004	W=-0.01+0.05(A)	10.681	0.0001
Fraction 9	Mortality	0.912	0.832	0.736	61.14±6.99	-0.09±0.37	O=61.14-0.09(M)	8.753	0.008
	Adult emergence	0.971	0.942	0.937	-0.04±0.02	0.07±0.01	W=-0.04+0.07(A)	12.802	0.0001
Fraction 10	Mortality	0.970	0.941	0.907	91.81±5.63	-0.09±0.41	O=91.81-0.09(M)	16.308	0.0001
	Adult emergence	0.855	0.732	0.705	-0.14±0.09	0.12±0.02	W=-0.14+0.12(A)	5.224	0.0001
Fraction 11	Mortality	0.902	0.814	0.707	61.18±22.46	0.01±0.05	O=61.18+0.01(M)	2.724	0.011
	Adult emergence	0.858	0.735	0.709	0.01±0.01	0.01±0.002	W=0.01+0.01(A)	5.271	0.0001
Fraction 12	Mortality	0.947	0.897	0.838	22.67±3.04	0.04±0.09	O=22.67+0.04(M)	7.461	0.001
	Adult emergence	0.976	0.953	0.948	0.001±0.006	0.005±0.00	W=0.001+0.005(A)	14.232	0.0001

Where AR = adjusted R square; K = constant; R_C = regression coefficient; R_E = regression equation; S. E= Standard error; O= oviposition and W = weight loss

Table 5: Chemical composition of Fraction 1 of *A. wilkesiana* oil extract

No.	Compounds	RI (Cal.)	RI (Lit.)	Percentage composition
1	Benzaldehyde	53812	5149	3.215
2	1-Hexen-3-yne, 2-methyl-	28126	2638	0.221
3	Benzyl alcohol	1081743	5466	73.438
4	1,2,4-Benzenetriol	10425	11328	0.140
5	1,3,5-Benzenetriol	27414	11332	0.345
6	[1,2,5]Oxadiazolo[3,4-b][1,4]diazocine-5,7(4H,6H)-dione,8,9-dihydro	65928	49888	1.152
7	Octadecanoic acid	262140	144272	10.383
8	Tetradecanoic acid, ethyl ester	157714	117465	3.289
9	Phytol	125326	155849	2.569
10	Cyclohexene, 4-(4-ethylcyclohexyl) -1-pentyl-	49468	123186	1.135
11	Ethyl 9,12,15-octadecatrienoate	86560	165627	2.277
12	Bicyclo[4.1.0]heptane, 2-chloro-	37389	13707	0.282
13	Octadecanoic acid, ethyl ester	76008	171414	1.552

6. Conclusion

The findings from this study having proven the botanical effective as cowpea protectant suggest future research on the active components in *A. wilkesiana* to establish its mode of action, their effect on digestive and detoxifying enzymes in humans and the environment.

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