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Entomocidal Efficacy of Crude Oil and Fractions of *Mormodica charantia* (l) Oil Extract Against Cowpea Bruchid *Callosobruchus maculatus* (Fab) Infesting Stored Cowpea Seeds

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ABSTRACT

The entomocidal potency of crude and different fractions of Momordica charantia oil extracts was evaluated under laboratory condition 28± 2 °C and relative humidity of 75± 5%. Bioassay of crude oil and different fractions 1;2;3;4;5;6;7;8 and 9 of the plant oil extracts was conducted for its insecticidal efficacy against Callosobruchus maculatus (Fab.) at 10, 20, 30, 40 and 50µl dosages/20g cowpea seeds. All the treatments were potent against the survival of the test beetles as dosages increase with increase in time of exposure. Likewise, it was evident that Fraction 3 appeared to be the most effective as they caused 100% beetle mortality at 96 hours post-treatment. Probit analysis showed that Fraction 3 of the plant oil extract required 40.67 and 71.15µl to achieve50 and 95% highest insect mortality within 48hours. Fraction 3 of M. charantia oil extract of the oil that may possibly have equally played the observed bioactivity accountable for the repellence activity of the beetle by the fraction. However, it is imperative to confirm the active ingredient present in the plant oil fraction and examine its toxicity to mammals.

Key words: *Callosobruchus maculatus*, Repellency, *Momordica charantia*

INTRODUCTION

Callosobruchus maculatus is the most prominent insect pest of cowpea, *Vigna unguiculata*. Cowpea is a staple food consumed in most Nigerian homes as a source of inexpensive protein in both human and animal diet (Ofuya, 2001). Damage done by *Callosobruchus maculatus* impacts negatively on cowpea by affecting its texture, taste, appearance and reduced its nutritional and economic values Oni *et.al* (2011). Ajayi *et al.* (2015) opined that insects alone is capable of causing more than 65% loss of cowpea and if left uncheck can cause total loss of cowpea.

Botanical insecticides have long touted as attractive alternative to obviate the danger associated with synthetic chemical insecticides probably because they are medicinal and eco-friendly (Akinkuolere *et.al*., 2006). ln Nigeria, farmers religiously imbibed the use of this hazardous synthetic chemical insecticides based on its quick action both on field and in storage not considering the human and environmental health hazards. Botanicals of various species have been investigated and suggested as promising alternative in the control of insect pests (Oni *et.al*., 2018; Ogungbite, 2015).

Momordica charantia (l) is a medicinal plant. lts medicinal report as hypoglycemic, antioxidative, alleviation of kidney damage, anti-fertility effect, antiviral activity, antimicrobial activity and anticancer activity was reported (Birdee and Yeh,2010; Abdullahi *et.al*., 2011; Basch *et.al*., 2003; Nerurkar *et.al*., 2006; Leelaprakash *et.al*, 2011 and Ray *et.al*. ,2010). We are not aware of any report on insecticidal activity of this plant on any urban insect. It is on this note that the entomocidal potency of crude and different fractions, as well as the active compound present in *Momordica charantia* oil extract against *Callosobruchus maculatus* infesting stored cowpea is being investigated.

MATERIALS AND METHODS

Collection of insects, culture, cowpea seeds

Cowpea bruchid *(C. maculatus)* used for this research were collected from infested cowpea seeds bought from Erekesan market, Akure, Nigeria. The insects were reared on clean disinfected cowpea seeds, variety Ife-brown obtained from the International Institute of Tropical Agriculture (IITA) Ibadan, Nigeria. This research work was carried out at the Entomology Research Laboratory of the Department of Crop, Soil and Pest Management Federal University of Technology, Akure, Nigeria at temperature 28± 2ºC and relative humidity 75± 2ºC arranged undisturbed until when needed in plastic containers covered with muslin –cloth.

Plant collection and identification

M. charantia 'Ejinrin' in Yoruba language, in Nigeria), fresh leaves and stems of were sourced from teaching and research farm of same institution and the identification was done at the Department of Crop, Soil and Pest Management, Federal University of Technology, Akure. Nigeria It was air-dried in the open laboratory and milled into fine powder using Vitamix 5200 blender, sieved in 3mm mesh and kept in a labeled plastic container prior to use.

Extraction of plant oil

The powdered *M. charantia* plant was subjected to cold extraction using ethanol as solvent, to extract the crude oil. About 150g of the pulverized plant was soaked in an extraction bottle containing 500ml of absolute ethanol 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 air-dried for traces of solvents to evaporate off. The extract was fractionalized into different fractions at the Research Laboratory Department of Biochemistry, Federal University of Technology, Akure. The fractions were then kept in the refrigerator prior to use.

Fractionalization of the oil extract

A silica gel 80-120 mesh (23g) was wet packed in a chromathographic column and diluted with ethanol.The methanolic extract (20ml) was placed at the top of the gel and eluted with ethanol. Fractions of 5ml at a time were collected in 10ml bottle and labelled1; 2; 3; 4; 5; 6; 7; 8 and 9successively. The fractions were then kept in room temperature prior Thin Layer Chromatography (TLC) .

Thin Layer Chromatography

Thin layer chromatography was performed on pre-coated 7 x 7 cm and 0.25 mm thick plates prepared by using silica gel G for TLC were left overnight for air drying. These plates were dried and developed in suitable solvents for rapid screening, methanol: n-hexane: acetone in the ratio 7:2:1. The plates were run in the above solvent systems and dried at room temperature.The plates was sprayed with 20% tetraoxosulphate (vi) acid, then heated in an oven at 105ºC for 15minutes .Different bands were observed and corresponding RF values are determined.(Barakat, 2011; Zeeshan *et al.,* 2012; Yang *et al.,* 2014).

Toxicity assay

M. charantia oil extracts pure compounds against the test insects was determined by Zhou *et al.,* (2012). Fractions 1; 2; 3; 4; 5; 6; 7; 8 and 9 of the oil was prepared into dosages of 10, 20, 30, 40 and 50 µl. Each fraction was mixed thoroughly with twenty grams of cowpea seeds separately weighed into 250ml plastic containers with lid. Equally 10, 20, 30, 40 and 50 µl of the crude oil of *M. charantia* oil were thoroughly mixed separately with cowpea seeds in the plastic container and 0.5ml ethanol were added into each concentration to serve as a spreader and left for 1hour to allow the solvent to evaporate. Two negative control experiments were set up, one with solvent alone (1.0ml ethanol) and the other one with neither solvent nor extract (untreated control), while a positive control was also set up using 0.001ml DDVP (Dichlorovos). Ten unsexed pairs of newly emerged *C. Maculates* were introduced into each plastic container and beetle mortality was observed at 24: 48: 72 and 96 h post treatment. Each treatment was replicated three times.

Repellency test

The repellency effect of crude and fractions of oil extract of *M. charantia* against *C. maculatus* was assessed by dividing filter papers into two equal parts and one side was treated with 10, 20, 30, 40 and 50µl of each concentration of the crude oil and fractions of *M. charantia* the second half of the filter paper was treated with 2µl of ethanol and was used as control. Both the treated half and the control half were air-dried for 15minutes to evaporate the solvents. 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 seam oriented in one of four different randomly selected directions to avoid any insecticidal stimuli affecting the distribution of insects. Ten unsexed newly emerged *C. maculatus* insects was released into the center of each filter paper disk and the petri dish was covered. There are three replicates for this experiment. Counts of insects present on each strip were made after 4: 6 and 24 hours. The percentage repellency (PR) of each fraction was then calculated using the formula.

$$PR (\%) = [(N_c - N_t)/(N_c + N_t)] \times 100/1$$

Where N_c is the number of insects present in the negative control half and N_t is the number of insects present in the treated half. As a positive control a commercial repellent dichlorovos or DDVP was used under the same conditions as the fractions.

Table 1: Percentage mortality of adult *C. maculatus* treated with different dosages of crude and fractions *of M. charantia* oil extract

Crude /Fractions	Dosage (µl)	Percentage mortality in hours			
		24	48	72	96
Crude oil	10	8.33±2.41^{ab}	20.00±1.89^{bc}	43.33±1.33fg	58.33±1.67d
	20	18.33±1.67^{cd}	26.67±1.33^{cd}	46.67±1.33fg	65.00±1.87^{f}
	30	20.00±0.00^{cd}	41.67±1.41^{ef}	56.67±1.33gh	71.67±1.67f
	40	23.33±1.33^{d}	43.33±2.67^{ef}	65.00±1.89h	86.67±1.33gh
	50	25.00±1.89^{de}	55.00±1.89^{f}	66.67±1.33h	98.33±1.67h
Fraction 1	10	6.67±1.33^{ab}	23.33±1.33^{c}	33.33±1.33ef	41.67±1.67c
	20	11.67±1.67^{bc}	26.67±1.33^{cd}	40.00±0.00f	51.67±1.67d
	30	20.00±0.00^{d}	41.67±2.67^{ef}	43.33±1.67fg	63.33±1.33d
	40	25.00±1.89^{d}	56.67±1.67^{f}	60.00±1.89h	76.67±2.67f
	50	31.67±1.67^{de}	56.67±1.33^{f}	66.67±1.33h	83.33±1.33g
Fraction 2	10	13.33±1.33^{bc}	20.00±1.89^{bc}	31.67±1.67e	46.67±1.33cd
	20	30.00±1.89^{de}	33.33±1.67^{de}	46.67±1.33g	58.33±1.67de
	30	41.67±1.67^{e}	45.00±1.89^{ef}	63.33±1.67hi	71.67±1.67f
	40	51.67±1.67^{f}	57.67±1.67^{fg}	71.67±1.67i	78.33±1.67fg
	50	58.33±1.67^{f}	61.67±1.67^{g}	76.67±1.67i	88.33±1.67g
Fraction 3	10	33.33±1.33^{de}	56.67±1.33^{fg}	63.33±1.33hi	71.67±1.41f
	20	46.67±1.33^{ef}	63.33±1.33^{gh}	71.67±1.67hi	75.00±1.89fg
	30	50.00±1.89^{f}	73.33±1.67^{hi}	78.33±1.67ij	83.33±1.33g
	40	56.67±1.67^{fg}	78.33±1.67^{hi}	83.33±1.67j	88.33±2.67g
	50	61.67±1.67^{g}	80.00±0.00^{i}	95.00±1.89k	100.00±0.00h
Fraction 4	10	6.67±1.33^{a}	26.67±1.33cd	51.67±1.67g	58.33±1.67d
	20	13.33±1.33^{bc}	33.33±1.33^{de}	58.33±1.67g	68.33±1.41e
	30	20.00±0.00^{cd}	48.33±1.41^{ef}	66.67±1.33h	81.67±1.67gh
	40	23.33±1.33^{cd}	51.67±1.67^{fg}	75.00±2.00hi	88.33±2.67gh
	50	28.33±1.67^{de}	60.00±1.89^{g}	91.67±1.67k	100.00±0.00h
Fraction 5	10	3.33±1.33^{a}	9.67±1.33ab	20.00±0.00cd	33.33±1.33b
	20	6.67±1.33^{ab}	18.00±0.00^{bc}	36.67±1.67ef	50.33±1.33cd
	30	11.67±1.67^{bc}	26.67±1.67^{cd}	41.00±1.89fg	53.33±1.67d
	40	16.67±1.67^{cd}	32.33±1.33^{d}	45.00±1.89fg	56.67±1.67d
	50	18.33±1.67^{cd}	38.00±1.89^{e}	53.33±1.33g	68.33±1.67e
Fraction 6	10	3.33±1.33^{a}	10.00±1.77b	13.33±1.33c	36.67±1.33h
	20	6.67±1.33^{ab}	13.33±1.33^{bc}	31.00±0.00d	53.33±1.33d
	30	18.33±1.67^{cd}	26.67±1.67^{cd}	31.67±1.67e	58.33±1.67de
	40	21.67±1.67^{cd}	28.33±1.67^{cd}	45.00±1.89fg	63.33±1.41e
	50	26.67±1.67^{de}	36.67±1.67^{de}	48.33±1.67g	75.00±1.89f
Fraction7	10	6.67±1.33^{a}	15.00±1.89b	31.67±1.67e	43.33±1.33c
	20	13.33±1.33^{bc}	28.33±1.67^{cd}	48.33±1.67g	60.00.±0.00d
	30	11.67±1.67^{cd}	31.67±1.33^{de}	53.33±1.67g	63.33±1.33de
	40	18.33±1.67^{cd}	36.67±1.33^{de}	55.00±0.00gh	66.67±1.33e
	50	23.33±1.67^{d}	43.33±1.67^{e}	60.00±1.89h	76.67±1.33f
Fraction 8	10	0.00±0.00^{a}	10.00±0.00ab	23.33±1.67c	48.33±1.67cd
	20	0.00±0.00^{a}	11.67±1.67^{b}	28.33±1.67cd	53.33±1.67d
	30	10.00±0.00^{b}	18.33±1.67^{bc}	41.67±1.67fg	55.00±1.89d
	40	11.67±1.67^{bc}	23.33±1.67^{c}	46.67±1.67g	66.67±1.67ef
	50	15.00±1.89^{bc}	30.00±0.00^{e}	56.67±2.41g	71.67±2.41f
Fraction 9	10	6.67±1.33^{a}	18.33±1.67^{a}	31.67±1.67e	45.00±1.89c
	20	10.00±0.00^{bc}	25.00±1.89^{cd}	38.33±1.67^{ef}	55.00±1.89^{df}
	30	13.33±1.67^{bc}	33.33±1.67^{de}	51.67±1.67g	63.33±1.67f
	40	18.33±1.67^{cd}	31.67±2.41^{de}	56.67±1.67gh	65.00±1.89f
	50	20.00±0.00^{cd}	40.00±0.00^{e}	61.67±1.67h	73.33±1.67fg
Negative control 1		0.00±0.00^{a}	0.00±0.00^{a}	3.33±1.33^{a}	3.33±1.33^{a}
Negative control 2		0.00±0.00^{a}	6.67±1.33^{a}	6.67±1.33^{a}	6.67±1.33^{a}
Positive control		73.33±1.33^{h}	100.00±0.00^{j}	100.00±0.00^{k}	100.00±0.00^{h}

Each value is the 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

Statistical analysis

All the data were subjected to one-way analysis of variance at 5% significant level and means were separated with New Duncan's Multiple Range Test version 17. Data obtained from beetle's mortality were subjected to regression analysis to calculate the lethal doses LD_{50} and LD_{95} of the oil extracts using probit analysis (Finney, 1971).

RESULTS

Mortality of *C. maculatus* exposed to crude oil and different fractions of *M. charantia* oil extracts

Regardless of the dosage and the period of exposure, the crude and different fractions of *M. charantia* oil extracts significantly affected the survival of adult insects. Significant differences existed between the treatments at ($p< 0.05$) in percentage adult mortality (Table 1).A comparison of the crude and all tested fractions indicated that fractions 3 and 4 oil extracts has significantly highest adult mortality (100%) at 50 µl /96hr.This was not significantly different from crude extract that caused 98.33% insect mortality at similar dosage and exposure time.10µl dosage of all treatments and exposure periods proved far less effective for causing below 50% insect mortality. On the contrary, fractions 1; 2; 5; 6; 7; 8 and 9 recorded above 50% adult mortality at 20 -50µl/ 96hr of exposure respectively.

Percentage Repellent activity of fraction 3 of *M. charantia* against *C. maculatus.*

Table 2 showed the percentage repellent of *C. maculatus* by fraction 3 of *M. charantia* oil extract. The result varied with dosage and time of exposure to tested fraction. Within 24hours of observation, none of the dosages repelled up to 50% of the insect. Regardless of the period of exposure, the effect of 10; 20 and 30µl were not significantly different from negative control, but significantly ($P<0.05$) different from the positive control. However, within 24hours of exposure, the plant dosages 40 and 50 µl were not significantly ($P>0.05$) different from the positive control.

Table 2: Percentage Repellent activity of fraction 3 of M. *charantia* oil against *C. maculatus*

Dosages (µl)	Percentage repellence in hours		
	4	8	24
10	6.67 ± 2,67[a]	13.33 ± 2.67[a]	20.00 ± 0.00[a]
20	6.67 ± 2.67[a]	13.33 ± 2.67[a]	26.67 ± 2.67[a]
30	20.00 ± 2.54[a]	33.33 ± 2.67[a]	46.67 ± 2.67[b]
40	46.67 ± 2.67[b]	66.67 ± 2.67[b]	86.67 ± 2.67[c]
50	53.33 ± 2.67[b]	73.33 ± 2.67[b]	93.33 ± 2.67[c]
Negative control	13.33 ± 2.67[a]	13.33 ± 2.67[a]	20.00 ± 0.00[a]
Positive control	80.00 ± 2.54[c]	100.00 ± 0.00[c]	100.00 ± 0.00[c]

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.

Lethal dosage required to achieve 50 and 95% mortality of *C. maculates* exposed to crude oil and different fractions *M. charantia* after 48h post-treatment

The amount of the dosage required to achieve 50 and 95% mortality of *C. maculates* varied among crude and fractions 1; 2; 3 and 4of *M. charantia* oil extract. (Table 3). Fraction 3 proved the most effective treatment, as it require 40.67 and 71.15µl dosages to attain 50 and 95% insect mortality within 48hr post treatment.

Lethal dosage required to achieve 50 and 95% mortality of *C. maculatus* exposed to crude oil and different fractions *M. charantia* after 72h post-treatment

Lethal dosage required to achieve 50 and 95% mortality of the beetle was presented in Table 4. The table indicated that higher amount of fractions 5; 6; 7; 8 and 9 of *M. charantia* oil were needed to achieve 50 and 90% mortality as reflected in their fiducial limits. However, fraction 7 proved more effective on the beetle than other fractions as it requires 45.30 and 77.72µl / 72hr to achieve 50 and 90% insect mortality.

Table 3: Lethal dose (L_{D50} and L_{D95}) of *M. charantia* crude oil and fractions required to achieve 50 and 95% mortality after 48h post-treatment.

Treatments	Slope ±SE	Intercept ±SE	X^2	LD_{50} FL@ 95%	LD_{95} FL@95%	Sig.
Crude	1.37±0.14	-0.92±0.07	30.22	45.23(43.34- 47.35)	77.27(75.53-79.14)	0.004
Fraction 1	1.47±0.14	-0.86±0.07	35.44	43.76(41.56-45.33)	76.74(74.68-78.08)	0.001
Fraction 2	1.68±0.11	-1.30±0.07	40.93	42.72(41.22-43.45)	74.47(72.86-76.33)	0.001
Fraction 3	1.02±0.14	0.13±0.06	9.7	40.67(38.37-42.67)	71.15(69.19-73.98)	0.718
Fraction 4	1.59±0.19	-0.87±0.09	12.05	41.33(39.41-43.89)	75.08(73.14-77.22)	0.360

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

Table 4: Lethal dosage of (L_{D50} and L_{D95}) *M. charantia* fractions required to achieve 50 and 90% insect mortality at 72 h post treatment

Treatments	Slope ±SE	Intercept ±SE	X^2	LD_{50} FL@ 95%	LD_{95} FL@95%	Sig.
Fraction 5	3.77±0.36	-3.59±0.29	9.01	55.29(53.67-57.43)	84.53(82.35-86.57)	0.772
Fraction 6	3.83±0.35	-3.45±0.27	12.33	52.33(51.27-54.35)	80.45(78.54-82.27)	0.501
Fraction 7	2.22±0.31	-1.71±0.24	10.31	45.30(43.33-47.00)	77.72(73.35-79.45)	0.684
Fraction 8	1.32±0.15	0.34±0.11	59.02	65.43(53.23-57.33)	90.34(88.54-92.27)	0.001
Fraction 9	1.72±0.11	-1.19±0.07	67.24	60.67(59.22-62.34)	89.36(88.29-91.58)	0.001

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

DISCUSSION

The use of plant materials has remained the major weapons amongst the tropical zones, farmers even before the discovery and commercial sources of nowadays synthetic chemical insecticides (Oni, 2014). Botanicals of different species have proven insecticidal against wide range of stored product insects and being suggested as promising alternative to the use of chemical insecticide probably because, they are believed to have low or no mammalian toxicity (lsman, 2006)

The result of this study provides empirical evidence that *M. charantia* oil extract have entomocidal potential to varying degrees in conferring protection of cowpea against infestation of C. *maculatus.* ln order words, the effectiveness of the oil extract was dependent of the treatments /dosages/time of exposure.

It is evident that the ability of any plant material to act as an insecticide depend on its ability to cause the mortality of the insects and thus the need to fractionate the plant oil into different fractions to justify the particular fraction of entomocidal potential. All treatments at 20-50µl/exposure periods effected high insect mortality when compared to 10µl dosage with lower mortality figures.

Moreover, at 48h, probit analysis reflected that fraction 3 oil extract, proved the most effective in that it exhibits the highest insect mortality. Similarly, the repellency of fraction 3 oil extract in this research varied with dosage/exposure period, this was in agreement with Oni *et.al* 2018; Yang *et.al* 2014 and Adesina *et.al* 2016. The high insect mortality in this research could be due to the ability of the fractions to disrupt normal respiratory process and its feeding on seeds coated with oil fraction thus leading to systemic poisoning (Ashamo *et.al* (2013); Ileke and Olotuah,(2013) ;Oni (2014) ; Oni *et.al* (2018). Fraction 3 *M. charantia* oil extracts offered great potential than other treatments in this study as toxicants capable of proffering protection to stored cowpea seeds against infestation by *C. maculatus*. The plant is readily available and native to the tropical region and widely grown in India and other part of the Indian subcontinent, Southeast Asia, China, Africa and the Carribean (Warrier *et al*; 1995).

Furthermore, this research results suggest the need to characterize the oil fractions, to establish the active compounds of insecticidal potential present in the plant and the toxicology assay to ascertain safe consumption of treated stored products by mammals.

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