

Toxicity and Repellent Activity of *Momordica charantia* (L.) Extracts against the Cowpea Weevil, *Callosobruchus maculatus* (Fab.) (Coleoptera: Chrysomelidae)

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ABSTRACT

Green pesticides are one of the focuses to solve environmental problems caused by the intensive use of synthetic chemical pesticides. The aim of the present study is to investigate the toxicity of acetone, *n*-hexane, and methanol extract of *Momordica charantia* (L.) leaf to adult and egg stages of *Callosobruchus maculatus* (Fab.) at 0, 10, 15, 20, and 25% concentrations. Repellent effect of the leaf extracts to adults was also tested at the same concentrations. Adult mortality was monitored after 24, 48, 72 and 96 h from exposure to the leaf extracts. Adult emergence from pre- and post-oviposition treated seeds was used as index of ovicidal activity of the tested extracts. The obtained data indicated that all extracts significantly ($P < 0.05$) killed adults and inhibited adult emergence of the insect at all concentrations and exposure-periods at a concentration-dependent manner. The 25% *n*-hexane extract caused 100% adult mortality at 96 h after treatment. The order of extract toxicity was *n*-hexane > methanol > acetone. Regarding post-oviposition treated experiment, *n*-hexane extract caused complete inhibition of adult emergence at all concentrations while methanol and acetone extracts caused 100% inhibition of adult emergence at 15-25% concentrations. On the other hand, all extract-treated cowpea seeds in repellent tests revealed complete repellent effect against the insects. The efficacy of extracts of *M. charantia* in this study indicated that the plant contained some active components against the tested insect.

Keywords: Bioactivity, *Callosobruchus maculatus*, *Momordica charantia* plant extracts.

INTRODUCTION

Cowpea, *Vigna unguiculata* (L.) Walp, is the most important food legume grown in the tropical and subtropical parts of Africa. It plays a critical role in the diets of many households in Africa, Latin America and Asia, providing nutrients that are deficient in cereals

(Jackai and Daoust, 1986). Damage of stored grains by insect pests has caused a great economic loss due to nutritional value and viability of grain crops in developing countries (Ivbijaro, 1984). *Callosobruchus maculatus* (Fab.) is a field-to-store pest of cowpea. Its life history, development and destructive activities on cowpeas have been described in early studies (Jackai and Daoust, 1986; Ojekwu and Merema, 2001; Adiewu and Declan, 2002).

The use of synthetic chemical insecticides has been the major means of reducing storage losses due to insect pests (Adiewu and Declan, 2002). Toxicity of chemical insecticides to man, non-target organisms, long-term persistence and environmental pollution is of global concern (Obeng-Ofori, 2007). Many organochlorine and

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organophosphate pesticides are already banned, while the use of some is being restricted (Forget, 1993). Currently, research in pest management focuses on eco-friendly methods for control of insect pests. It has been reported that botanical products breaks down rapidly to harmless metabolites and may less likely to build up genetic resistance in targeted species (Isman and Machial, 2006; Sidawi *et al.*, 2014).

This study aimed at evaluating *M. charantia* for control of *C. maculatus* in stored cowpea seeds. *M. charantia*, commonly called bitter melon is a tropical and sub-tropical herbaceous vine of the family Cucurbitaceae. It is widely grown in South and South East Asia, Africa, China and Caribbean for medicinal and food purposes (Hassanah *et al.*, 2002), *M. charantia* is a slender annual vine with long-stalked simple and alternate leaves 4-12cm across, and 3-7 deeply separated lobes. Each plant bears separated yellow male and female flowers which produced emerald-green gourd-like fruit that turned orange-yellow when turned ripe (Hassanah *et al.*, 2002). Previous studies have revealed its anti-plasmodial property (Subramaniam *et al.*, 2012), blood sugar-lowering effect (Sridhar *et al.*, 2008), anti-bacterial activity against *Escherichia coli*, *Staphylococcus*, *Pseudomonas*, *Salmonella* (Makhija *et al.*, 2011).

Toxicity, repellent and anti-feeding effects of extracts of *M. charantia* against some agricultural insect pests and insect of medical importance (mosquito) have been documented (Zhi *et al.*, 2007; Fiaz *et al.*, 2012; Subramaniam *et al.*, 2012; Hameed *et al.*, 2013). However, little is known about insecticidal activity of *M. charantia* against stored products pests. Previous studies tested leaf powder of *M. charantia* against *C. maculatus* and reported some level of efficacy (Boeke *et al.*, 2004; Adesina *et al.*, 2012). This study evaluated the bioactivity of the leaf extracts of *M. charantia* to *C.*

maculatus. Plant extracts contained concentrated phytochemicals of the whole plant. However, therefore hypothesized that extracts of *M. charantia* might have stronger bioactivity against adult and egg stages of *C. maculatus*. Furthermore, it has been established that different solvents might yield different extracts and extraction compositions (Anwar and Przybylski, 2012; Hijazi *et al.*, 2013). A second hypothesis was that extracts of *M. charantia* from different extracting solvent might have varying level of bioactivity against the adults and egg stages of *C. maculatus*. Hence, *n*-hexane, methanol and acetone extracts of the leaf of *M. charantia* were screened for toxicity and repellent bioactivity against *C. maculatus*.

MATERIALS AND METHODS

Cowpea seeds:

Cowpea (Ife-brown cultivar) seeds were purchased from local grocery store in Akure, Ondo State Nigeria located between latitude 7° 15' N and Longitude 5° 12' E. The seeds were kept in polythene bags, sealed and kept in a Thermo cool deep freezer maintained at -20°C for 72 h until they were used for insect cultures or the bioassays (Adedire and Ajayi, 2003; Johnson and Valero, 2003).

Insect Culture:

A fresh culture was started from an old culture of more than 10 generations in the laboratory by placing ~25 pairs of 2-day-old *C. maculatus* in a 1 L kilner jar containing ~200 g of cowpea seeds held at 28 ± 2°C, 70 ± 5% relative humidity (RH), and a photoperiod of L:D 12:12 h (Adedire and Ajayi, 2003). From this stock, pure culture was raised on cowpea grains to obtain new generations following the method described by Asawalam (2006). Freshly emerged adults of *C. maculatus* (0 – 24 h) were used for the experiments.

Plant Collection and Extracts Preparation:

Momordica charantia leaves were collected from a local farm in Akure. They were rinsed under running water and air-dried in the laboratory. The dried leaves were milled into powder using an electric blender (Binatone®, Model BLG400 China) (Ajayi *et al.*, 2012). Extracts of the leaves powder, acetone and methanol, were prepared by soaking method, and hexane extract (oil) by Soxhlet extraction as described by Harbone (1984) using analytical grade solvents of 99% purity. The solutions of plant powder and solvents were filtered with Muslin cloth at ~72 h after soaking. The filtrates were concentrated in rotary evaporator. Concentrated extracts were lyophilized using Millrock Laboratory series lyophilizer (Model LD85, 35"W × 42"D × 52"H, 230V, 60Hz, USA) to remove traces of solvents. Extracts obtained were kept in dark bottles and stored in refrigerator at -4°C until used. Extraction of essential oil from the leaf powder was done in a 1000 mL Soxhlet extraction system at 65°C using *n*-hexane as described by Harbone (1984) and modified by Adedire and Ajayi (2003). *n*-Hexane was separated from the extract using rotary evaporator at 65°C and thereafter, the extract was exposed to air at 160 rpm from 130cm diameter fan for 24 h to get rid of Hexane traces. The extract thus obtained was kept in dark bottle and stored in a dark cupboard at room temperature until used.

Toxicity tests:

Twenty grams of disinfested whole cowpea seeds were weighed into plastic plates (4cm (h), 8cm Ø). Five concentrations, 0, 10, 15, 20, and 25% of all extracts were tested using the respective extraction solvent as the diluents. For acetone and methanol extracts, 1, 1.5, 2.0, 2.5g of each extracts were weighed into 10 ml Pyrex conical flask and completed to 10 ml with acetone or methanol to obtain 10%, 15%, 20%, and 25% w/v concentrations of acetone or methanol extracts. The

same measurement was used with *n*-hexane extract (oil) and the volume was completed to 10 ml with *n*-hexane to obtain the respective concentration.

One (1) ml of each concentration of the three extracts was added to 20g cowpea seeds in the plastic plates. These were thoroughly mixed by agitation to ensure a uniform coating of the seeds. The treated seeds were allowed to air-dry for 30 min after which 10 unsexed adults (0-24 h old) of *C. maculatus* were introduced into each replicate (Zapata and Smagghe, 2010). **Control experiments:** Solvent-treated and untreated cowpea were set up. One (1) ml of solvent was added to solvent-treated experiment, while untreated cowpea had no treatment. All treatments concentrations were replicated five times. Mortalities were recorded at 24, 48, 72 and 96 h after treatment. Adult insects were assumed to have died upon failure to respond when probed with sharp object at the abdomen. Percentages of adult mortalities were calculated and corrected with mortality in control using Abbott's formula (Abbott, 1925).

Ovicidal activity of *M. charantia* extracts on eggs of *Callosobruchus maculatus*:

Survival of eggs laid on pre-oviposition and post-oviposition treated cowpea seeds were assessed. Cowpea seeds, 100 grams were treated with 10%, 15%, 20% and 25% of the three extracts solutions at the rate of 1ml/20g seeds. The setup was exposed to air 30 min to allow the solvents to dry off. Ten pairs of 2-day-old adults of *C. maculatus* were introduced to lay eggs on the treated cowpea seeds for 24 h. From each treatment, 100 eggs, maximum of 2 per seed were counted into plastic plates. Treatment concentrations were replicated five times.

Ten pairs of 2-day-old adults of *C. maculatus* were introduced into containers of 100 g untreated for 24 h. Cowpea seeds with a total of 100 eggs, maximum of 2 per seed were counted into plastic plates. The total

weight of egg laden cowpea that was less than 20g in a plate was made-up to 20g with uninfested cowpea seeds without eggs to ensure uniform weight. Treatments were applied as stated above. All experiments were conducted at the above mentioned conditions for insect culture. Observation for adult emergence started at 30 days post-treatment and lasted for ten days. Number of adults that emerged was used as index of extract toxicity to the treated eggs. The percentages of adults that emerged were recorded.

Repellency potential of *M. charantia*:

Experiments to determine repellency potential of *M. charantia* were conducted as described by Adebowale and Adedire (2006) with modification. Melted candle wax was poured into 14cm Ø Petri dishes and allowed to solidify. Six holes were made on the solidified candle wax. Plates containing twenty grams of cowpea seeds were treated with 1 ml of 10, 15, 20 and 25% solutions of each of the three extracts and exposed to air for 30 min for the solvents to dry off. Five seeds from each treatment concentration were dropped in each hole made on the candle wax. Solvent-treated and untreated seeds were included. Ten mated females of *C. maculatus* were introduced at the center of each plate. The choice of female for this experiment was on the basis that adult male and female of *C. maculatus* do not feed after emergence. After mating, the mated female looked for cowpea to deposit eggs (Rees, 2004). Hence, female was responsible for locating suitable host for the eggs. The set-up was replicated five times. The number of insects found in each hole was recorded at 24 h after treatment.

Data Analysis:

The experiment was Completely Randomized Designed (CRD) with five replicates per treatment. Data in percentages were arcsine transformed to normalize the data, and subjected to analysis of variance (ANOVA). The means were separated by Tukey's HSD test using

SPSS Version 16.

RESULTS

Toxicity to adult *Callosobruchus maculatus*:

The three extracts of *Momordica charantia* caused significant mortality of adult *C. maculatus*. Mortality varied between treatments (extracts and controls), concentration and exposure-period (Table 1). *n*-Hexane extract was most effective at all exposure periods and concentrations tested while acetone extract was the least. One-way ANOVA showed significant differences ($P < 0.05$) between the percentages of adults mortalities obtained with the three extracts at all concentrations and exposure periods. However, Tukey's HSD revealed no significant differences between extracts toxicities at some concentrations, and between the concentrations of each extract. At 24 h post-treatment, the percentages of adults mortalities with acetone and methanol were not significantly different at 10 and 15% ($P = 0.816$), 20% ($P = 1.000$), and 25% ($P = 0.835$). At 48 h, acetone and methanol were not significantly different at all concentrations (10% ($P = 1.000$), 15% ($P = 0.793$), 20% ($P = 0.164$) and 25% ($P = 0.055$)). There was no significant differences between acetone and methanol toxicity with 10% concentration at 72 h ($P = 0.407$) and 96 h ($P = 0.062$). Hexane and methanol extracts were not significantly different with 25% ($P = 0.093$) and 15% ($P = 0.165$) at 24 and 72 h post-treatment, respectively. Generally, mortalities increased with increase in concentrations and exposure periods. Percentages of adult mortalities in solvents and untreated experiment ranged between 0.2 – 8.0% (Table 1).

Ovicidal and repellent potential of *Momordica charantia* extracts on eggs of *Callosobruchus maculatus*:

Figs. 1 and 2 showed the results of toxicity of *M. charantia* extracts to eggs of *C. maculatus*.

Concentrations and times of application of treatments had pronounced effects on egg survival to adults. Adults emerged from all the treatments in pre-oviposition coated seeds, but the percentage of emergence reduced with increased concentrations of the extracts (Fig. 1). Adults emerged only at 10% concentrations with acetone and methanol in post-oviposition coated seeds (Fig. 2). One-way analysis of variance showed significant differences ($P < 0.05$) between percentages of adults emergence in treatments and the two controls in both pre- and post-oviposition experiments (Figs. 1 and 2). *n*-Hexane extract was most effective in both pre- and post-oviposition treatments at inhibiting adult emergence. In pre-coated seeds tests, adult emergence ranged between 8.2 and 20%, 18.4 and 26.0% and, 23.6 – 32.2% in hexane, methanol and acetone from lowest to highest treatment concentrations, respectively (Fig. 1). Percentage of adults emergence in solvent and untreated experiments ranged between 93.8 – 94.8% and 95.6 - 97% respectively (Fig. 1).

In post-oviposition treated experiment, 100% adults emergence inhibition occurred at 15, 20, and 25% of all treatments. In 10% acetone and methanol extract-treated seeds, 7.6% and 6% adults emerged, respectively. Adults emergence ranged between 92 and 96% in solvent and untreated controls, respectively (Fig. 2).

All *M. charantia* extracts at all concentrations tested repelled *C. maculatus* (Table 2). The insects concentrated in the holes containing solvent-treated and untreated seeds.

DISCUSSION

The toxicity of acetone, *n*-hexane, and methanol extracts of the leaf of *M. charantia* to *C. maculatus* assayed in this study varied among the three extracts, exposure periods, and treatment concentrations. *n*-Hexane extract was most toxic at all concentrations and

exposure periods to both eggs, and adult stages of *C. maculatus*. Acetone extract was least toxic to the two life stages. No insects were found on extract-treated cowpea seeds in the repellent test against adult *C. maculatus*. Earlier studies revealed steroidal saponins, insulin-like peptides, tannins, glycosides, and alkaloids as the secondary metabolites in *M. charantia* (Hassanah et al., 2002; Bakare et al., 2010). Steroidal saponins and alkaloids were known to be toxic and repellent to insects (Schumtterer, 1984; Grover and Yadav, 2004; DeGeyter et al., 2012). Interestingly, triterpenes which was found suitable for use as pesticides due to its toxicity to wide range of insect pests (Schumtterer, 1984), was discovered as the major insecticidal properties in *M. charantia* in the form of triterpene mono-glucoside and triterpene di-glycoside) (Yausi, 2002). Extracts toxicities and repellency to *C. maculatus* observed in this study might be due to the combined activities of the biochemical components of *M. charantia*.

Adesina et al. (2012) assessed efficacy of leaf powder of *M. charantia* at 0.5 - 2.0g/20g cowpea seeds against adult and egg stages of *C. maculatus*. They reported low adult toxicity, maximum of 30% at 2.0g/20g seeds. The difference between the toxicity results in this study and that of Adesina et al. (2012) showed that extracts of the leaf contained concentrated components of the plant Therefore, the insects had contact with the toxic components in extracts more readily than the powder. Usha Rani and Devanand (2011) reported 100% mortality of *Sitophilus oryzae* (L.) and *Tribolium castaneum* (Herbst.) with 100mg/20g acetone extract of *M. charantia* at 72 h post-treatment. This study recorded 100% mortality of adults of *C. maculatus*, but with 25%v/v *n*-hexane extract at 96 h after treatment. Susceptibility of insects to insecticides depended on insect species, age, sex, mode of feeding and the mode of action of insect control agents (Lahlou,

2004). Any of these might account for the difference between the adult toxicity in this study and that of Usha Rani and Devanand (2011). Adesina *et al.* (2012) and Akinkurolere (2012) reported reduction of adults of *C. maculatus* emergence with *M. charantia* leaf powder though the number of eggs used was not specified. On the contrary, adult emerged at all concentrations applied in pre-oviposition coated experiments. The least effective at reducing adults emergence in this study was 10% acetone extract from which 32.2% adults emerged as against 95.6% that emerged from untreated control. This was about 66.3% reduction of adults emergence from the least effective extracts and the lowest concentrations tested in this study. This indicated that leaf extracts of *M. charantia* inhibited adult emergence of *C. maculatus* more than the powder. Adult emergence in pre-oviposition coated experiment significantly ($P < 0.05$) reduced with increased treatment concentration. However, post-oviposition coating resulted in complete inhibition of adult emergence at all treatment concentrations except 10% of acetone and methanol extracts. In pre-oviposition coated seeds, extract might have reacted with the seed coat which might reduce the extracts toxicities. Furthermore, female *C. maculatus* released waxy organic substance on cowpea seeds to glue the egg to the seeds (Rees, 2004) which might impair egg contact with the extracts on seed coat. Again, the aeropyle and the chorion of the eggs in pre-oviposition coated seed were in contact with oxygen in the surrounding air. This eggs exchange respiratory gases with oxygen unhindered. However, the coating of eggs with the seeds (post-oviposition extract-coated)

exposes larger part of the surface area of the egg to the extracts. The extracts might enter and block egg aeropyles thus causing suffocation. Extracts might also diffuse from aeropyle and egg surface to the chorion, through which the toxic components of the extracts might get to the oocyte and damage the egg. Therefore, the underline factor of the differences between the percentages of adult emergence obtained in pre- and post-oviposition treated seeds might be the time of treatment application. All extracts tested in this study repelled *C. maculatus*. The anti-feedants in *M. charantia*: momordicacine II, triterpene mono-glucoside and triterpene di-glucoside (Yasui, 2002) might with other components interact to repel the insects.

This study confirmed that leaf extracts of *M. charantia* were more toxic to *C. maculatus* than the powder. The three extracts were toxic to adults and egg stages; and repelled the adults with *n*-hexane extract as the most effective among the three extracts. The results further confirmed that *M. charantia* contained some components that were toxic to insects which if explored might yield effective biopesticides. Mush (Adesina *et al.*, 2012; Akinkurolere, 2012) had not documented about toxicity of this plant against pests of edible stored products presumably because of its bitter taste. If the bitterness made it suitable to store future food, it might be used to preserve seeds for planting. Therefore, further research is necessary to determine the effect of *M. charantia* crude extracts or pure components, on acceptability of future food grains, food quality of stored grains, seed viability and stored grain microbes.

Table 1. Toxicity of different solvent extracts of *Momordica charantia* against *Callosobruchus maculatus* adults after different periods of exposures.

Concentrations (%)	Solvent extract	Corrected cumulative % mortality after different periods of exposures (hours)			
		24	48	72	96
10	Acetone	12.00 ^a	20.00 ^a	29.11 ^a	36.33 ^a
	n-Hexane	26.00 ^b	38.00 ^b	49.78 ^b	66.67 ^b
	Methanol	14.00 ^a	20.00 ^a	20.67 ^a	50.67 ^a
15	Acetone	24.00 ^a	36.00 ^a	45.77 ^a	51.72 ^a
	n-Hexane	38.00 ^b	48.00 ^b	66.45 ^b	93.78 ^c
	Methanol	26.00 ^a	38.00 ^a	59.34 ^b	74.67 ^b
20	Acetone	28.00 ^a	38.00 ^a	46.89 ^a	60.72 ^a
	n-Hexane	40.00 ^b	58.00 ^b	81.11 ^c	97.78 ^c
	Methanol	28.00 ^a	44.00 ^a	70.00 ^b	80.67 ^b
25	Acetone	34.00 ^a	44.00 ^a	58.22 ^a	71.89 ^a
	n-Hexane	44.00 ^b	70.00 ^b	98.00 ^c	100 ^c
	Methanol	36.00 ^{ab}	54.00 ^a	70.22 ^b	89.11 ^b
Solvent	Acetone	0.00 ^a	0.00 ^a	4.00 ^a	6.00 ^a
	n-Hexane	0.00 ^a	0.00 ^a	2.00 ^a	2.00 ^a
	Methanol	0.00 ^a	0.00 ^a	4.00 ^a	4.00 ^a
Untreated	Acetone	0.00 ^a	0.00 ^a	4.00 ^a	8.00 ^a
	n-Hexane	0.00 ^a	0.00 ^a	4.00 ^a	4.00 ^a
	Methanol	0.00 ^a	0.00 ^a	6.00 ^a	6.00 ^a

Each value is a mean of five replicates. Values with the same letter within a column in a row are not statistically different by ANOVA and Tukey's HSD test ($P < 0.05$).

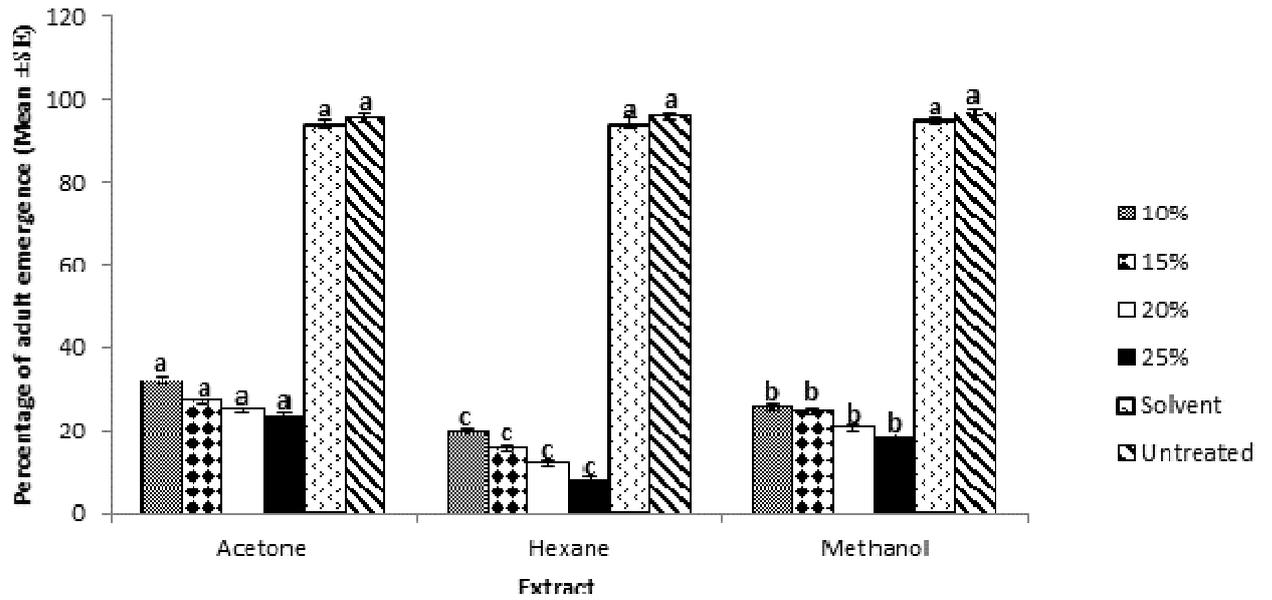


Fig. 1: Adult *C. maculatus* emergence from pre-oviposition treated cowpea seeds. Analysis compared the three extracts at each concentration tested.

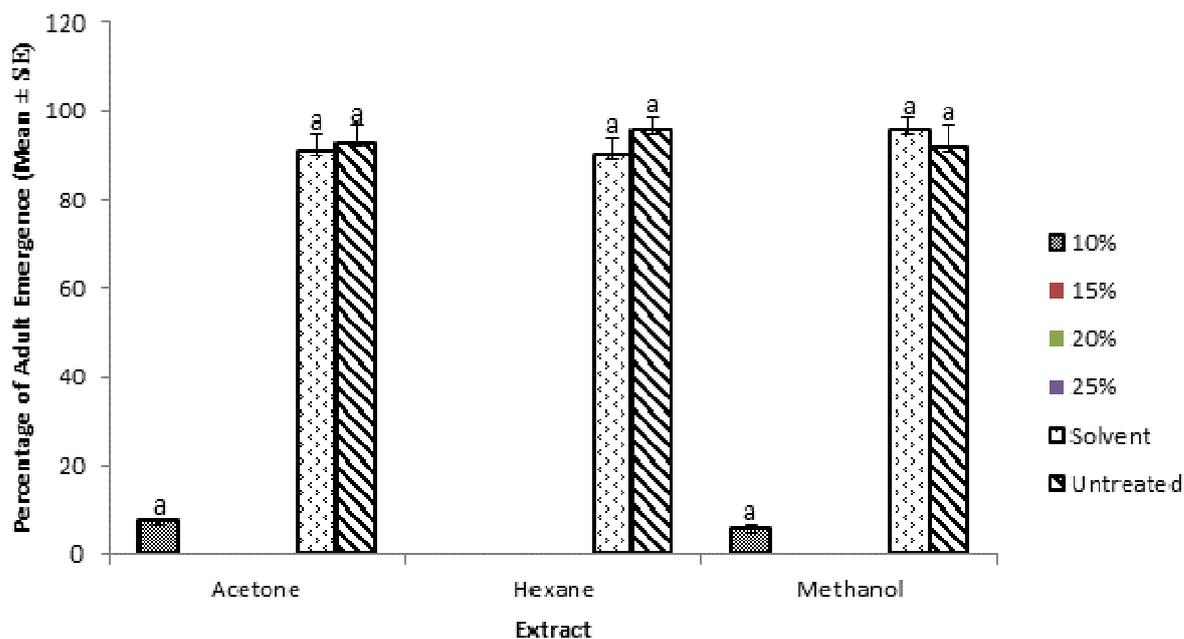


Fig. 2: Adult *C. maculatus* emergence from post-oviposition treated cowpea seeds. Analysis compared the three extracts at each concentration tested.

Table 2. Response of adult *Callosobruchus maculatus* to cowpea seeds treated with *M. charantia* extracts under laboratory conditions

Extract	Treatment concentration (%)					
	10	15	20	25	Solvent	Untreated
Acetone	0.33 ^a	0.00 ^a	0.00 ^a	0.00 ^a	4.67 ^a	4.67 ^a
<i>n</i> -Hexane	0.00 ^a	0.00 ^a	0.00 ^a	0.00 ^a	4.67 ^a	5.33 ^a
Methanol	0.00 ^a	0.00 ^a	0.00 ^a	0.00 ^a	4.33 ^a	5.33 ^a

Each value is a mean of five replicates. Values with the same letter within a column are not statistically different by ANOVA and Tukey's HSD test ($P < 0.05$).

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السمية والنشاط الطارد لمستخلص *Momordica charantia* (L)

ضد خنفساء البازيلاء

Callosobruchus maculatus (Fab) (Chrysomelidae) غمدية الاجنحة :

أولوفنملايو أجاى *

ملخص

المبيدات الخضراء هي موضع اهتمام لحل المشاكل البيئية الناجمة عن الاستعمال المكثف للمبيدات الكيميائية المصنعة . الهدف من هذه الدراسة هو البحث عن مستخلصات الاسبينون، n - هكسان، ميثانول. الأوراق *Momordica charantia* (L) وسميتها لأطوار الحشرات الكاملة وبيض خنفساء البازيلاء بالتركيزات صفر، 10، 20، 15، 25%. القوة الطاردة لمستخلصات الأوراق للحشرات البالغة تم اختبارها بنفس التركيزات السابقة . تم مراقبة موت الحشرات البالغة بعد 24، 48، 72، 96 ساعة. من بداية التعرض لمستخلص الأوراق. خروج الحشرات الكاملة قبل وبعد وضع البيض على البذور المعاملة تم استعماله كمؤشر (كدليل) على القوة الابادية للمستخلصات المختبرة على البيض . النتائج المتحصل عليها اظهرت أن جميع المستخلصات أظهرت نسبة موت عند ($P < 0.05$) للحشرات الكاملة وتم تثبيط فقس الحشرات الكاملة مع جميع التركيزات المستعملة واوقات التعرض وفق اعتماد على التركيز. مستخلص 25% n - هكسان نتج عنه نسبة موت 100% بعد 96 ساعة من المعاملة . ترتيب سمية المستخلصات كانت n - هكسان < ميثانول < اسبتون. بخصوص تجربة وضع البيض ، تسبب n - هكسان بتثبيط كامل لفقس الحشرات الكاملة مع جميع التركيزات بينما مستخلصات ميثانول، اسبتون تسببت بتثبيط 100% لفقس الحشرات الكاملة مع التركيزات 15-25% . من ناحية اخرى أظهرت قوة طاردة كاملة ضد الحشرات . كفاءة مستخلصات *Momordica charantia* (L) في هذه الدراسة اظهرت النتائج أن النبات يحتوي على بعض المواد النشطة ضد حشرة خنفساء البازيلاء .

الكلمات الدالة: النشاط البيولوجي ، *Callosobruchus maculatus* ، مستخلصات نبات ، *Momordica charantia* (L)

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