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Toxicity and oviposition inhibitory effect of extract and powder of *Momordica charantia* leaf against *Callosobruchus maculatus* Fab. (Coleoptera: Chrysomelidae) on stored cowpea seed

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ABSTRACT: The pulse beetles, *Callosobruchus maculatus* is a principal field-to store pest of cowpea in the tropics and at present synthetic insecticides constitute a major means of control. The insecticidal effect of ethanolic extracts and powders of *Momordica charantia* leaf against the cowpea beetle, *Callosobruchus maculatus* Fab. were carried out in the laboratory. The efficacy of powder and ethanolic extract of *M. charantia* leaf as toxicants against *C. maculatus* were investigated using contact toxicity and oviposition deterrence. The powders and extracts were applied at various dosages of 0.2, 0.4, 0.6, and 0.8 g and 0.2, 0.4, 0.6 and 0.8 ml per 20 g of cowpea seeds. Beetle mortality was monitored for 96 h. The mortality of the insect increased with increase in dosage and period of exposure. Within 24 h post treatment, 0.8 g dosage of the plant powder recorded the highest beetle mortality of 35% but its effect was not significantly ($p>0.05$) different from that of 0.4 and 0.6 ml. At 96 h of post-treatment, all the dosages of the plant powders recorded above 75% insect mortality with 0.8 g of the powder achieving the highest insect mortality of 85% and its effect was not significantly ($p<0.05$) different from 0.2, 0.4 and 0.6 g. At 96 h post treatment, 0.8 ml of the extract achieved the highest mortality of 95% which was not significantly ($p<0.05$) different from that of 0.6 ml. All dosages of the extract recorded up to 80% bruchid mortality within 96 h post treatment. At all treatment levels, insect mortality was significantly ($p<0.05$) different from the control. It was observed that oviposition was reduced in both powder and extract treatments when compared to the control. The results show that both powder and extract of *M. charantia* were effective in controlling *C. maculatus* and could serve as alternative to over-dependence on synthetic insecticide for preservation of stored cowpea seeds against *C. maculatus.*

Key word: *Callosobruchus maculatus,* dosages, insecticidal, *Momordica charantia*, mortality, treatment.

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). Also, Okosun and Adedire (2010) reported that cowpea is an important leguminous crop, providing plant protein for human and animals' consumption.

Cowpea is susceptible to attack by *Callosobruchus maculatus* in storage. They cause heavy losses and physical damage. According to Oparaeke *et al.* (2000), yield losses in cowpea due to insect pests in Nigeria were estimated to be above 80%. *C. maculatus* infestation causes direct damage to cowpea grains, causing loss of grains (Babarinde et al., 2015).

Currently, synthetic insecticides are the chief means of insect pests control both in the field and in storage (Ojo et al., 2018). They have shown efficacy against a wide range of pest species of agricultural crops. Chemical control is generally practiced by farmers for higher gains, but its injudicious utilization has created many problems. Sole reliance on chemical control leads to problems of pests resistance, resurgence of pests, pesticide residues, destruction of beneficial fauna (non-target) and environmental pollution, human poisoning, destruction of natural enemies of pests, crop pollination problem due to honey bee losses, domestic animal poisoning, contamination of livestock products, fish and wildlife losses and contamination of underground water and rivers (Ewete and Alamu1999; Asawalam and Adesiyan, 2001; Obembe, 2017). As yields are however generally low (Olatunde et al., 1991), sometimes total yield losses and crop failure occur (Singh and Jackai 1985) due to the activities of a wide spectrum of insect pests which ravage the crop in the field at different growth stages. Consequently, farmers spray their farms as many as eight to ten times during the growing season (Omongo et al., 1997).

As an alternative to the chemical insecticides, botanical insecticides have been discovered by researchers. Botanical insecticides are naturally occurring chemicals, extracted from plants which break down readily in the soil and are not stored in plant or animal tissue. Often, their effects are not long lasting as those of synthetic pesticides (Arong et al., 2011). Botanical insecticides are generally pest–specific and are relatively harmless to non-target organisms. These natural insecticides have proved to be effective, biodegradable, low cost, low technological base, selective and environmentally friendly (Obembe, 2017; Adededire et al., 2011; Ojo et al., 2018). Also, the possibility of insect developing resistance to botanical insecticide is less (Scott et al., 2005). Furthermore, plant extracts act as mortality agents, repellents, anti-feedants, attractants, oviposition deterrents and sterility agents (Lale, 2002).

Over 2000 species of plants are known to possess insecticidal activities (Sariah, 2010; Arong et al., 2011). Such plant materials include powders, water extracts, oil and wood ash from plants like Neem tree (*Azadirachta indica*), groundnuts (*Arachid hypogea),* nutmeg (Myristica fragrans) and coconut. Others are leaf extracts of ginger (*Zingiber offficinale)*, garlic (*Allium sativum)*, African Black Pepper *(Piper guineensis)* tobacco (*Nicotiana tabacum*), cashew (*Anacardium occidental*), (Ivbijaro, 1983; Grainger and Ahmed, 1988; Hall and Harman, 1991; Adedire et al., 2011; Musa et al., 2015; Babarinde et al*.,* 2016).

M. charantia is popularly used in various systems of traditional medicine for various activities such as antidiabetic, antioxidant, antitumor, antiviral and analgestic (Shibu et al., 2017). An earlier study has revealed the insecticidal activity of *M. charantia* against mustered saw fly *Athalia proxima* (Kumar et al., 1979). Jayapal et al., (2012) also reported the larvicidal and pupicidal properties of the leaf crude extract of *M. charantia* against potent malarial vector, *Anopheles stephensis*. The present research aimed at evaluating the effectiveness of *Momordica charantia* leaf extracts and powders in the control of cowpea weevil, *Callosobruchus maculatus.*

MATERIALS AND METHODS

Study area

The study was conducted in Plant Science Department Laboratory, Ekiti State University, Ado Ekiti, Nigeria under prevailing atmospheric conditions of 28±2^{0}C temperature and 70 to 75% relative humidity.

Insect culture

The initial culture of *C. maculatus* used for the experiment was obtained from local market in Iworoko Ekiti, Ekiti State, Nigeria, with naturally infested cowpea seeds. The insects were cultured at room temperature of 28±2^{0}C and humidity of 70 to 75% inside a Kilner jar covered with muslin cloth to disallow the escape of the insect and as well disallow the entry of intruding insects that may act as parasitoid. The culture was maintained by replacing the damaged seeds with new un-infested seeds.

Collection of plant materials and preparation of extracts

Momordica charantia leaves were obtained from the Faculty of Science, Ekiti State University Ado Ekiti, Nigeria. The leaves were air-dried in the laboratory for two weeks in other to reduce the moisture content, so as to prevent moldiness. The air-dried leaves were ground into fine powder using an electric blender. The powder was stored in black cellophane bag until used for either extraction or powder bioassay with the bruchids. About 50 g of *M. charantia* powders was measured into thimbles and extracted using 95% ethanol in a Soxhlet apparatus at a temperature of 60 to 80^{0}C for about 4 to 5 hours. The extract was exposed to slow blowing fan to remove any trace of ethanol and was thereafter poured into a bottle and stored in a refrigerator until needed for entomological bioassay.

Effect of plant extracts and powder on weevil mortality

Four dosages (0.2, 0.4, 0.6, and 0.8 ml) extract of *M. charantia* were mixed with 20 g of cowpea seed in separate Petri dishes. The cowpea grains were shaken vigorously to ensure uniform coating of the grains with the extracts. Ten males and ten females *C. maculatus* adult which were freshly emerged from the culture were released into the Petri dishes and covered in order to prevent the exit and entry of other insects. Also, 0.2, 0.4, 0.6 and 0.8 g of *M. charantia* leaf powder were mixed with 20 g of cowpea seeds in separate Petri dish and 10 males and 10 females adult *C. maculatus* which freshly emerged in the culture were released into Petri dish and covered tightly in order to prevent the entry and exit of insects. Ten males and ten females *C. maculatus* introduced in a Petri

Table 1. Mortality (%) of *Callosobruchus maculatus* exposed to different dosages of *Momordica charantia* powders.

Treatments (g)	% mortality in hours			
	24	48	72	96
0.2	17.50±4.27[ab]	47.50±2.39[b]	55.00±1.44[b]	75.00±1.44[b]
0.4	27.50±4.27[b]	50.00±2.04[b]	60.00±2.04[bc]	80.00±2.04[b]
0.6	32.50±2.39[b]	55.00±1.44[b]	62.50±2.39[bc]	82.50±2.39[b]
0.8	35.00±3.23[b]	57.52±2.39[b]	67.50±2.39[c]	85.00±1.44[b]
0.0	0.00±0.00[a]	0.00±0.00[a]	0.00±0.00[a]	0.00±0.00[a]

Each value is mean ± standard error of three replicates. Values followed by the same letter (s) are not significantly (p>0.05) different from each other using New Duncan's Multiple Range Test.

Table 2. Mortality (%) of *Callosobruchus maculatus* exposed to different dosages of *Momordica charantia* extract.

Treatments (ml)	% mortality in hours			
	24	48	72	96
0.2	30.00±3.23[b]	55.00±3.23b	62.50±1.25b	80.00±2.04b
0.4	32.50±2.39bc	57.50±2.39bc	65.00±1.44b	82.50±1.25b
0.6	40.00±2.04bc	60.00±2.04bc	67.50±1.25b	87.50±2.39bc
0.8	47.50±2.39c	70.00±2.04c	77.50±1.25c	95.00±1.44c
0.0	0.00±0.00[a]	0.00±0.00[a]	0.00±0.00[a]	0.00±0.00[a]

Each value is mean ± standard error of three replicates. Values followed by the same letter (s) are not significantly (p>0.05) different from each other using New Duncan's Multiple Range Test.

dish with no extract or powder served as control for the experiment. Each dosage of the extract and powder bioassay and the control was replicated four times.

Mortality of the insects was observed and recorded at 24 h interval. This was done by gently probing the insect with a sharp pin on the abdomen. Insect that did not react to the probing were considered dead (Adedire et al., 2011). This continued for 96 hours after which the survivors from the treated and untreated cowpea seeds were removed. Thereafter, the experiment was allowed to stay for another 3 days and data were collected on the number of eggs laid.

Experimental design and data analysis

Data obtained from all the parameters were subjected to one-way analysis of variance at 5% significant level and means were separated with New Duncan's Multiple Range Tests using SPSS version 17. In addition, data obtained from beetles' mortality were subjected to regression analysis to calculate the LD_{50} and LD_{95} of the powder and extract after 96 hours of application using probit analysis.

RESULTS

Percentage mortality of *C. maculatus* exposed to different concentration of *M. charantia* powder

Table 1 presents the toxic effects of *M. charantia* powder against *C. maculatus*. The mortality of the insect varied with the dosage of the plant powder and the period of exposure. Within 24 hours post treatment, 0.8 g dosage of the plant powder recorded the highest beetle mortality of 35% which was not significantly (p>0.05) different from other treatments. At 72 hours of application, all the dosages of the plant powder recorded above 50% insect mortality with 0.8 g of the powder achieving the highest insect mortality of 67.5% and its effect was significantly (p<0.05) different from 0.2 g dosage. All the dosages of the plant powders recorded above 70% mortality of the insect within 96 hours post treatment. Although, none of the dosages was able to achieve 100% mortality within 96 hours post treatment. The effects of the treatments were significantly (p<0.05) different from the control throughout the period of exposure.

Percentage mortality of *C. maculatus* exposed to different dosages of *M. charantia* extract

Table 2 presents the toxicity of *M. charantia* extract against *C. maculatus*. At 24 hours post treatment, only 0.8 ml of the extract was able to achieve above 45% mortality of the insect but its effect was not significant (P>0.05) different from other treatment except 0.2 ml dosage which recorded 30% bruchid mortality. At 72 hours after application, 0.8 ml of extract achieved the highest insect mortality rate of

Table 3. Lethal dose of *Momordical charantia* required to achieve 50 and 95% mortality of *Callosobruchus maculatus* within 96 h of application.

Treatments	Slope ± S.E	Intercept ±S.E	X^2	LD_{50} (95% FL)	LD_{95}(95%FL)	Sig.
Oil	1.86±0.14	-1.20±0.07	88.235	4.44(3.67-5.99)	9.76(6.42-10.64)	0.0001
Powder	1.78±0.14	-1.21±0.07	87.102	5.00(4.01-7.36)	11.31(7.73-16.26)	0.0001

S.E: standard error; X^2: Chi square; LD: lethal dose; FL: fiducial limit.

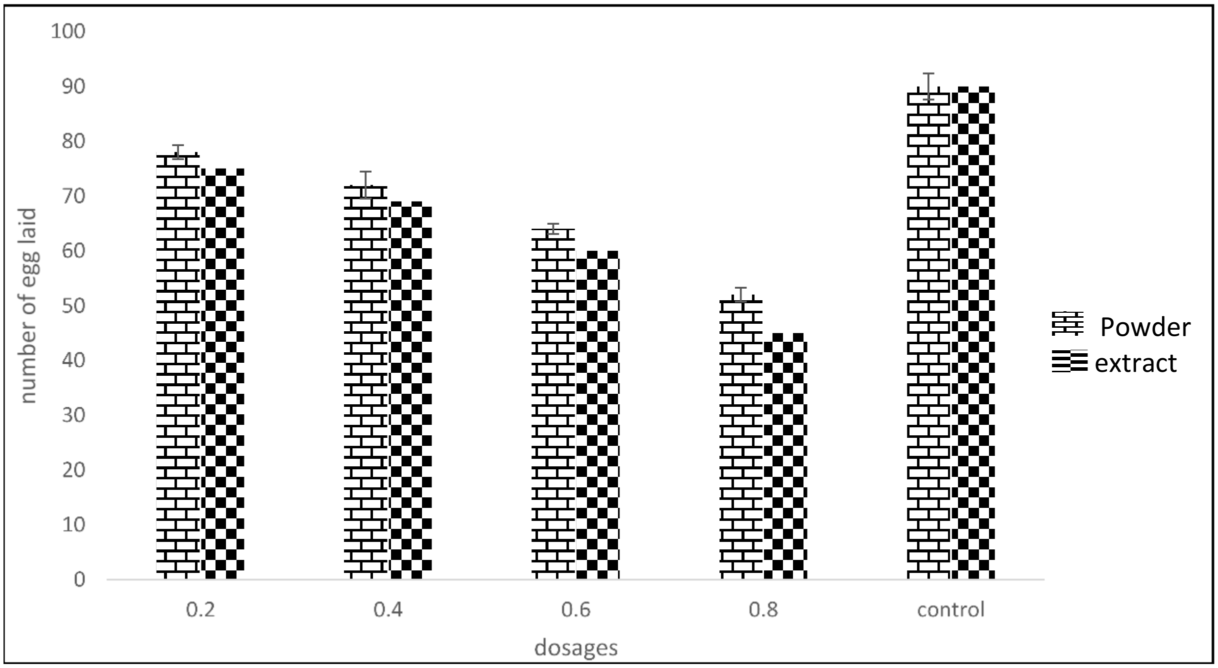

Figure 1. Number of egg laid by *Callosobruchus maculatus* exposed to powder and extract of *Momordica charantia* leaf.

77.5% which was significantly (p<0.05) different from other treatments. At 96 hours post treatment, 0.8 ml of the extract achieved the highest mortality of 95%. None of the dosages was able to achieve complete beetle mortality. All the extract dosages recorded up to 80% bruchid mortality within 96 hours post treatment which was significantly (p<0.05) different from the control.

Lethal dosage of *M. charantia* required to achieve 50 and 95% mortality of *C. maculatus* within 96h of exposure

The lethal dosages of *M. charantia* extract and powder required to achieve 50 and 95% mortality of the insect within 96 hours post treatment are presented in Table 3. The negative coefficient of the extract and powder indicated that the higher the dosage of the extract and powder, the higher the mortality of the insect. Also, chi square values that are greater than zero indicated the high level of relationship between the dosages of the treatments and the mortality of the insect. There was significant relationship between the mortality of the insect and dosages of extract and powder as the p-value of the calculated chi square was less than 0.05. Only 4.44 ml and 5.0 g dosages of the plant extract and powder were required to achieve 50% mortality of the beetle within 96 hours post treatment respectively. Furthermore, only 9.76 ml and 11.31 g of extract and powder of the botanical respectively were required to achieve 95% mortality of the insect within 96 hours post treatment.

Effect of Extract and powder of *M. charantia* on *C. maculatus* oviposition

Figure 1 presents the effect of extract and powder of M. charantia on adult C. maculatus. The oviposition of the insect decreases as the dosages of the extract and powder increases. There were significant (p<0.05) differences between the treatment and the control. There was significantly lowest oviposition of the bruchid on cowpea seeds treated with 0.8 ml extract than the oviposition in other treatments. The extract was more effective than powder as lesser number of eggs were laid on cowpea seeds treated with the extracts than what was observed on the powder-treated seeds and the untreated control.

DISCUSSION

The result from this study shows that ethanolic extract and powder of Mormodica charantia leaf have insecticidal properties against Callosobruchus maculatus. This shows that M. charantia could successfully be used for the control of C. maculatus. The extract from the plant were observed to be toxic on C. maculatus at various dosage of (0.2, 0.4, 0.6 and 0.8 ml), and the leaf powder at various dosage of (1 g, 2 g, 3 g and 4 g). On oviposition inhibitory effect, M. charantia extract proves more effective than M. charantia powder. Previous researchers have demonstrated that extracts/oils are relatively efficacious against virtually all life stages of insects (Don-Pedro, 1998, Adedire, 2003). The results from this research are similar to the observation of Adedire et al. (2011), who obtained 97.50% mortality of C. maculatus in cowpea seeds treated with acetone extract from cashew kernels at 0.5% v/w. Kayode and Obembe (2012) had also reported the effective protection of cowpea seeds against C. maculatus with aqueous extracts from seven tropical trees.
The finding is in conformity with the report of Yalamanchilli and Punukolu (2000) who observed that the oil from the leaves of Curcuma domestica could effectively protect cowpea seeds against C. chinensis at 2.0% concentration. Babarinde and Ewete (2008) also reported the toxicity and oviposition deterrence of three Khaya species against C. maculatus. The effectiveness of this botanical against C. maculatus can be due to the presence of active constituents of the plant (Asawalam et al., 2007). Shibu et al., (2017) reported that the secondary metabolites of M. charantia are alkaloids, flavonoids, tanins, saponins and disogenin. Since both M. charantia leaf powder and extract were toxic against C. maculatus, either of them can serve as alternatives to over-dependence on synthetic insecticides and can be recommended for use by local farmers for the protection of cowpea seeds meant for consumption and sales.

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