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Effects of Three Medicinal Plant Products on Survival, Oviposition and Progeny Development of Cowpea Bruchid, *Callosobruchus maculatus* (Fab.) [Coleoptera: Chrysomelidae] Infesting Cowpea Seeds in Storage

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Abstract

Powders from three medicinal plants were evaluated for their efficacy as contact and fumigant insecticides on cowpea bruchid, *Callosobruchus maculatus* (Fab.) in the laboratory at ambient tropical conditions of temperature and relative humidity. The plant powders tested included *Capsicum frutescens* L. (fruit and seed), *C. annum* Miller (fruit and seed) and *Citrus sinensis* Osbeck (peel). The powders were applied at rates 0.0 (control), 2g and 3.0g/20g of cowpea seeds either directly for contact with the insect pest or in plastic containers to assess fumigant toxicity of their volatiles. Results of contact toxicity assay showed that powders of *C. frutescens* and *C. annum* seeds were more effective against the adult *C. maculatus* evoking 100% mortality within 2 days of application at 3g/20g of cowpea seeds. There was no progeny development of the bruchid in samples treated with *Capsicum* species. The survival of the bruchid from eggs to adults when treated with the plant powders showed that there was significantly (P<0.05) more % progeny development in the control (69.32%) compared to others. However, the results of fumigant assays showed that *C. sinensis* had the highest insecticidal activity causing ~~2~~81.25% mortality of *C. maculatus* within 4 days of application at rate 3g/20g of cowpea seeds. This study showed that all the tested plant products were toxic to cowpea bruchid and the powders can be mixed with cowpea seeds to prevent hatching of the eggs thereby helping in their management.

Key Words: Insecticidal activity, progeny development, *Callosobruchus maculatus*, *Capsicum frutescens*, *Capsicum annum*, *Citrus sinensis*, cowpea seed.

1. Introduction

Cowpea, *Vigna unguiculata* (L.) Walp, belongs to the family Leguminosae, subfamily Papilionaceae and Tribe Phaseolae (Gbaye and Holloway, 2011). It is a legume widely cultivated in tropical and subtropical countries and largely produced in West Africa, Brazil and India. Cowpea is a staple component of the diet in several developing nations and a major source of protein to combat malnutrition in young children in lieu of expensive animal protein in such countries.

One of the major problems encountered in agriculture in developing countries is post harvest losses which usually occur during storage (Adedire *et al*., 2011). The cowpea bruchid, *Callosobruchus maculatus* has been recognized for decades as the major post harvest insect pest of cowpea seeds. It is a cosmopolitan species (Ofuya, 2001; Ileke and Bulus, 2012a). Initial infestation of cowpea starts in the field just before harvest and the insects are carried into the store where the population builds up rapidly (Appert, 1987; Ofuya, 2001; Ileke *et al*., 2012). The huge post-harvest losses and quality deterioration caused by this insect is a major obstacle to achieving food security in developing countries such as Nigeria. Annual production loss of 5% to *C. maculatus* in Nigeria alone would amount to 40,000 tonnes of cowpea seeds cost about $100 million (Singh and Ntare, 1985). The larvae are the major destructive stage because adult cowpea bruchid do not feed (Ofuya, 2001; Gbaye and Holloway, 2011).

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Efficient control of stored products insect pests has long been the aim of entomologists throughout the world. Synthetic insecticides have been used for many years to control stored product insect pests (Salem *et al*., 2007; Ashouri and Shayesteh, 2010). Development of resistance of insect pests to these conventional storage insecticides, increased concern by consumers over insecticide residues, ecological consequences and increasing cost of application call for new approaches to control stored products insect pests that are readily available, affordable and less detrimental to the environment (Adedire and Lajide, 2003; Udo, 2005; Ileke and Oni, 2011; Ashouri and Shayesteh, 2010; Ileke and Bulus, 2012b). A number of plants used locally for medicinal purposes, have also demonstrated potential as insect control agents (Arannilewa *et al.,* 2006; Oni, 2011). For example, cowpea seeds mixed with *Alstonia boone*i and *Eugenia aromatic* have been found to reduce infestation by cowpea beetle (Ofuya *et al*., 2007; Ileke *et al*., 2012). Plants such as *Capsicum frutescens, C. annum* fruit and *Citrus sinensis* peel have previously been shown to cause mortality of adult *Dasyses rugosella* in yam tuber (Ashamo, 2010). Oni (2011) examined contact toxicity of *Capsicum* species to adult *Sitophilus zeamais* and *C. maculatus.* In the present investigation, survival, oviposition and progeny development of cowpea bruchid, *C. maculatus* exposed to *Citrus sinensis* peel, *Capsicum frutescens* and *C. annum* fruit and seeds powders were evaluated.

2. Materials and Methods

This study was conducted in the Environmental Biology and Fisheries Research Laboratory, Faculty of Science, Adekunle Ajasin University, Akungba Akoko, Ondo State, Nigeria.

2.1. Insect culture

The insects used to establish a laboratory colony of *C. maculatus* came from a batch of infested cowpea seeds, *Vigna unguiculata* (L.) Walp variety Ife brown collected from Agricultural Development Project, Akure, Ondo State, Nigeria. Beetles were reared subsequently by replacement of devoured and infested cowpea seeds with fresh un-infested cowpea seeds in 2-L kilner jars covered with muslin cloth to allow air circulation. Insect rearing and the experiments were carried out at ambient temperature of 28±2^{o}C and 75±5% relative humidity.

2.2. *Plant collection*

The plants evaluated in this work were *Capsicum frutescens* (fruit and seed), *Capsicum annum* (fruit and seed) and *Citrus sinensis* (peel). They were obtained in fresh form, free of insecticides from Oja-Oba market, Akure, Ondo State, Nigeria and authenticated by the Plant Science and Technology Department of Adekunle Ajasin University, Akungba Akoko, Ondo State. These plant materials were rinsed in clean water to remove sand and other impurities, cut into smaller pieces before air dried in a well ventilated laboratory and ground into very fine powder using an electric blender. The powders were further sieved to pass through 1mm^2 perforations. The powders were packed in plastic containers with tight lids and stored in a refrigerator at 4^{o}C prior to use.

2.3. Collection of Cowpea Seeds

Cowpea seeds used for this study were obtained from a newly stocked seeds free of insecticides at Agricultural Development Program (ADP), Akure, Ondo State, Nigeria. Firstly, the seeds were cleaned and disinfested by keeping at -5^{o}C for 7 days to kill all hidden infestations. This is because all the life stages, particularly the eggs are very sensitive to cold (Koehler, 2003). The disinfested cowpea seeds were then placed inside a Gallenkamp oven (model 250) at 40^{o}C for 4 hours (Jambere *et al*., 1995) and later air dried in the laboratory to prevent mouldiness (Adedire *et al*., 2011) before they were stored in plastic containers with tight lids.

2.4. Effect of contact toxicity of plants powders on adult mortality, oviposition and progeny development of Callosobruchus maculatus

Fine powders of *Capsicum frutescens, Capsicum annum, Citrus sinensis* were admixed with cowpea seeds at the rates of 2 and 3g /20g of cowpea seeds in 250ml plastic containers. Ten pairs of adult *C. maculatus* (2 to 3 days old) sexed according to the methods described by Halstead (1963); Appert (1987); Odeyemi and Daramola (2000) were introduced into the treated. Male *C. maculatus* have comparative shorter abdomen and the dorsal side of the terminal segment is sharply curved downward and inward. In contrast the females have comparatively longer abdomen and the dorsal side of the terminal segment is only slightly bent downward. The female also has two dark visible spots on their elytra (Odeyemi and Daramola, 2000). Untreated cowpea seeds were similarly infested. Four replicates of the treated and untreated controls were laid out in Complete Randomized Block Design in insect cage. Insect mortality was assessed every 24 hours for four days. Adults were assumed dead when probed with sharp objects and made no responses. At the end of day 4, all insects, both dead and alive were removed from each container. The experiment was kept inside the insect cage for another 30 days to allow for the emergence of the first filial (F_1) generation. The number of adults that emerged from each replicate was counted with an aspirator and recorded. The percentage adult emergence was then calculated using the method described by Odeyemi and Daramola (2000).

$$\% \text{ Progeny development} = \frac{\text{No of adult emerged}}{\text{No of eggs laid}} \times \frac{100}{1}$$

2.5. Fumigant effect of plants powders on adult mortality, oviposition and progeny development of C. maculatus

Ten grams of the cowpea seeds were weighed into 50ml transparent plastic tubes that had been cut opened at the bottom and sealed with muslin cloth. Fine powders of *Capsicum frutescens, Capsicum annum, Citrus sinensis* weighing 2g and 3g concentrations were put into another half-cut 25ml plastic tubes. The 50ml tube and 25ml tube were then joined together with the aid of gum (Ileke and Bulus, 2012a). Ten pairs of adult *C. maculatus* (2 to 3 days old) sexed according to the methods described above were introduced to the tube containing 10g of cowpea seeds and tightly sealed (Ileke and Bulus, 2012a). Untreated cowpea seeds were similarly infested. Four replicates of the treated and untreated controls were laid out in Complete Randomized Block Design in insect cage.

Bruchid mortality was assessed every 24 hours for four days. Adults were assumed dead when probed with sharp objects and made no responses. At the end of day 4, all insects, both dead and alive were removed from each container. The experiment was kept inside the insect cage for another 30 days to allow for the emergence of the first filial (F_1) generation. The number of adults that emerged from each replicate was counted with an aspirator and recorded. Percentage adult emergence was calculated as described above.

2.6. Statistical analysis

Data were subjected to analysis of variance and where significant differences existed, treatment means were separated using the Tukey's test.

3. Results

3.1. Effectiveness of plants powders as contact insecticides

The effectiveness of the various plant powders on the survival of cowpea bruchid, *C. maculatus* at different periods after treatment is presented in Tables 1 and 2. The results revealed that in each treatment, the mortality of *C. maculatus* increased gradually with time of exposure. Chilly pepper, *C. frutescens* seed powder caused 100% mortality of *C. maculatus* at rate 2g/20g of cowpea seeds within 2 days of exposure (Table 1). The corresponding value for *C. sinensis, C. frutescens* fruit and *C. annum* fruit and seed powders were 34.5%, 87.5%, 51.25% and 71.25% mortality of adult cowpea bruchid respectively. The contact toxicities of these plant powders increased with increase in dosage as well as increase in the period of exposure to plant powders (Table 2). At rate 3g/20g of cowpea seeds, 100% mortality was obtained in sample treated with *C. frutescens* seed at 1 day after application of powder. The results indicated that various plant powders tested as contact insecticides significantly (P<0.05) reduced number of tested insect. In general, *Capsicum* species seeds powders were more toxic than other tested plant powders.

Table 1. Percentage mortality of adult *Callosobruchus maculatus* treated with various powders at rate 2g/20g of cowpea seeds for contact toxicity.

Powder	Mortality % ± SE mean after			
	1 day	2 day	3 day	4 day
Citrus sinensis (p)	17.25± 1.44b	34.50± 1.44b	68.25± 2.39b	80.00± 1.25b
Capsicum frutescens (f)	45.00± 2.04c	87.50± 5.20e	100.00± 0.00c	100.00± 0.00c
Capsicum frutescens (s)	70.00± 1.25d	100.00± 0.00f	100.00± 0.00c	100.00± 0.00c
Capsicum annum (f)	20.00± 4.05b	51.25± 2.39c	100.00± 0.00c	100.00± 0.00c
Capsicum annum (s)	41.25± 2.39c	71.25± 2.39d	100.00± 0.00c	100.00± 0.00c
Control	0.00± 0.00a	0.00± 0.00a	0.00± 0.00a	0.00± 0.00a

Each value is a mean ± standard error of four replicates. Means within the same column followed by the same letter(s) are not significantly different at P>0.05 using Tukey's test.
Keys: f- fruit, s - seed, p – peel.

Table 2. Percentage mortality of adult *Callosobruchus maculatus* treated with various powders at rate 3g/20g of cowpea seeds for contact toxicity.

Powder	Mortality % ± SE mean after			
	1 day	2 day	3 day	4 day
Citrus sinensis (p)	28.75± 1.25b	47.50± 1.44b	78.75± 1.25b	88.75± 1.25b
Capsicum frutescens (f)	50.00± 1.25c	71.25± 2.39c	100.00± 0.00c	100.00± 0.00c
Capsicum frutescens (s)	100.00± 0.00e	100.00± 0.00d	100.00± 0.00c	100.00± 0.00c
Capsicum annum (f)	32.75± 1.25b	60.00± 1.25c	100.00± 0.00c	100.00± 0.00c
Capsicum annum (s)	71.75± 1.25d	100.00± 0.00d	100.00± 0.00c	100.00± 0.00c
Control	0.00± 0.00a	0.00± 0.00a	0.00± 0.00a	0.00± 0.00a

Each value is a mean ± standard error of four replicates. Means within the same column followed by the same letter(s) are not significantly different at P>0.05 using Tukey's test.
Keys: f- fruit, s-seed, p - peel

3.2. Fumigant effect of various plant powders

Table 3 and 4 showed the fumigant effect of various plant powder against *C. maculatus*. At day 4 after post treatment, *C. sinensis* was able to cause 66.75% mortality of adult *C. maculatus* while the corresponding values for *C. frutescens* and *C. annum* seeds powders were 55% and 40% mortality of bruchid respectively at 2g/20g of cowpea seeds. (Table 3). The toxicities of these plant powders to cowpea bruchid increased with an increase in concentration and period of exposure to plant powders. *C. sinensis* powder caused 81.25% mortality of *C. maculatus* while the corresponding value for *C. frutescens* seed powder was 72.5% mortality of *C. maculatus* at rate 3g/20g of cowpea seeds (Table 4).

Table 3. Percentage mortality of adult *Callosobruchus maculatus* treated with various powders at rate 2g/20g of cowpea seeds for fumigant toxicity.

Powder	Mortality % ± SE mean after			
	1 day	2 day	3 day	4 day
Citrus sinensis (p)	20.00± 1.25b	238.75± 1.25c	45.00± 2.04d	66.75± 1.25d
Capsicum frutescens (f)	0.00± 0.00a	0.00± 0.00a	10.00± 1.25b	20.00± 1.25b
Capsicum frutescens (s)	10.00± 1.25b	22.50± 1.44b	31.25± 1.25c	55.00± 2.04d
Capsicum annum (f)	0.00± 0.00a	0.00± 0.00a	0.00± 0.00a	12.50± 1.44b
Capsicum annum (s)	0.00± 0.00a	10.00± 1.25b	20.00± 1.25bc	40.00± 1.25c
Control	0.00± 0.00a	0.00± 0.00a	0.00± 0.00a	0.00± 0.00a

Each value is a mean ± standard error of four replicates. Means within the same column followed by the same letter(s) are not significantly different at P>0.05 using Tukey's test.
Keys: f- fruit, s-seed, p – peel.

Table 4. Percentage mortality of adult *Callosobruchus maculatus* treated with various powders at rate 3g/20g of cowpea seeds for fumigant toxicity.

Powder	Mortality % ± SE mean after			
	1 day	2 day	3 day	4 day
Citrus sinensis (p)	45.00± 2.04c	67.50± 2.04d	78.75± 1.25e	81.25± 2.39d
Capsicum frutescens (f)	0.00± 0.00a	12.50± 1.44b	25.00± 1.25c	31.25± 2.39b
Capsicum frutescens (s)	20.00± 1.25b	38.75± 1.25c	51.25± 2.39d	72.50± 3.15cd
Capsicum annum (f)	0.00± 0.00a	0.00± 0.00a	8.75± 1.25b	20.00± 1.25b
Capsicum annum (s)	8.75± 1.25b	20.00± 1.25b	38.75± 1.2d	62.00± 1.25c
Control	0.00± 0.00a	0.00± 0.00a	0.00± 0.00a	0.00± 0.00a

Each value is a mean ± standard error of four replicates. Means within the same column followed by the same letter(s) are not significantly different at P>0.05 using Tukey's test.
Keys: f- fruit, s-seed, p - peel

3.3. Effect of various pant powders applied as contact and fumigant insecticides on oviposition and progeny development of C. maculatus

Table 5 shows the oviposition and percentage progeny development of *C. maculatus* after being exposed to various plant powders as contact insecticide at two concentrations after 4 days. Progeny development was significantly suppressed by various plant powders with *Capsicum* species and completely inhibited the emergence of *C. maculatus* (100% efficiency).

Table 5. Fecundity of *Callosobruchus maculatus* treated with various plant powders as contact insecticides

Plant Powder	2g/20g of cowpea seeds No of egg laid	% no of Progeny development	3g/20g of cowpea seeds No of egg laid	% no of Progeny development
Citrus sinensis (p)	36.25± 1.70c	15.84	31.25± 2.39c	9.60
Capsicum frutescens (f)	10.50± 1.32b	0.00	2.75± 1.25ab	0.00
Capsicum f rutescen (s)	0.00± 0.00a	0.00	0.00± 0.00a	0.00
Capsicum annum (f)	12.75± 2.02b	0.00	7.25± 2.39b	0.00
Capsicum annum (s)	0.00± 0.00a	0.00	0.00± 0.00a	0.00
Control	88.75± 1.25d	69.32	88.75± 1.25d	69.32

Each value is a mean ± standard error of four replicates. Means within the same column followed by the same letter(s) are not significantly different at P>0.05 using Tukey's test.
Keys: f- fruit, s-seed, p - peel

In Table 6, fecundity of *C. maculatus* showed that more eggs were laid on samples treated with plants powders applied as fumigant. Oviposition and % progeny development were high in cowpea seeds treated with *Capsicum* species fruit powders.

Table 6. Fecundity of *Callosobruchus maculatus* treated with various plant powders as fumigant insecticides

Plant Powder	2g/20g of cowpea seeds No of egg laid	% no of Progeny development	3g/20g of cowpea seeds No of egg laid	% no of Progeny develop ment
Citrus sinensis (p)	11.25± 2.39a	11.33	9.50± 3.15a	10.32
Capsicum frutescens (f)	38.75± 1.25bc	32.47	30.00± 2.04bc	28.76
Capsicum frutescens (s)	25.00± 2.04ab	21.67	12.00± 0.00a	19.49
Capsicum annum (f)	41.25± 2.39c	35.86	37.50± 3.15c	31.11
Capsicum annum (s)	32.50± 3.15bc	27.17	21.25± 2.39ab	24.58
Control	77.50± 1.44d	80.00	77.50± 1.44d	80.00

Each value is a mean ± standard error of four replicates. Means within the same column followed by the same letter(s) are not significantly different at P>0.05 using Tukey's test.
Keys: f- fruit, s-seed, p - peel

4. Discussion

Results reported in this study show that *Capsicum* species and *C. sinensis* powders have insecticidal effects on cowpea bruchid, *C. maculatus* at all levels of treatment but varied with the method of application, exposure period and plant powder concentrations. The two *Capsicum* species seed and fruit powders applied as contact insecticides were very effective against *C. maculatus* causing 100% mortality of adult *C. maculatus* at rate 3g/20g of cowpea seeds within 4 days of application. They also reduced oviposition and completely inhibited progeny development. This shows that *Capsicum* species probably have oviposition deterrent, ovicidal and lavicidal properties. The observed activity may be due to the "pepperich" nature and pungency of the *Capsicum* species (Ashamo, 2010). The pungency of *Capsicum* species was attributed to capsacin (Miyakado *et al*., 1979; Ashamo, 2010). This result is in agreement with the results of Ivbijaro and Agbaje (1986), and Asawalam *et al*. (2007). They both found that *C. frutescens* considerably reduced all stages of *C. maculatus*. The result of this investigation are also similar to the observation of Ashamo (2010) who obtained 100% mortality of adult *Dasyses rugosella* in yam tuber treated with powders and oils of *Capsicum* species. Oni (2011) reported that *Capsicum* species seeds and fruits powders significantly toxic to *Sitophilus zeamais* and *C. maculatus* in stored maize and cowpea seeds, respectively.

The high mortality and low progeny development caused by the powder of *C. sinensis* can be attributed to strong choky odour disrupting respiratory activity of the beetles. Sweet orange peel powders may probably have the same insecticidal properties when applied as contact and fumigant. The results obtained from this study agreed with those reported by Don Pedro (1996a; b) in studies with six *Citrus* species peel oils against *C. maculatus, S. zeamais* and *Dermestes maculatus*. He reported on fumigant action of toxic vapour of *Citrus* species peel oils against *C. maculatus, S. zeamais* and *Dermestes maculatus*.

In this study, the lethal effect of tested plant powders on cowpea bruchid could be as a result of contact toxicity. Insects breathe by means of trachea which usually opens at the surface of the body through spiracles (Adedire *et al*., 2011). These spiracles might have been blocked by the powders thereby leading to suffocation. The powders also prevented oviposition and progeny development when applied as contact insecticides. The choky effect of these powders also disrupt mating activities, sexual communication and inhibit locomotion an effect that have been reported by many researchers (Ofuya, 1992; Adedire 2002; Maina and Lale, 2004; Akinkurolere *et al*., 2006; 2009; Adedire *et al*., 2011; Ileke *et al*., 2012).

Adult bruchids do not feed on stored cowpea seeds but only deposit their eggs. Thus, the use of oviposition inhibitors would be advantageous for the management of cowpea bruchids. The powders of these plants could be mixed with stored cowpea seeds before storage.

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