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**Insecticidal Potential of Indigenous Plant Powders Against Beetle, *Callosobruchus maculatus* (F.)(Coleoptera:Chrysomelidae) in Stored Cowpea**

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**Abstract**

Experiments were carried out on the potentials of bitter leaf, cashew leaf, sweet orange peel and pawpaw leaf powders in the control of bean beetle, *Callosobruchus maculatus* (F.)(Coleoptera:Chrysomelidae), under laboratory conditions of  $28 \pm 3^{\circ}\text{C}$  temperature and  $71 \pm 4\%$  relative humidity. The plant powders were tested at the concentrations of 0.0, 2.5, 5.0 and 7.5% (w/w) on cowpea grains. The experiments were laid in completely randomized design with four replications. At 24 and 48 hours post-infestation, 5.0 and 7.5% concentrations of pawpaw leaf and orange peel powders caused significant difference ( $p < 0.05$ ) in adult mortality when compared with the untreated control. Grains treated with highest concentration of the plant powders prevented oviposition and progeny emergence of *C. maculatus* and reduced grain damage. It was found that bitter leaf powder was the most effective against the bean beetle in small scale storage.

**Keywords:** Botanicals, *Callosobruchus maculatus*, emergence, oviposition, grain damage

## Introduction

Cowpea, *Vigna unguiculata* (L.) Walp, is a household name in Nigeria where it is called ``wake`` or ``ewa`` and consumed by virtually every household on daily basis in different forms (Osipitanet *al.*, 2013) such as ``moin-moin`` and ``akara``. It is rich in amino-acids, especially lysine and tryptophan (Wudilet *al.*, 2013). Cowpea grains contain about 25% protein and the ability of cowpea plants to tolerate drought and poor soils make it an important crop in the savannah regions where these constraints restrict other crops (IITA, 2004). The preservation of cowpea grains is constrained by a number of factors which include insect infestation.

Bean beetle, *Callosobruchus maculatus* (F.)(Coleoptera:Chrysomelidae), is definitely a great threat to cowpea grains preservation in the tropics. The larvae and adults of the insect are responsible for cowpea grain damage. The use of synthetic

insecticides for controlling stored-product insects is associated with problems such as their persistent toxicity in grains, development of resistance in insect populations and effects on non-target organisms (Hammanet *al.*, 2012; Iramet *al.*, 2013). In recent years, concern about the consequences of the drawbacks associated with the use of synthetic insecticides necessitates the need to evaluate plants and their products against stored-product insects. Akinneyeet *al.* (2006) reported that plant materials and local traditional methods are much safer than chemical insecticides and suggested that their use needed exploitation. Ojo and Ogunleye (2014) showed that cowpea seeds mixed with plant powders would fill intergranular air spaces and prevent free movement of adults for mating and oviposition. Orange peel (containing volatile oils) is burned at night to drive off mosquitoes (Don-Pedro, 1985). The present study focused on the insecticidal

potentialsof powders of mature bitter leaf, *Vernoniaamygdalina*Dileli (Compositae), cashew, *Anacardiumoccidentale*L. (Anacardiaceae) leaf, sweet orange, *Citrus sinensis* L. (Rutaceae) peel and pawpaw, *Asiminatriloba* (L.) Dunal (Annonaceae) leaf as grain protectants for reduction of postharvest damage caused by bean beetle, *C. maculatus* in stored cowpea.

## Materials and Methods

### Insect culture

Fifty pairs of *C. maculatus*adults were picked from existing stock in the Crop Protection laboratory, University of Ilorin, Nigeria and used to infest cowpea grains in a 500 ml Kilner jar. After 7 days, the insects were sieved from the grains and fresh adults that emerged later were used for the study. The culture was maintained at ambient temperature of  $28 \pm 3^{\circ}\text{C}$  and relative humidity of  $71 \pm 4\%$ .Two pairs (sex ratio of 1:1) of freshly emerged adults (1-2 days old) were used fortheexperiment.

### Collection and preparation of plant powders

Mature bitter leaf, *V. amygdalina*, cashew, *A. occidentale*,leaves, sweet orange, *C. sinensis*, peels and pawpaw, *A. triloba*, leaves were removed from their parent plants at various locations in Ilorin, Nigeria. These plant parts were washedthoroughly in running tap water, rinsed in distilled water and air-dried for 5 days.The dried plant parts were ground separately using an electric blender and sieved through a 40 holes/mm<sup>2</sup> mesh sieve to obtain fine powders.The plant powders were stored in separate plastic containers until required for use.

### Source and preparation of cowpea grains

Cowpea grains (variety IT96k-610) were obtained from the International Institute of Tropical Agriculture (IITA), Ibadan, Nigeria in February 2014. The grains were wrapped in a transparent polyethylene bag kept in a deep freezer at  $4^{\circ}\text{C}$  for 7 days to

free the grains from any insidious infestation. The cowpea grains were allowed moisture equilibration under laboratory conditions before they were used for the experiment.

### Sex determination

The method of Blumer and Beck (2008) was adopted in identifying the sexes. The female beetle has enlarged and dark plate covering the end of the abdomen on both sides while the male beetle has smaller plate which lacks stripes.

### Experimental procedure

Bitter leaf, cashew leaf, sweet orange peel and pawpaw leaf powders were mixed with cowpea grains at the concentrations of 2.5, 5.0 and 7.5% (w/w) in plastic containers (8 cm diameter). Each container was thoroughly shaken to ensure effective mixing of the grains with the powders before infesting with two pairs (1:1) of freshly emerged adults (1-2 days old) of *C. maculatus*. The open ends of the containers were covered with muslin material to

allow air circulation and prevent insect escape. The treated grains and an untreated control were arranged in completely randomized design in four replicates, including the control. The laboratory experiment was maintained at ambient temperature of  $28 \pm 3^{\circ}\text{C}$  and relative humidity of  $71 \pm 4\%$ .

### Data collection

The mean number of dead beetles per treatment was calculated by dividing total number of dead beetles by the number of replicates at 24 and 48 hours post infestation (HPI). The mean number of eggs per treatment was estimated by dividing total number of eggs laid on the surface of twenty randomly selected grains by the number of replicates at 5 days post infestation (DPI). The method of Asawalam and Onu (2014) was adopted with little modification to determine the mean number of progeny emergence. All dead and live insects were removed and discarded at 21 days after infestation to avoid overlap with  $F_1$  progeny emergence.

The grains were returned to their respective containers. The freshly emerged adults were recorded at 27 and 32 days post infestation (DPI) and the mean number of emerged adults was estimated.

### **Grain damage**

The mean number of grains damaged by the beetle was computed before the experiment was terminated at three months post infestation (MPI).

### **Data analysis**

Data collected were subjected to analysis of variance (ANOVA) using Gen-stat Statistical Package (Discovery Edition 3). Significantly different means were separated using Least Significant Difference at  $p=0.05$  level of significance.

### **Results**

#### **Effect on adult mortality**

Table 1 shows the insecticidal effects of the test plant powders on mean adult mortality of *C. maculatus* at varying degrees of potency. At 24 HPI, mortality

of *C. maculatus* adults was significantly ( $p<0.05$ ) higher on cowpea grains treated with 5.0% concentration of pawpaw leaf powder compared with the bitter leaf and cashew leaf powders. During the same period, there was no significant effect ( $p>0.05$ ) of treatment with bitter leaf and cashew leaf powders compared with the orange peel powder at 5.0 and 7.5% concentrations. Lower concentration (2.5%) of the plant powders had no significant effect on adult mortality when compared with the untreated control which had zero mortality during the period.

At 48 HPI, mortality of *C. maculatus* adults was significantly ( $p<0.05$ ) higher on cowpea grains treated with orange peel and pawpaw leaf powders compared with the bitter leaf and cashew leaf powders at 5.0% concentration (Table 1). There was significant effect in the mean adult mortality recorded in the 2.5% concentration of bitter leaf powder when compared to the zero mortality recorded in the untreated control. Also, at 48 HPI,

adult mortality of *C. maculatus* was significantly ( $p < 0.05$ ) higher for cowpea grains treated with pawpaw leaf powder compared with cashew leaf and orange peel powders at 7.5% concentration. The untreated control produced no adult mortality of *C. maculatus* during the investigation.

### **Effect on oviposition**

Table 2 shows the effect of different plant powders on oviposition of *C. maculatus*. The untreated control had significantly ( $p < 0.05$ ) higher mean number of eggs laid than the treated grains at all concentrations of bitter leaf, cashew leaf, orange peel and pawpaw leaf powders. No oviposition from the cowpea grains treated with 7.5% concentration of the plant powders. Results showed that the mean number of eggs laid in the grains treated with 5.0% concentration of pawpaw leaf powder and in the untreated control occurred in the proportion of 1:4 at 5 (DPI). The plant powders applied at lower concentrations gave significant ( $p < 0.05$ )

reduction in mean number of eggs laid on the cowpea grains while the higher concentration of 7.5% of the plant powders prevented oviposition.

### **Effect on progeny emergence**

Table 3 shows the mean progeny emergence of *C. maculatus* in grains treated with different plant powders. There was significantly higher ( $p < 0.05$ ) progeny emergence in the untreated control than the grains treated with plant powders. The number of insects was mostly higher in the lower concentration than the higher concentrations. Cowpea grains treated with all concentrations of bitter leaf powder produced no progeny emergence at 27 DPI. No progeny emergence on grains treated with all the powders at 7.5% concentration. Cowpea grains treated with cashew leaf powder at all concentrations were statistically the same in reducing the mean number of progeny emergence on the grains at 27 and 32 DPI. Cashew leaf powder applied at 2.5 and 5.0% concentrations also produced highest

number of progeny at 32 DPI. The performance of bitter leaf powder in either inhibiting progeny emergence at 27 DPI or significantly reducing the progeny emergence at 32 DPI, was better than other plant powders used. Although the plant powders applied at 2.5 and 5.0% concentrations were not as effective as the highest concentration of 7.5%, they were however, numerically better than the untreated control. The grains treated with 2.5% concentration of pawpaw leaf powder had the higher mean progeny emergence than 7.5% concentration which had zero mortality.

### **Effect on grain damage**

Table 4 shows the mean grain damage due to *C. maculatus* infestation in treated and untreated cowpea grains. Statistical

analysis showed that there was significant difference in the mean grain damage among the treated grains compared with the untreated grains. At 3 months post infestation (MPI), the mean grain damage recorded in 2.5 and 7.5% concentrations of cashew leaf powder was significantly ( $p < 0.05$ ) different from the mean grain damage recorded in the untreated control. Mean grain damage in the plant powders except pawpaw leaf powder decreased with increase in concentration of the plant powders. Cowpea grains treated with 5.0 and 7.5% concentrations of the bitter leaf and orange peel powders had significantly ( $p < 0.05$ ) lower mean grain damage than the mean grain damage recorded in other plant powders and the untreated control.

Table 1: Mean mortality of *Callosobruchus maculatus* adults on cowpea grains treated with different plant powders

Plant powders	Mean mortality of <i>C. maculatus</i> adults (24 HPI)			Mean mortality of <i>C. maculatus</i> adults (48 HPI)		
	Concentration of plant powders (%)			Concentration of plant powders (%)		
	2.5	5.0	7.5	2.5	5.0	7.5
Bitter leaf	0.25 <sup>a</sup>	0.50 <sup>bc</sup>	1.50 <sup>ab</sup>	1.00 <sup>a</sup>	1.50 <sup>b</sup>	2.75 <sup>ab</sup>
Cashew leaf	0.00 <sup>a</sup>	0.25 <sup>bc</sup>	0.75 <sup>bc</sup>	0.25 <sup>ab</sup>	1.00 <sup>bc</sup>	1.25 <sup>c</sup>
Orange peel	0.25 <sup>a</sup>	1.00 <sup>ab</sup>	1.25 <sup>ab</sup>	0.50 <sup>ab</sup>	4.00 <sup>a</sup>	2.50 <sup>b</sup>
Pawpaw leaf	0.50 <sup>a</sup>	1.50 <sup>a</sup>	2.00 <sup>a</sup>	1.00 <sup>a</sup>	3.00 <sup>a</sup>	3.25 <sup>a</sup>
Control	0.00 <sup>a</sup>	0.00 <sup>c</sup>	0.00 <sup>c</sup>	0.00 <sup>b</sup>	0.00 <sup>c</sup>	0.00 <sup>d</sup>
<b>LSD<sub>(0.05)</sub></b>	<b>0.62</b>	<b>1.00</b>	<b>0.83</b>	<b>0.75</b>	<b>1.17</b>	<b>0.70</b>
<b>S.E.M</b>	<b>0.37</b>	<b>1.08</b>	<b>0.94</b>	<b>0.63</b>	<b>2.00</b>	<b>2.34</b>

Values with the same superscript (s) in the same column are not significantly different at p=0.05 using Least Significant Difference

HPI=Hours post infestation



Table 2: Mean oviposition of *Callosobruchus maculatus* on cowpea grains treated with different plant powders

Plant powders	Oviposition of <i>C. maculatus</i> (5DPI)		
	Concentration of plant powders (%)		
	2.5	5.0	7.5
Bitter leaf	9.75 <sup>b</sup>	9.25 <sup>b</sup>	0.00 <sup>b</sup>
Cashew leaf	9.25 <sup>b</sup>	8.75 <sup>b</sup>	0.00 <sup>b</sup>
Orange peel	9.25 <sup>b</sup>	9.25 <sup>b</sup>	0.00 <sup>b</sup>
Pawpaw leaf	9.00 <sup>b</sup>	8.00 <sup>b</sup>	0.00 <sup>b</sup>
Control	23.00 <sup>a</sup>	32.75 <sup>a</sup>	19.50 <sup>a</sup>
<b>LSD<sub>(0.05)</sub></b>	<b>1.92</b>	<b>6.19</b>	<b>3.55</b>
<b>S.E.M</b>	<b>11.95</b>	<b>19.14</b>	<b>15.57</b>

Values with the same superscript in the same column are not significantly different at  $p=0.05$

using Least Significant Difference

DPI=Days post infestation

Table 3: Mean progeny emergence of *Callosobruchus maculatus* adults on cowpea grains treated with different plant powders

Plant powders	Mean emergence of <i>C. maculatus</i> adults (27 DPI)			Mean emergence of <i>C. maculatus</i> adults (32 DPI)		
	Concentration of plant powders (%)			Concentration of plant powders (%)		
	2.5	5.0	7.5	2.5	5.0	7.5
Bitter leaf	0.00 <sup>c</sup>	0.00 <sup>b</sup>	0.00 <sup>b</sup>	0.25 <sup>e</sup>	2.50 <sup>d</sup>	0.00 <sup>b</sup>
Cashew leaf	2.50 <sup>b</sup>	2.50 <sup>b</sup>	0.00 <sup>b</sup>	23.25 <sup>b</sup>	23.25 <sup>b</sup>	0.00 <sup>b</sup>
Orange peel	2.50 <sup>b</sup>	2.25 <sup>b</sup>	0.00 <sup>b</sup>	11.75 <sup>c</sup>	2.50 <sup>d</sup>	0.00 <sup>b</sup>
Pawpaw leaf	7.00 <sup>a</sup>	2.50 <sup>b</sup>	0.00 <sup>b</sup>	10.25 <sup>cd</sup>	11.25 <sup>c</sup>	0.00 <sup>b</sup>
Control	8.00 <sup>a</sup>	9.25 <sup>a</sup>	8.00 <sup>a</sup>	36.50 <sup>a</sup>	49.00 <sup>a</sup>	33.75 <sup>a</sup>
<b>LSD<sub>(0.05)</sub></b>	<b>1.28</b>	<b>4.31</b>	<b>1.83</b>	<b>9.18</b>	<b>3.43</b>	<b>8.03</b>
<b>S.E.M</b>	<b>5.93</b>	<b>7.25</b>	<b>6.92</b>	<b>22.73</b>	<b>7.31</b>	<b>24.11</b>

Values with the same superscript (s) in the same column are not significantly different at  $p=0.05$  using Least Significant Difference

DPI=Days post infestation

Table 4: Grain damage caused by *Callosobruchus maculatus* in stored cowpea grains

Plant powders	Mean grain damage caused by <i>C. maculatus</i> (MPI)		
	Concentration of plant powders (%)		
	2.5	5.0	7.5
Bitter leaf	5.75 <sup>b</sup>	5.25 <sup>c</sup>	5.25 <sup>c</sup>
Cashew leaf	54.50 <sup>a</sup>	53.25 <sup>b</sup>	44.75 <sup>a</sup>
Orange peel	11.00 <sup>b</sup>	8.75 <sup>c</sup>	6.25 <sup>c</sup>
Pawpaw leaf	20.00 <sup>b</sup>	52.75 <sup>b</sup>	24.25 <sup>b</sup>
Control	60.50 <sup>a</sup>	72.00 <sup>a</sup>	46.25 <sup>a</sup>
<b>LSD<sub>(0.05)</sub></b>	<b>21.31</b>	<b>11.10</b>	<b>9.40</b>
<b>S.E.M</b>	<b>45.34</b>	<b>53.07</b>	<b>35.53</b>

Values with the same superscript (s) in the same column are not significantly different at  $p=0.05$  using Least Significant Difference

MPI=Months post infestation

## DISCUSSION

All the plant powders were observed to have insecticidal effect on *C. maculatus* adults, which increased with increase in the concentrations of the powders used. Danjumaet *al.* (2009) had earlier reported that increase in concentration of *Nicotiana tabacum*,

*Allium sativum* and *Zingiber officinale* from 0.5 to 1.0 g resulted in an increase in the mortality of *Sitophilus zeamais* to 96.67% within 6-7 days of their introduction to maize. In this report, cashew leaf powder was the least effective among the powders used, while pawpaw leaf powder was observed to be the most effective in causing higher mortality among *C.*

*maculatus* adults at 5.0 and 7.5% concentrations within 48 HPI. The insecticidal activity of pawpaw (*V.amygdalina*) leaf powder might be attributed to the report of Jewel (2008), that the plant possesses toxic properties. Adeniyet *al.* (2010) had earlier reported that crude extracts of *V.amygdalina* leaf at 4.0% concentration resulted in higher toxicity to *Acanthoscelides obtectus*. Cowpea grains treated with pawpaw leaf powder reduced progeny emergence when the concentration was increased from 2.5 to 5.0% concentrations. Bitter leaf powder was most effective in preventing adult emergence of *C. maculatus* while pawpaw leaf powder had the least effect on progeny emergence at 2.5 and 5.0% concentrations.

The study revealed that all the plant powders applied to the grains reduced grain damage when exposed to *C. maculatus*. The reduction in grain damage could be attributed to the varying degrees of potency of the powders in reducing

progeny emergence and increasing adult mortality. Among the plant powders studied, bitter leaf powder was observed to have caused the least grain damage indicating that it was more effective against *C. maculatus* than other plant powders. Kabeh and Jalingo (2007) reported that bitter leaf contained hydrocyanic acid and oxalic acid. The insecticidal potential of the plant powders could be due to the concentration, type of plant powder and exposure period. In this study, significant differences were observed on adult mortality and progeny emergence of *C. maculatus* on treated and untreated grains, indicating that the plant powders had significant effect on the behaviour of the insect. This study has shown that coating the cowpea grains with plant powders is effective in reducing post-harvest damage. It was observed that the abrasive action of the plant powders may have been responsible for lower number of eggs laid by the insect (Belmain and Stevenson (2001). The resultant high

mortality of adult *C. maculatus* observed on cowpea seeds treated with plant powders could be due to contact toxicity resulting in blockage of the spiracles (Steve, 2010).

The insecticidal potential of sweet orange peel has been implicated in adult mortality of *C. maculatus* and in reducing grain damage. Heaps of orange peels can be converted for use as protectant against pests, thereby solving the problem of environmental pollution caused by the waste as observed by Emeasor and Okorie (2008). Orange peels contain secondary metabolites that show insecticidal activity against several coleopterans and dipterans (Belmain and Stevenson, 2001; Salvatore *et al.*, 2004; Shrivastava *et al.*, 2010). Morawej and Abbar (2008) reported that fumigant toxicity of the orange peel oil of *C. sinensis* against stored product insect pests.

It has been observed that mechanical effects of large quantities of plant powders (Rajapakse, 2006), the active ingredients of the powders and physiological processes of the beetle could have effect on oviposition. Insect spiracles may have been blocked by the powders (Komabonta and Falodu, 2013) and thereby causing suffocation. Previous researchers showed that when plant powders and their extracts were mixed with grains in storage, there appeared to be oviposition inhibition and suppressed adult emergence and reduced seed damage caused by stored product insects (Bakkaliet *al.*, 2008; Tripathiet *al.*, 2009). The high mortality observed among *C. maculatus* adults on grains treated with pawpaw leaf powder showed that pawpaw leaf powder is a promising control agent against *C. maculatus* suggesting its potential for its future use against *C. maculatus* as botanical insecticides.

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