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Comparative toxicities of *Zingiber officinale*, *Eugenia aromatica* and *Piper nigrum* powders on *Callosobruchus maculatus* (Coleoptera: Chrysomelidae)

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ABSTRACT
Toxicity of three plants powders; Zingiber officinale, Eugenia aromatica and Piper nigrum, on adult Callosobruchus maculatus was conducted under laboratory conditions. Plant powders at 2.5, 5.0, 7.5, and 10.0 g per 100g of cowpea seeds were infested with 20 insects/replicate in transparent plastic bowls and observed for mortality at 24, 48, 72 and 96 hours after treatment under ambient laboratory conditions. The Lethal Concentration 50% (LC_{50}) was determined. Residual efficacy of the powders on F_1 and F_2 progenies of C. maculatus was also evaluated. Powder of E. aromatica at 2.5g/100g seeds was the most toxic to adult C. maculatus with 61.65% and 90.00% mortalities after 24 and 48 hours, respectively. At 5.0g/100g seeds of E. aromatica, 100.00% mortality was observed and it was significantly higher than mortalities observed on Z. officinale (5.00%) and P. nigrum (26.65%) after 48 hours. The LC_{50} values showed that E. aromatica was 322 times more toxic than Z. officinale within 24 hours whereas P. nigrum did not cause mortality at that time. There was no C. maculatus progeny emergence and development in cowpea treated with E. aromatica after three months of storage. Zingiber officinale and P. nigrum, had 177.67 and 85.67 F_1 progeny emerged at 10.0g/100g cowpea compared with 294.67 and 222.33 that emerged in the control, respectively. This study showed that E. aromatica powder extract at 2.5g/100g cowpea seeds was more effective than Z. officinale and P. nigrum in controlling C. maculatus on cowpea in storage.

Key words: Bean beetle, *Zingiber officinale*, *Eugenia aromatica*, *Piper nigrum*, LC_{50}, *C. maculatus* progeny emergence and residual bioactivity

INTRODUCTION

Insect pests are among the most important factors limiting successful production and storage of cowpea in several cowpea-growing areas of the world (Belmam and Stevenson, 2003). Cowpea, *Vigna unguiculata* (L.) Walpers, is a major food crop that generates income for many small holder farmers and traders in sub-Saharan Africa (Langyintuo, 2003). Cowpea has high protein and lysine content which makes the crop a natural supplement to staple diets of cereals, roots and tubers commonly grown in many poor countries (Adedire *et al*., 2011). In Nigeria, it is consumed in the form of bean pudding, bean cake, baked beans, fried beans, and bean soup among others (Mbah and Silas, 2007). Cowpea is usually stored for food reserve and also for seed material for planting.

The cowpea beetle, *Callosobruchus maculatus* Fabricius (Coleoptera: Chrysomelidae), is a major field-to-store pest of cowpea in Nigeria. *Callosobruchus maculatus* attacks cowpea pods in the field and continues in stored seeds, thereby causing quantitative and qualitative losses manifested by seed perforation, reductions in weight, nutritional value, and market value. Estimates of storage losses are highly variable ranging widely from 4 - 90% (Umeozor, 2005) due to perforations by this insect, thus, reducing the degree of usefulness and making the seeds unfit either for planting or human consumption (Ali *et al*., 2004).

Although synthetic insecticides have made a tremendous impact over the years in the field of stored product protection, it has become necessary to minimize the amount of toxic materials released into the environment as a result of their adverse effects such as insecticide residues on food and the development of insect pest resistance (Akob and Ewete, 2007; Adekola and Oluleye, 2007). Also, the abuse and misuse of chemical pesticides have led to several health effects including acute and chronic poisoning in man, sudden deaths, blindness and skin irritation (Asawalam and Emosairue, 2006). Therefore, there is an urgent need to increase the search for cheap, easily biodegradable, safe and readily available plant/natural products for post-harvest pest control (Ukeh, 2009). It is against this background that the comparative toxicity of powder extracts of three common plants namely *Eugenia aromatica* (cloves), *Zingiber officinale* (ginger), and *Piper nigrum* (black pepper), were tested as protectants of stored cowpea seeds against the cowpea beetle *C. maculatus*.

MATERIALS AND METHODS

Source of cowpea seeds and plant powders

Cowpea seeds were obtained from a local market in Bariga, Lagos State, Nigeria, and disinfested in a dry oven for 24 hrs at a temperature of 40^{o}C in order to kill any adult insect, larvae or eggs present in the cowpea. The disinfested cowpea seeds were left under ambient laboratory conditions at temperature 28^{o}C ± 2^{o}C and relative humidity 72% ± 5% for 24 hrs for their moisture contents to stabilize before being used for the experiments. Fruits of dried cloves – *Eugenia aromatica*, ginger – *Zingiber officinale* and black pepper – *Piper nigrum* were sun-dried, decorticated and pulverized into powder using a manually operating mill. Powders were then stored under ambient laboratory conditions until they were ready for use.

Insect culture

A culture of *C. maculatus* was established from infested cowpea seeds obtained from the market. The infested cowpea seeds were kept in a plastic container covered with muslin cloth to enable aeration and prevent the insects from escaping. After 7-10 days of mating and oviposition, all adult beetles were removed. The new generation of insects that emerged was then used for the experiment. This culture was maintained under ambient conditions at the Entomology laboratory of the Department of Zoology, University of Lagos.

Toxicity of plant powders to adult Callosobruchus maculatus

For each plant material (*E. aromatica*, *Z. officinale* and *P. nigrum*), 2.5, 5.0, 7.5 and 10.0 g of powders were separately introduced into 100 g of cowpea seeds in a plastic container measuring (17.3 x 12 x 7.5) cm. The seeds were shaken thoroughly to ensure even mixing of the powder and the lid of each jar was covered with a muslin cloth, secured with a rubber-band. Control for each set of treatments consisted of cowpea seeds without plant powders. Three replicates of each treatment and untreated cowpea seeds were set up. After 24 hours, 20 adult *C. maculatus* (n=20) aged one-day old were introduced to each plastic container cage using a fine brush. Adult mortalities were recorded at 24, 48, 72 and 96 hrs after treatment.

Data on percentage adult mortality was corrected using Abbott's (1925) formula:

$$Pt = \frac{Po - Pc}{100 - Pc} \times 100$$

Where Pt = Corrected mortality,

Po = Observed mortality on treatment,

Pc = Control mortality.

Residual effect of plant powders on Callosobruchus maculatus progeny emergence

At the end of the experiment on toxicity, all live and dead insects were removed and the cowpea seeds were left in the jars which separately contained plant powders at 2.5, 5.0, 7.5 and 10.0 g/100 g of cowpea seeds. Control for each set of treatments consisted of untreated cowpea seeds. All treated and untreated cowpea seeds were observed weekly for F_1 and F_2 *C. maculatus* emergence for a period of three months. After emergence of the first generation of adults (F_1), the emergents were counted and removed. Observation continued until the second generation (F_2) emerged.

Data Analysis

All statistical analyses were carried out using SPSS statistical program version 20. Data obtained on mortality and emergence of *C. maculatus* were subjected to Analysis of Variance (ANOVA) after the data were transformed by square root of (X+0.5). Means showing significant difference were separated using Tukey's (HSD) test at $P<0.05$. Probit analysis was used to determine LC_{50} (Lethal Concentration 50) values.

RESULTS

Mortality tests of Zingiber officinale, Eugenia aromatica and Piper nigrum on Callosobruchus maculatus

The mortality of *C. maculatus,* in cowpea treated with the powders of *Z. officinale*, *E. aromatica* and *P. nigrum,* after 24, 48, 74 and 96 hours are presented in Table 1. Only two out of the three plant powders gave mortality after 24 hours of treatment. *Piper nigrum* did not give mortality of the beetles in all concentrations used at 24 hours after treatment whereas *E. aromatica* applied at 10g and 7.5g per 100g of cowpea each gave 100.00% mortality, which was significantly higher than those of the other two powders ($p<0.05$). On the other hand, *Z. officinale* applied at 10g gave 3.35% mortality in 24 hours. However, there were no significant differences ($p>0.05$) in mean mortality of *C. maculatus* with all concentrations of *Z. officinale* after 24hours (Table 1).

At 48 hours after treatment, *E. aromatica* achieved a significantly high mortality of 100% at 5g, 7.5g and 10g/100 g cowpea seeds concentration, whereas Z. *officinale* and *P. nigrum* gave 8.35% and 26.65% mortality at 10g and 5g/100 g cowpea seeds respectively (Table 1). At 72 hours after treatment, *P. nigrum* achieved 50.00% mortality of beetles with 7.5g/100 g seeds, which suggested the powder was slow acting. Similarly, 5.0g of *Z. officinale* gave a significant mortality of 31.65% in 72hours. At 96hours after treatment, *Piper nigrum* gave 80%, 83%, 85% and 75.00% mortalities in 2.5g, 5g, 7.5 g and 10 g/ 100 g cowpea seeds concentrations respectively, showing increase in mortality by time. *Eugenia aromatica* maintained a significantly high mortality of 100% in all dosages (Table 1).

Table 1: Mortality of *Callosobruchus maculatus* treated with powders of *Zingiber officinale, Eugenia aromatica and Piper nigrum* at 24, 48, 72 and 96 hours after treatment

Hours after treatment/ Plant Powder concentrations (g)	***Z. officinale*** **X ±SE**	***E. aromatica*** **X ±SE**	***P. nigrum*** **X ±SE**
24 hours			
0.0 (Control)	0.00±0.00a	0.00±0.00a	0.00±0.00a
2.5	0.00±0.00a	12.33±3.93b	0.00±0.00a
5.0	0.67±0.33a	19.67±0.33b	0.00±0.00a
7.5	0.00±0.00	20.00±0.00	0.00±0.00
10.0	0.67±0.67a	20.00±0.00b	0.00±0.00a
48 hours			
0.0 (Control)	0.33±0.33a	0.00±0.00a	0.67±0.33a
2.5	0.34±0.34a	17.33±1.45b	2.01±0.57a
5.0	0.67±0.34a	20.00±0.00b	4.70±1.78a
7.5	0.00±0.00a	20.00±0.00c	3.70±1.22b
10.0	1.34±0.88a	20.00±0.00b	4.03±0.59a
72 hours			
0.0 (Control)	0.67±0.33a	0.00±0.00a	0.67±0.33a
2.5	1.35±0.89a	20.00±0.00b	3.68±1.66a
5.0	5.71±1.69a	20.00±0.00b	8.06±3.09a
7.5	1.68±0.67a	20.00±0.00b	9.40±6.89ab
10.0	3.69±0.89a	20.00±0.00b	6.38±0.90a
96 hours			
0.0 (Control)	0.67±0.33a	0.33±0.33a	1.00±0.00a
2.5	5.04±2.11a	19.73±0.27b	15.15±2.02b
5.0	8.05±1.02a	19.73±0.27b	15.82±1.68b
7.5	5.03±0.58a	19.73±0.27b	16.16±2.10b
10.0	7.72±1.46a	19.73±0.27c	14.14±1.17b

Means followed by different letters across a row are significantly different at $P < 0.05$ using Tukey’s (HSD) test.

Bioassay determination of LC_{50} values of plant powders on *Callosobruchus maculatus*

Table 2 shows the LC_{50} values of *Z. officinale, E. aromatica and P. nigrum* on *C. maculatus* at 24, 48, 72 and 96 hours. Although the LC_{50} values reduced over exposure periods from 723.30 at 24 hours to 57.71 at 96 hours for *Z. officinale*, there was an increase to 9752.55 at 48 hours after treatment. In *E. aromatica*-treated cowpea, there was a reduction in LC_{50} from 2.24 at 24 hours to 1.62 at 48 hours. The toxicity factor showed that *E. aromatic*a was 322 times more toxic than *Z. officinale* within 24 hours whereas *P. nigrum* did not give mortality at that time. However, *P. nigrum* had its lowest LC_{50} value - 13.58 at 72 hours after treatment.

Residual effect of plant powders on emergence of F_1 and F_2 progeny of *Callosobruchus maculatus*

The residual effects of *Z. officinale, E. aromatica* and *P. nigrum* on two successive generations of *C. maculatus* in storage are presented in Tables 3 and 4. Powders of *E. aromatica* did not support emergence of F_1 and F_2 progenies of *C. maculatus* in all treatments whereas the control gave 358.67 and 460.00 F_1 and F_2 progenies, respectively. Conversely, the mean numbers of adult *C. maculatus* that emerged from *Z. officinale* treatment increased by the second filial generation from 177.67 F_1 to 1318.33 F_2 progeny at concentration 10g/100g cowpea. Similarly, at concentration 10g/100g of *P. nigrum*, the mean number of bruchid emergents increased from 85.67 F_1 to 1466.67 F_2 progeny.

Table 2: Toxicity of the plant powders to *Callosobruchus maculatus* at 24, 48, 72 and 96 hours after treatment

Plant type/ Hours after treatment	LC_{50}	Regression Equation	Standard Error	Slope	Df
Zingiber officinale					
24 hrs	723.30	y = -2.947 + (-3.192)x	1.086	-3.192	2
48 hrs	9752.55	y = -2.087 + (-3.981)x	0.648	-3.981	2
72 hrs	617.90	y = -1.215 + (-2.241)x	0.430	-2.241	2
96 hrs	57.71	y = -0.649 + (2.241)x	0.994	-2.241	2
Eugenia aromatica					
24 hrs	2.24	y = -2.173 + (-3.515)x	1.349	-3.515	2
48 hrs	1.62	y = -1.417 + (-0.604)x	5.800	-0.604	2
72 hrs	-	-	-	-	-
96 hrs	-	-	-	-	-

Table 3: The F_1 adult emergents of *Callosobruchus maculatus* on cowpea seeds treated with three plant powders

Plant material Powder concentrations (g)	F_1 progenies		
	Zingiber officinale	***Eugenia aromatica***	***Piper nigrum***
0 (Control)	294.67±105.33a	315.67±52.27b	222.33±46.72a
2.5	322.33±61.01a	0.00±0.00a	218.67±43.11a
5.0	142.33±59.68a	0.00±0.00a	148.33±71.45a
7.5	390.33±138.09a	0.00±0.00a	77.00±57.41a
10	177.67±18.52a	0.00±0.00a	85.67±13.86a

Mean values followed by different letters, within the same column are significantly different (at $p<0.05$) from each other using Tukey's (HSD) test.

Table 4: The F_2 adult emergents of *Callosobruchus maculatus* on cowpea seeds treated with three plant powders

Plant material Powder concentrations (g)	Mean No. (X ± SE) emerged F_2 progenies		
	Zingiber officinale	***Eugenia aromatica***	***Piper nigrum***
0 (Control)	57.33±302.54a	460.00±36.56b	992.00±261.61a
2.5	563.33±95.49a	0.00±0.00a	1027.00±59.43a
5.0	179.67±272.89a	0.00±0.00a	882.67±260.67a
7.5	783.67±335.93a	0.00±0.00a	697.33±398.62a
10.0	1318.33±49.36a	0.00±0.00a	1466.67±193.59a

Mean values followed by different letters, within the same column are significantly different (at $p<0.05$) from each other using Tukey's (HSD) test.

DISCUSSION

The powders of plant material applied at varying concentrations showed different levels of toxicity against *C. maculatus*. Of the three plant powders tested, *E. aromatica* was the most toxic with 61.65% and 90.00% mortalities of adult *C. maculatus* after 24 and 48 hrs respectively at 2.5g/100g cowpea seeds. At the rate of 5.0g/100g seeds, *E. aromatica* achieved 100.00% mortality whereas *Z. officinale* and *P. nigrum* gave 5.00% and 26.65% mortality, respectively after 48 hours. The lower LC_{50} values of *E. aromatic*a further corroborated that *E. aromatica* powder was more toxic and faster in action against the bean beetle than the other two powders. The observed activity may be due to the "pepperish" nature and pungency of *E. aromatica* (Asawalam and Emosairue, 2006). The result of this study is in agreement with Chukwulobe and Echezona (2014) who found that *E. aromatica* powder compared favorably with the synthetic pesticide - primiphos methyl, in causing high mortality of the red flour beetle, *Tribolium castaneum* as well as suppressing the population growth of the beetle on plantain chips. Our study also agrees with that of Olotuah (2014) who showed that ethanolic extract of essential oil of *E. aromatica* was highly toxic to a number of storage insect pests including *C. maculatus*, *Sitophilus zeamais*, *S. oryzae* and *T. castaneum.*

In this present study, *P. nigrum* gave higher mortality of beetles than *Z. officinale* over time. This agrees with Abdullahi and Muhammad (2004) who reported that powders of *P. nigrum* had pronounced toxic effects on *C. maculatus* compared to treatment with *Z. officinale* powders. The choky effect of these powders may have caused disruption in mating activities, sexual communication and inhibited locomotion (Ofuya, 1992; Adedire *et al*., 2011).

The results of the residual toxicity bioassay suggest that if cowpea has to be stored for up to 3 months and above, they must be treated to avoid infestation by *C. maculatus*. In this study, no beetles (F_1 and F_2 progenies) emerged from cowpea treated with *E. aromatica* after three months in storage. This confirms the reports of earlier authors. For instance, Adedire and Lajide (1999) found that *E. aromatica* powder had significant contact and fumigant actions against *C. maculatus* and suggested that the mechanism of action is by inhibition of oviposition and direct toxicity to eggs (ovicidal) and adults. It was reported that *E. aromatica* powder still manifested significant contact and fumigant insecticidal activity against the cowpea seed beetle for up to four years after the dry flower buds were powdered (Ofuya and Dawodu, 2002).

In this study, it was observed that the cowpea seeds in *Z. officinale* and *P. nigrum* treatments had become moldy and caked up after the emergence of the second filial generation of *C. maculatus* in three months of storage. This result suggests that while *E. aromatica* maintained significant residual effectiveness on *C. maculatus*, *Z. offficinale* and *P. nigrum* lost their residual bioactivity over time. The moldiness observed on the cowpea treated with *Z. officinale* and *P. nigrum* at the emergence of F_2 progenies of *C. maculatus*, possibly confirms the finding of Pantenius (1988) which indicated that insect feeding damage encouraged higher moisture content and development of micro-organisms (fungi) and possible contamination with aflatoxins. Akob and Ewete (2007) also observed moldiness on maize grains infested by *Sitophilus zeamais* after six months of storage.

This study showed that *E. aromatica* powder is a more effective botanical compared to *Z. officinale* and *P. nigrum*, as clearly seen from the results. *Eugenia aromatica* also gave residual protection of cowpea seeds after three months in storage. Therefore, *E. aromatica* can be recommended for use as a protectant of cowpea in storage. Further research can be conducted to isolate and identify the active principles conferring the efficacious bioactivity on *E. aromatica.* This could eventually lead to our own indigenous product development that could compete favourably with synthetic insecticides. More so that plant products have comparative advantages over synthetic products in that they are readily available, affordable and biodegradable. With the development and use of plant-derived insecticides, accidental poisoning and deaths of humans and non-target organisms will be greatly minimized.

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