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INSECTICIDAL ACTIVITIES OF SOME PLANT EXTRACTS AGAINST THE COWPEA BEETLE, *Callosobruchus maculatus* F. (Coleoptera: Chrysomelidae)

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ABSTRACT

Ethanolic leaf extracts of *Euphorbia balsamifera* and *Lawsonia inermis* were tested on their efficacy against *Callosobruchus maculatus* in stored cowpea under laboratory condition of 28 ± 2°C and 75 ± 5% r.h. in the laboratory of the Department of Biology, Umaru Musa Yar'adua University, Katsina, Nigeria. The objective of this study was to evaluate the efficacy of ethanolic leaf extract of *E. balsamifera* and *L. inermis* against *C. maculatus* in stored cowpea seeds. Each of the two plants extract was tested by exposing five pairs of adult weevils to 20 g of cowpea mixed with various conc. of 2.5×10^4, 5.0×10^4 and 10.0×10^4 ppm separately in three replicates. No plant extract was added to the control. Results showed that *E. balsamifera* caused 96.67% adult mortality and *L. inermis* exerted 90.00% adult mortality after 14 days post treatment. The findings of this study also showed that *L. inermis* plant extract was the most virulent with the lowest LC_{50} and LC_{90} values of 2.7×10^3 and 3.0×10^3 ppm respectively. There was fewer numbers (2.13) of eggs in 10.0×10^4 *L. inermis* than in *E. balsamifera* of the same conc. which was 2.60. Among the two plant extracts, *E. balsamifera* applied at 2.5×10^4 ppm resulted in the highest (12.25 ± 0.25) adult emergence while the least (3.00 ± 0.05) was obtained from 10.0×10^4 ppm *L. inermis*. Results also indicated that there was significant difference ($P < 0.05$) between weight loss of cowpea seeds in ethanolic extracts of the two botanicals and the control. Therefore, these plants extracts could be suggested for development as biopesticides against *C. maculatus*.

Keywords: Bio-control, *Callosobruchus maculatus*, Cowpea, *E. balsamifera*, *L. inermis*, Plant extracts

1. INTRODUCTION

The cowpea weevil, *Callosobruchus maculatus* is the main insect pest of cowpea (*Vigna unguiculata*), in Latin America and Africa (Murad, *et al*., 2008). It is a worldwide pest, and its larvae develop within various cultivated legumes, such as black eyed beans, *V. unguiculata* (Nabaei *et al*., 2012). Ojebode *et al.* (2016) reported that *C. maculatus* is very destructive on account of its short life cycle. Their damage causes loss of weight, nutritional and commercial values of cowpea seeds and viability of stored grains (Nabaei *et al*., 2012; Suleiman, 2016). Larvae feed and develop inside the seed which become unsuitable for human consumption and when adults emerge they leave a neat circular exit hole. Each adult consumes approximately 25% of the seed from which it develops (Asawalam and Anaeto, 2014).

The control of *C. maculatus* in stores has been accomplished by synthetic chemical pesticides like Permethrin (Suleiman and Suleiman, 2014). The extensive use of these chemicals has given rise to so many problems such as insecticide resistance, health risk to consumers and environmental contamination. These problems have necessitated the replacement of synthetic insecticides with natural compounds which are eco-friendly and safe to protect stored grains from insect infestations (Vanmathi *et al*., 2012; Suleiman, 2014). Previous findings demonstrated the use of botanical insecticides as safe and effective protectants of stored cowpea against *C. maculatus* infestation and damage (Suleiman and Suleiman, 2014; Asawalam and Anaeto, 2014). Over the past decade, four major types of botanicals such as pyrethrum, rotenone, neem and essential oils have been successfully used for insect management (Kedia *et al*., 2015).

Euphorbia balsamifera (Balsam spurge) is a perennial shrub in the Euphorbia genus growing 3 – 6 m from the ground. It is native to Nigeria and other African countries and Asia. Previous studies have reported that, leaf powder of *E. balsamifera* have been reported effective against the fecundity of adult cowpea weevil and recording high level mortality (Suleiman and

Suleiman, 2014). It also has been reported to cause significant mortality on *Sitophilus zeamais* (Suleiman *et al.*, 2012).

Lawsonia inermis has also been reported to be lethal on cowpea weevil as well as *S. zeamais* (Suleiman *et al.*, 2013; Suleiman and Suleiman, 2014; Chudasama *et al.*, 2015). The objective of this study was therefore to evaluate the efficacy of ethanolic leaf extracts of *E. balsamifera* and *L. inermis* against *C. maculatus* in stored cowpea seeds.

2. MATERIALS AND METHODS

2.1 Rearing of *Callosobruchus maculatus*

All experiments were conducted in the Biology Laboratory 3 of Umaru Musa Yar'adua University, Katsina (UMYUK), Nigeria. Adults of *C. maculatus* were obtained from infested cowpea seeds from a local store in Katsina Central Market and sieved out from the infested cowpea seed. Fresh, healthy cowpea seeds were used as food media for the insects.

The seeds were subjected to dry heat treatment in an oven at 40^{0}C for 48 hours to disinfect the seeds from any insects, mites or microorganisms that might be present. A sample of 250 g of the disinfected cowpea seeds was placed in each of five rearing bottles of 50 cm^3 capacity after which 50 pairs of the adult *C. maculatus* were introduced. The rearing bottles were covered with muslin cloth and secured with rubber band to prevent escape of the insect and allow gaseous exchange. The bottles containing the insects were then kept in an incubator at 28 ± 2^{0}C and 70 ± 5% r.h. for 7 days of oviposition period after which the beetles were sieved out leaving the cowpea seed only. The bottles containing the seeds were maintained under the constant condition until the emergence of adults. The newly emerged adults were used for the experiments.

2.1 Collection and Preparation of Plant Materials

Fresh leaves of *E. balsamifera* and *L. inermis* were collected from their natural habitat (bushes) around Umaru Musa Yar'adua University, Katsina (latitude 12° 53' N and longitude 7° 35' E) and taken to the Department of Biology, UMYUK, for identification. The leaves were then rinsed with distilled water and shade-dried. The dried leaves were ground using laboratory blender (Model 8010ES) and sieved using 1 mm laboratory sieve as outlined by Rugumamu (2014). The powders were then separately kept in polythene bags at room temperature in the laboratory.

One hundred grams of plant powders were then dissolved in 400 ml of ethanol and kept in the laboratory shelf for 48 hours at room temperature. The extracts of the two plants were filtered separately using muslin cloth and what man No.1 filter papers as described by Khaliq *et al.* (2014). The filtrate was then concentrated by evaporating excess solvents using rotary evaporator followed by air-drying the extracts and stored in the refrigerator at 4°C in accordance with the methods of Abou-Elnaga (2015) prior to use for the laboratory experiments.

2.3 Adult mortality assessment

Extracts of the two botanicals were diluted to 0.5, 1.0 and 2.0 g/ 20 ml ethanol (2.5, 5.0, and 10.0%) equivalent to 2.5 x10^4, 5.0 x x10^4 and 10.0 x 10^4 ppm, respectively. Four replicates of 2ml of the diluted extract were added separately to 20 g of disinfested cowpea seeds and mixed thoroughly in petri dish. Another 2ml of ethanol was used in the control and air – dried (de Oliveira *et al*., 2012). Ten of 1 to 7 days old adults of *C. maculatus* obtained from the rearing set up were introduced into each of the petri dishes containing the treated and untreated seeds and covered with white muslin cloth secured with rubber bands and then placed in an incubator at 30^{0}C and 70% r.h. Dead beetles in each replicate were removed and recorded daily for 14 days and adult mortality was assessed as:

$$\%\ \text{Mortality} = \left(\frac{\text{Number of Dead Weevils}}{\text{Total Number of Weevils}}\right) \text{X } 100$$

2.4 Determination of number of eggs laid

On the 15th day, all the beetles (dead and alive) were removed and the grains were maintained under the same condition until the emergence of F_1. Samples of 10 cowpea seeds were then randomly taken from each of the treated and untreated cowpea seeds and the number of eggs deposited on them were observed using hand lens, counted and recorded.

2.5 Adult emergence

Cowpea seeds were inspected daily and the emerging progenies from each petri dish were removed, counted and recorded. Observation continued for 14 days from the day the first emergence from both treated and untreated cowpea.

2.6 Damage Assessment

After 28 days of treatment, the percentage weight loss of the seed was evaluated by weighing the cowpea seeds in the petri dish using weighing balance and the differences from the initial weight and final weight were transformed into percentage weight loss as follows:

$$\% \text{ Grain Weight Loss} = \left(\frac{\text{Initial Grain Weight} - \text{Final Grain Weight}}{\text{Initial Grain Weight}}\right) \text{X } 100$$

2.7 Statistical Analysis

The data collected was subjected to analysis of variance (ANOVA) and significantly different means were separated using Bonferroni's multiple comparisons test using Graph Pad Prism version 7.01. Also, data obtained from weevil's mortality were subjected to regression analysis to calculate the LC_{50} of the extracts using probit analysis. All analyses were carried out at 5% ($P < 0.05$) level of significance.

3. RESULTS

Results from this study show that *E. balsamifera* and *L. inermis* tested against *C. maculatus* were toxic against *C. maculatus*. Percentage mortality varied according to botanical species and concentrations of the botanicals applied. Highest (96.67%) adult mortality of *C. maculatus* was caused by 10.0 x 10^4 ppm *E. balsamifera*, while the least (70.00%) was caused by 2.5 x 10^4 ppm *L. inermis* (Table 1). Adult mortality of *C. maculatus* was significantly different ($P < 0.05$) between the different conc. of the test botanicals applied. Adult mortality of *C. maculatus* in the control was 20% only.

Lethal dose of the tested botanicals is presented in Table 2. The result shows that *L. inermis* leaf powder was the most virulent with the lowest LC_{50} and LC_{90} values of 2.7 x 10^3 and 3.0 x 10^3 ppm, respectively. The LC_{50} and LC_{90} values of *E. balsamifera* were calculated as 2.9 x 10^3 and 3.1 x 10^3 ppm, respectively (Table 2).

Table 3 presents the mean number of eggs laid by *C. maculatus* on cowpea seeds at different conc. The percentage eggs laid in all treatments was significantly lower than in the control control. However, an increase in the concentration of the botanical extract used decreased the number of eggs laid. There was fewer numbers (2.13) of eggs in 10.0 x 10^4 *L. inermis* than in *E. balsamifera* (2.60) of the same conc. At the lower conc. of 2.5 x 10^4, the number of eggs laid increased to 5.00 and 3.33 for *E. balsamifera* and *L. inermis*, respectively, while 17.00 eggs were counted in the control. The number of eggs deposited was significantly different ($P < 0.05$) between the test botanicals and the control.

Table 4 shows the results obtained from the effect of two plant extracts on adult emergence of *C. maculatus* on cowpea grain.

It was observed that, adult emergence had an inverse relationship the level of concentration of the plant treatments. Among the two plant extracts, *E. balsamifera* applied at 2.5x10^4 ppm resulted in the highest (12.25 ± 0.25 ppm) adult emergence while the least (3.00 ± 0.05 ppm) was obtained from 10.0x10^4 ppm *L. inermis* treatment. There were 18.25 ± 0.01 emerged adults in the control. The number of adults emerged was significantly different ($P < 0.05$) between treated cowpea and the control.

Weight loss of cowpea seeds treated with ethanolic leaf extracts of *E. balsamifera* and *L. inermis* due to *C. maculatus* infestation is presented in Table 5. Untreated control recorded the highest (11.83% ± 0.22) weight loss when compared to other treatments. Weight loss for all the treatment increased as the concentration of botanicals decreased. At 2.5 x 10^4, the weight loss was higher (8.52% ± 1.59) in *E. balsamifera* than in *L. inermis* (8.17% ± 1.68), while at 10.0 x 10^4 it was higher (7.58% ± 0.80) in *L. inermis* than in *E. balsamifera* (7.27% ± 0.74). There was significant difference ($P < 0.05$) between weight loss of cowpea seeds in ethanolic extracts of the two botanicals and the control.

4. DISCUSSION

The results of this study indicate that, all the level of the two botanicals extracts tested were toxic to *C. maculatus* which resulted in high mortalities of the adult beetles. Percentages of adult mortality were found to increase with increase in concentrations of the two botanicals. Similar trend was reported by Danga *et al*., 2015 with the use of *Plectranthus glandulosus* (Lamiaceae) and *Callistemon rigidus* (Myrtaceae) Leaf Extract. The finding of current study seems to be consistent with other studies that used leaf powders of many botanicals against *C. maculatus* including family of Euphorbiaceae and Lythraceae (Mundi *et al*., 2012; Asawalam and Anaeto, 2014; Suleiman and Suleiman, 2014).

Higher concentrations of the treatments recorded lower number of eggs laid in all the two botanicals under trials. These corroborate the finding of Jose and Adesina (2014) who reported that higher concentration of *L. inermis* resulted in lower number of eggs as compared to lower dosed. Suleiman and Suleiman (2014) also reported that leaf powders of *E. balsamifera* and *L. inermis* reduced the number of eggs laid by *C. maculatus*. Similarly, Kosar and Srivastava (2016) reported that, lowest mean egg laid was observed in experimental sets treated with higher dose of plant extract belonging to the family Euphorbiaceae.

Similarly, at the higher concentration of plant extract there was low progeny emergence. Similar findings were observed by Suleiman *et al.* (2011) and Mundi *et al.* (2012). The ability of the evaluated plant extracts to significantly suppress adult emergence indicated that the plant might possess ovicidal and larvicidal properties (Jose and Adesina, 2014).

The weight loss of cowpea seeds at the end of the experiment was higher in the control compared to the botanical treatments. Similar results were reported by Suleiman and Suleiman (2014) that leaf powders of *E. balsamifera* and *L. inermis* greatly reduced cowpea seed damage 28 days after treatment. This might be due to limited contact of *C. maculatus* with the treated seed. This finding is in agreement with Jose and Adesina (2014) who reported that all the plant extracts formulation that kill *C. maculatus* at the end of experiment did not significantly loss their weight compared to control treatment as a result of toxicity effect exhibited by the botanicals used which led to the reduction in F_1 progeny, low egg and adult emergence that causes weight loss.

5. CONCLUSION

In this study, *C. maculatus* in stored cowpea seeds were exposed to ethanolic extracts of two plants, *E. balsamifera* and *L. inermis*. Results indicated that, percentage mortality varied according to plant species and concentrations of the plant extracts. *L. inermis* had lower LC_{50} and LC_{90} values, hence the most toxic plant extract to *C. maculatus*. Accordingly, higher concentrations of the treatments recorded lower number of eggs laid and progeny emergence. Therefore, these botanicals can be used as option for the control of *C. maculatus* in stored cowpea. However, further research is encouraged to study their pesticidal activities against other insect pests.

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Table 1: Effect of ethanolic extracts of *Euphobia balsamifera* and *Lawsonia inermis* on adult mortality of *Callosobruchus maculatus* on cowpea seeds after 7 days of treatment.

Botanicals	Mean Adult Mortality (% ± S.E.)/Conc. (ppm)		
	2.5×10^4	5.0×10^4	10.0×10^4
E. balsamifera	80.00 ± 5.77	90.00 ± 5.77	96.67 ± 8.00
L. inermis	70.00 ± 5.77	80.00 ± 5.77	90.00 ± 5.77
Control	20.00 ± 0.60	20.00 ± 0.60	20.00 ± 0.60

Table 2: LC_{50} and LC_{90} of ethanolic leaf extract of *Euphobia balsamifera* and *Lawsonia inermis* against adult *Callosobruchus maculatus*

Botanicals	LC_{50}	LC_{90}	Regression equation = a + bx
E. balsamifera	2.9×10^3	3.1×10^3	6.37 + 1.64x
L. inermis	2.7×10^3	3.0×10^3	4.94 + 1.24x

Table 3: Mean number of eggs laid by *Callosobruchus maculatus* on cowpea seeds at different conc. of ethanolic extracts of *Euphobia balsamifera* and *Lawsonia inermis*

Botanicals	Number of eggs laid (% ± S.E.)/Conc. (ppm)		
	2.5×10^4	5.0×10^4	10.0×10^4
E. balsamifera	5.00 ± 1.13	3.81 ± 0.86	2.60 ± 0.34
L. inermis	3.33 ± 0.21	3.20 ± 0.40	2.13 ± 1.14
Control	17.00 ± 0.14	17.00 ± 0.14	17.00 ± 0.14

Table 4: Effect of ethanolic extracts of *E. balsamifera* and *Lawsonia inermis* on adult Emergence of *Callosobruchus maculatus* on cowpea seeds after 28 days post treatment.

Botanicals	Number of emerged Adults (% ± S.E.)/Conc. (ppm)		
	2.5×10^4	5.0×10^4	10.0×10^4
E. balsamifera	12.25 ± 0.25	10.00 ± 0.46	6.75 ± 0.83
L. inermis	8.52 ± 0.22	4.83 ± 0.09	3.00 ± 0.05
Control	18.25 ± 0.01	18.25 ± 0.01	18.25 ± 0.01

Table 5: Effect of ethanolic extracts *Euphobia balsamifera* and *Lawsonia inermis* on weight loss of cowpea seeds caused by *Callosobruchus maculates*

Botanicals	Mean weight loss (% ± S.E.)/Conc. (ppm)		
	2.5×10^4	5.0×10^4	10.0×10^4
E. balsamifera	8.52 ± 1.59	7.42 ± 1.16	7.27 ± 0.74
L. inermis	8.17 ± 1.68	7.73 ± 0.42	7.58 ± 0.80
Control	11.83 ± 0.22	11.83 ± 0.22	11.83 ± 0.22

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