BAJOPAS Volume 13 Number 1, June, 2020 **http://dx.doi.org/10.4314/bajopas.v13i1.5**

Bayero Journal of Pure and Applied Sciences, 13(1): 25 - 31
Received: September, 2019
Accepted: May, 2020
ISSN 2006 – 6996

BIOEFFICACY OF ETHANOLIC LEAF EXTRACTS OF SOME BOTANICALS AGAINST COWPEA BEETLE, *Callosobruchus maculatus* F. [COLEOPTERA: CHRYSOMELIDAE]

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ABSTRACT

Ethanolic leaf extracts of Euphorbia balsamifera Aiton, Lawsonia inermis L., Mitracarpus hirtus (L.) DC. and Senna obtusifolia L were tested on their efficacy against Callosobruchus maculatus in stored cowpea under laboratory conditions of 28 ± 2°C and 75 ± 5% r.h. The study was conducted in the laboratory of the Department of Biology, Umaru Musa Yar'adua University, Katsina (UMYUK), Nigeria, between July 2018 to February 2019. Four plant extract was tested by exposing five pairs of adult weevils to 20 g of cowpea mixed with ethanolic leaf extracts of the test botanicals at 2.5 x 10^4, 5.0x$10^{4,}$ and 10.0 x 10^4 ppm separately in four replicates. No plant extract was added to the control. Results showed that all the four plants extract caused significant adult mortality of C. maculatus after 96 hours post-treatment. E. balsamifera causedtotal mortality of thebeetles at all doses,while similar results were obtainedat the highest dose of 10.0 x 10^4 ppmonly ofL. inermis and M. hirtus.The findings of this study also showed that E. balsamifera plant extract was the most virulent with the lowest LC_{50}value of 2.0x 10^2ppm. There was no adult emergence in all the treatments except in the control.Similarly, there was no seed weight loss was recorded in all the four treatments.The plant species could, therefore, be suggested as biopesticides against C. maculatus infesting stored cowpea.

Keywords: Biopesticides, Callosobruchus maculatus, Cowpea seed, Mortality rate, Plant extracts

INTRODUCTION

The cowpea weevil, *Callosobruchus maculatus* is the leading cause of damage on cowpea (*Vigna unguiculata*L. Walp.,), 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). Despite its short life cycle,*C. maculatus* is a very destructive insect pest that causesperforations and weight losses, leading to losses in nutritional as well as commercial values of cowpea seeds (Suleiman, 2016: Ojebode *et al*.,2016).

Larvae feed and develop inside the seed which becomes 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 and health risk to consumers. These problems have necessitated the replacement of synthetic insecticides with natural compounds that are safe and effectivein protecting stored cowpea grains from insect infestations (Vanmathi *et al.,* 2012).

Recently, researchers have shown an increased interestin using biological control agents for insect pests' control. However, previous findings demonstrated the effective use of botanical insecticides as safe and effective protectants of stored cowpea against *C. maculatus* infestations and damages (Asawalam and Anaeto, 2014; Ojebode *et al*.,2016; Mbatchou *et al*.,2018).

Over the past decade, four major types of botanicals such as pyrethrum, rotenone, neem, and essential oils have been successfully used for *C. maculatus* management (Kedia *et al*., 2015).

Several plant substances have served as repellents with hightoxicity against *C. maculates* (Zandi-Sohani *et al.,* 2012; Pandey *et al.,* 2012;Tiroesele *et al.,* 2015; Sani and Suleiman, 2017; Suleiman and Sani, 2017). Extracts and powder of some plant species were reported to contain secondary metabolites such as alkaloids, flavonoids, saponins, tannins, steroids and phenolic compounds that can reduce fecundity, oviposition and larval development of *C. maculates* (Adedire *et al.,* 2011; Dimetry *et al.,* 2015; Kosar and Srivastava 2016; Ojebode *et al.* 2016).

In the present study, the effectiveness of ethanolic extracts of*Euphorbia balsamifera* Aiton, *Lawsonia inermis* L., *Mitracarpus hirtus* (L.) DC. and *Senna obtusifolia* L (Table 1) were testedagainst *C. maculatus* under Laboratory conditions.Therefore, this study is aimed to study the efficacy of four ethanolic plant extracts (*E. balsamifera, L. inermis, M. hirtus,*and *S. obtusifolia*) against *C. maculates*.

MATERIALS AND METHODS

Rearing of *C. maculatus*

All experiments were conducted in the Department of Biology Laboratory III 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. The insects were sieved out from the infested cowpea seed.

Fresh, healthy cowpea seeds were obtained and subjected to dry heat treatment in an oven at 40^{o}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 500 cm^3 capacity after which 50 pairs of adult *C. maculatus* were introduced. The rearing bottles were covered with the muslin cloth and secured with rubber bands to prevent the 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 same condition until the emergence of adults. The newly emerged adults (1 to 3 days old) were used for the experiments(Adedire *et al.,* 2011;Suleiman and Suleiman, 2014).

Collection and preparation of the extracts

Fresh leaves of *E. balsamifera* and *L. inermis, M. hirtus, and S. obtusifolia* were collected from their natural habitat (bushes) around UMYUK (latitude 12º 53′ N and longitude 7º 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 blended using a laboratory blender and sieved using a 1 mm laboratory sieve as outlined by Rugumamu (2014).

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 four plants were filtered separately using a muslin cloth and what man No.1 filter papers (Khaliq *et al.*,2014; Suleiman *et al*., 2018a). The filtrate was then concentrated by evaporating excess solvents using a rotary evaporator followed by air-drying the extracts and stored in the refrigerator at 4°C before use for the experiments.

Adult mortality assessment

Extracts of the four botanicals were diluted to 0.5, 1.0 and 2.0 g/ 20 ml ethanol equivalent to 2.5 $x10^4$, 5.0 $x10^4$ and 10.0 x 10^4 ppm, respectively. Four replicates of 2ml of the diluted extracts were added separately to 20 g of disinfested cowpea seeds and mixed thoroughly in a petridish. Another 2ml of ethanol was used in the control and air-dried (de Oliveira *et al*., 2012). Ten of 0 to 3 days old adults of *C. maculatus* obtained from the rearing container 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 28 ± 2^{o}C and 70 ± 5% r.h. Dead beetles in each replicate were removed and recorded daily for 96 hours and adult mortality was assessed as follows:

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

Examination of adult emergence

All beetles, dead and alive, were removed from both treated and untreated seeds immediately after assessment of adult mortality (96 hours after treatment). The cowpea seeds were maintained in the incubator until the emergence of the adult beetles. The emerging progenies from each petri dish were removed, counted, and recorded. Observations continued for 30 days from the day of the first emergence of adults in untreated cowpea(Adedire *et al.,* 2011; Sani and Suleiman, 2017).

Assessment of weight losses of treated cowpea seeds

After 30 days of treatment, the percentage weight loss of the seed was evaluated by re-weighing the cowpea seeds after sieving dust. The difference between initial and final weight was transformed into percentage weight loss as follows:

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

Statistical analysis

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

Table 1: Plant samples evaluated for Efficacy against *C. maculatus.*

Scientific Name	Common Name	Family	Part used
Euphorbia. Balsamifera	Balsam spurge	Euphorbiaceae	Leaves
Lawsonia inermis	Henna	Lythraceae	Leaves
Mitracarpus hirtus	Girdlepod	Rubiaceae	Leaves
Senna. Obtusifolia	Coffeeweed	Fabaceae	Leaves

RESULTS

Adult Mean Mortality of *C. Maculatus*in Cowpea Seeds Treated with fourplant Extracts

Results obtained in this study showed that ethanolic extracts of all the four plant species caused significant adult mortality of *C. maculatus*. Mortality in the control was recorded at0.00%. *E. balsamifera* caused 100% adultmortality after 96 hours of exposure at all three different concentrations (Table 2).The adult mortality of the beetles in cowpea seeds treated with *L. inermis* and *M. hirtus* and *S. obtusifolia* ranged from 80.00±0.72 to 100±0.00, 66.67±1.37 to 100±0.00 and 53.33±2.66 to 73.33±0.74, respectively. All three concentration of *E. balsamifera* causes 100% of *C. maculatus* over a period of 96 hours post-treatment. However, similar mean mortality values recorded in the lowest and median concentrations of *M. hirtus* treatment also recorded in median and highest concentrations of *S. obtusifolia* respectively (Table 2). Interestingly, mortality increased with an increase in the concentration of the extracts.

Table 2: Adult mean mortality of *C. maculatus* on cowpea seeds treated with ethanolic extracts of four plants species after 96 hours post treatment

Botanicals	Concentration (ppm)	Mean Mortality (% ± S. E.)
E. balsamifera	2.5×10^4	100.00 ± 0.00
	5.0×10^4	100.00 ± 0.00
	10.0×10^4	100.00 ± 0.00
L. inermis	2.5×10^4	80.00 ± 0.72
	5.0×10^4	100.00 ± 0.00
	10.0×10^4	100.00 ± 0.00
M. hirtus	2.5×10^4	66.67 ± 1.37
	5.0×10^4	73.33 ± 0.74
	10.0×10^4	100.00 ± 0.00
S. obtusifolia	2.5×10^4	53.33±2.66
	5.0×10^4	66.67 ± 1.37
	10.0×10^4	73.33 ± 0.74
Control	0.00×10^4	0.00 ± 0.00

The Emergence of Adult *C. Maculatus* in Cowpea Seeds treated with Four Plant Extracts

There was no adult emergence in all the treatments throughout the study period. However, 56.67±5.77 adults of the beetles emerged from theuntreated seeds (Table 3).

Table 3: Effect of four plants extracts on adult emergence of *C. maculatus* on cowpea seeds after 30 days post treatment

Botanicals	Concentration (ppm)	Adult Emergence after 30 days (% ± S. E.)
E. balsamifera	$2.5X10^4$	0.00±0.00
	$5.0X10^4$	0.00±0.00
	$10.0X10^4$	0.00±0.00
L. inermis	$2.5X10^4$	0.00±0.00
	$5.0X10^4$	0.00±0.00
	$10.0X10^4$	0.00±0.00
M. hirtus	$2.5X10^4$	0.00±0.00
	$5.0X10^4$	0.00±0.00
	$10.0X10^4$	0.00±0.00
S. obtusifolia	$2.5X10^4$	0.00±0.00
	$5.0X10^4$	0.00±0.00
	$10.0X10^4$	0.00±0.00
Control	$2.5X10^4$	56.67±5.77
	$5.0X10^4$	56.67±5.77
	$10.0X10^4$	56.67±5.77

Effect of Four Plants Extract on Weight Loss of Cowpea Seed

As can be seen from Table 4, there was no weigh loss and damage in the cowpea seed treated with four plant extracts after the expirinment. Loss of weight was only observed in the untreated seed.

Table 4: Effect of ethanolic extracts offour plants extracts on weight loss of cowpea seeds caused by *C. maculatus*

Botanicals	Concentration	Percentage Weight loss of cowpea seed after 30 days (% ± S. E.)
E. balsamifera	$2.5X10^4$	0.00±0.00
	$5.0X10^4$	0.00±0.00
	$10.0X10^4$	0.00±0.00
L. inermis	$2.5X10^4$	0.00±0.00
	$5.0X10^4$	0.00±0.00
	$10.0X10^4$	0.00±0.00
M. hirtus	$2.5X10^4$	0.00±0.00
	$5.0X10^4$	0.00±0.00
	$10.0X10^4$	0.00±0.00
S. obtusifolia	$2.5X10^4$	0.00±0.00
	$5.0X10^4$	0.00±0.00
	$10.0X10^4$	0.00±0.00
Control	$2.5X10^4$	11.83±0.22
	$5.0X10^4$	11.83±0.22
	$10.0X10^4$	11.83±0.22

Lethal Concentration (LC_{50}) f Ethanolic Leaf Extracts of Four Plant Species tested on *C. Maculatus*

The lethal concentration of the tested botanicals required to kill 50% of the insect is presented in Table 5. The result shows that *E. balsamifera* leaf extract was the most virulent with the lowest LC_{50} as 2.0×10^2 ppm.

Table 5: LC_{50} of ethanolic leaf extracts of four plant species against adult *C. maculatus*

Botanicals	LC_{50} (ppm)	Regression Equation
E. balsamifera	2.0 x 10^2	0.78+0.41x
L. inermis	6.0 x 10^2	0.45+0.40x
M. hirtus	1.3 x 10^3	1.04+0.32x
S. obtusifolia	1.8 x 10^3	1.45+90x

DISCUSSION

The use of plant species as biological control agents against insect pests of stored products has long been recognized. Some of the plant species have been reported to reduce the fecundity as well as the population of insect pests of stored products (Adedire *et al.,* 2011; Suleiman *et al.,* 2012;Suleiman *et al.,* 2018b; Dimetry *et al.,* 2015;Danga *et al*., 2015; Ojebode *et al.* 2016).

In this study,all four botanicals extract tested were toxic to *C. maculatus* which resulted in high mortalities of the adult beetles.The mortality of the adult insects increasedwith an increase in the concentration of plant extracts applied. This is concurring with findings ofDanga *et al*., (2015) and Suleiman *et al.,*(2018a) that some plant powders and extracts of the test botanicals resulted in increased adult mortality of *Sitophilus zeamais* with an increase in concentrations. Also, the findings of this studyare consistent with other studies that leaf powders and extracts of many plants of the families Euphorbiaceae, Fabaceae and Lythraceae were toxic against *C. maculatus* (Mundi *et al*., 2012; Asawalam and Anaeto, 2014; Suleiman and Suleiman 2014; Danga *et al*., 2015; Obadofin *et al.,* 2015).

The total adult mortality of *C. maculatus* caused by *E. balsamifera, L. inermis* and *M. hirtus* atthe highest dose agrees with an earlier study conducted by Suleiman *et al.*(2018a) who recorded 100% mortality of adults *S. zeamais*treated in sorghum grains. Further, it was reported that powders and extracts of *E. balsamifera*, *L. inermis,* and *Senna tora* caused > 50.0% to 90.0% adult mortality of *C. maculatus* (Jose and Adesina, 2014; Suleiman and Suleiman, 2014; Mbatchou *et al.*,2018).

This high mortality of adults insects cause in treated plant extract might be due topresence of some secondary metabolites such as the steroids, phenolic compounds, tannins, terpenoids, flavonoids, alkaloids, saponins and glycosides which reported to havea wide range of biological activity with a great impact on insecticidal activities (Rahman and Talukder, 2006; Obadofin *et al.,* 2015; Dimetry *et al.,* 2015).

This study has revealed that all the four plant extracts completely inhibited adult emergence after 30 days post-treatment. This agreed with other researchers who reported that phytochemicals derived from plant sources possess ovicidal and larvicidal properties (Adedire *et al.,* 2011; Jose and Adesina, 2014; Tenne and Karunaratne, 2018).

The weight loss of cowpea seeds at the end of the experiment was only observed in the control but, there was neither weight loss nor damage in the cowpea seed treated with plant extract. A similar result was reported by (Adedire *et al.,* 2011). This was possible due to limited contact of *C. maculatus* with the treated seed and toxicity effect exhibited by the test plants which inhibited the production of F_1 progeny. Moreover,it has been reported that the adult beetles make emergence holes in the untreated seeds which lead to perforations and finally weight losses (Tiroesele *et al.,* 2015).

The lethal concentration of plants required to kill 50% (LC_{50})of adult *C. maculatus* showed that extracts of *E. balsamifera* had lower value and cause great efficacy by causing 100% adult mortality of *C. maculatus*in stored Cowpea, even at a concentration below the lowest amount used.This finding is in agreement with Suleiman *et al* (2018a) findings which showed *E. balsamifera* to be effective in killing adult weevil at a lower concentration within a short period. These findings further support the idea ofBiswas *et al* (2016) who found the effectiveness of *L. inermis*in killing red flower beetle (*T. castaneum*) at a lower concentration within a short time.

CONCLUSION AND RECOMMENDATION

The findings of this study indicated that ethanolic extracts of all the four plant species caused significant mortality on adult *C. maculatus*after 96 hours post-treatment. *E. balsamifera*had a lower LC_{50} value, hence the most toxic plant extract to *C. maculatus*. However,all four plant extracts completely inhibited adult emergence. Additionally, no weight losses were recorded in the cowpea seeds treated with the plant extracts.

Therefore, these botanicals could be used as an option for the control of *C. maculatus* in stored cowpea. However, further research is encouraged to study the organoleptic properties of cowpea seeds treated with the test botanicals to ensure safe consumption and its viability study.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

ACKNOWLEDGEMENT

The authors are thankful to Dr. Babangida Abdulkarim for his assistance in Statistical Analysis and for the general suppot.

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