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Research Article
Bio-efficacy of Some Plants Ethanolic Extracts Against Cowpea Weevil (*Callosobruchus maculatus* Fabricius) Infestation of Stored Cowpea Seeds

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Abstract

Background and Objective: A research was conducted to evaluate the insecticidal effect of ethanolic extracts obtained from the leaves of 3 plants against cowpea weevil; (*Callosobruchus maculates*) infestation of stored cowpea seeds. **Materials and Methods:** Ethanolic extracts at 6 different concentrations (0.5, 1.0, 2.0, 4.0 and 8.0 mg L^{-1} and control) were formulated from the leaves of *Hyptis suaveolens* (Lamiaceae), *Alstonia boonei* (Apocynaceae) and *Tephrosia vogelii* (Fabaceae) and were tested for their protecting abilities on cowpea seeds and their toxicity on cowpea bruchid, *C. maculatus* (F.). The data obtained were analyzed using Analysis of Variance with significant means separated using least significant difference. **Results:** The results obtained revealed significant difference ($p \leq 0.05$) in the insecticidal efficacy of the 3 plant extracts relative to control in inducing mortality and inhibiting oviposition and adult emergence thereby reducing seed damage. About 79.10% mortality was induced by 8.0 mg L^{-1} concentration obtained from *Tephrosia vogelii* extract at 48 h. All test plant extracts at high concentrations of (4.0 and 8.0 mg L^{-1}) caused significant increase in mortality but a significant reduction ($p \leq 0.05$) in oviposition, fecundity and progeny emergence relative to control. **Conclusion:** It was concluded that, all the extracts from the 3 test plants showed high insecticidal activity against *C. maculatus* and hence protect the stored cowpea seeds from its infestation. The trend in toxicity of the extracts is in the following order: *T. vogelii* > *H. suaveolens* > *A. boonei*. The effect is concentration dependent, increases with increase in concentration. The 8.0 mg L^{-1} concentration is recommended for used against *C. maculatus* infestation.

Key words: Cowpea, *Callosobruchus maculatus*, concentrations, ethanolic extracts, infestation

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Competing Interest: The authors have declared that no competing interest exists.

Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

Cowpea (*Vigna unguiculata* [L.] Walp) is a leguminous crop grown mostly for its nutritious seeds and leaves. It is produced and consumed largely by subsistence farmers in the semi-arid and sub-humid regions of Africa[1] where famine and malnutrition exists. It is an important cash and food crop for many poor people in Nigeria as it serves as the major integral component of their diets. Cowpea is relatively cheap and supplements the protein requirements of many families in Africa[2] where meat and other sources of animal protein are very expensive. Nigeria is the largest producer and consumer of cowpea in the world[3] and it was estimated by FAO that 3.3 million tons of cowpea dry grains were produced[4] in 2000. Despite the several economic values of cowpea and its relative importance to Nigeria's economic development, it fails to meet the qualitative and quantitative needs of the population. One major constraint to cowpea is its vulnerability to storage pests mostly insects pests[5].

The cowpea weevil, *Callosobruchus maculatus*, F., (Coleoptera: Bruchidae) is a serious pest of stored cowpea grains in Sub-Saharan Africa[6] in general and Nigeria in particular. Post harvest losses of cowpea 3-4 months in storage caused by *C. maculatus* infestation have been reported as high as 50% in Northern Nigeria[7] and 60% in Northern Ghana[8]. The loss of cowpea is a serious problem in Africa where as much as 20-50% of the grain is damaged by *C. maculatus*[9]. The infestation by the weevil cause tremendous reduction in weight, viability and marketability of cowpea seeds[10]. A number of management techniques are available to control stored products insect pests. The synthetic insecticides are commonly used to control pests more especially stored product pests. But these methods posed threat to non-targeted populations and manifold health and environmental problems, like development of resistance in insect pests, residues in food product, pest resurgence and carcinogenesis[11]. These problems lead to a search for more effective method of controlling cowpea weevil that is safer and eco-friendly. One alternative control method is the use of plant extracts[12-13] which favors natural enemies, necessary for the biological balance[14]. This study therefore aimed at testing the bio-efficacy of some plants extracts in the control of *C. maculatus* infestation of stored cowpea grains.

MATERIALS AND METHODS

Test plants: Fresh leaves of *Hyptis suaveolens* (Lamiaceae), *Alstonia boonei* (Apocynaceae) and *Tephrosia vogelii* (Fabaceae) were procured from vendors at Oyingbo market in Lagos and identified in the Herbarium of the Department of Botany, University of Lagos, Nigeria.

Source of insects: The total of 600 larvae and adults of *C. maculatus* were derived from a laboratory mass rearing facility. Insects were supplied with fresh cowpea seeds and were reared in wood cages according to the technique describe by Dabire *et al.*[15]. Toxicity tests were carried out on 1st, 3rd and 5th instars larvae and adults of *C. maculatus*.

Preparation of plants ethanolic extracts

Fresh leaves of the 3 test plants: *Hyptis suaveolens*, *Alstonia boonei* and *Tephrosia vogelii* were air dried at room temperature, pounded to fine powder by pestle and mortar according to the protocols described by Dabire *et al.*[16]. The ethanolic extracts were prepared by soaking 100 g of each powder in 150 mL of 95% ethanol and shaken in orbital shaker at 120 rpm. The preparations were left to stand for another 24 h and then filtered through a gauze and then Whatman No. 1 filter paper. The filtrates were concentrated to dryness at 40°C under reduced pressure on a rotary evaporator and were stored in a refrigerator at –4°C until the need arise. Different concentrations of 0.5, 1.00, 2.00, 4.00 and 8.00 mg L^{-1} were prepared from each of the plant materials.

Disinfestation of test cowpea seeds: Cowpea seeds were obtained from Bariga market, Lagos. They were identified at the International Institute of Tropical Agriculture (IITA), Ibadan. All damaged seeds and debris were sorted out from the grains after which disinfestations was carried out in an oven at 50°C for 6 h to kill all life stages of insects within the seeds. The seeds were then left respectively for 24 h to stabilize at ambient conditions.

Culture of test insects: *C. maculatus* were maintained on disinfested cowpea seeds. Fifty unsexed 7-14 day old adults of the test insects (*C. maculatus*) were introduced into 500 g of disinfested cowpea seeds in 1 L kilner jars respectively in three replicates in the laboratory. All adult insects were left for seven days to allow for oviposition, after which they were removed. They were then left undisturbed until adults were observed to emerge. At each peak of emergence, the adults were removed and used to set up new cultures. Series of fresh cultures were made from these to ensure regular supply of adult insects of known ages for use in subsequent experiments.

Hermetic storage of ethanolic extract of test plants on cowpea for a period of 6 months: Five kilograms of disinfested cowpea seeds were measured into plastic containers. Concentrations of 0.5, 1.00, 2.00, 4.00 and 8.00 mg L^{-1} of the ethanolic extracts of *Hyptis suaveolens*, *Alstonia boonei* and *Tephrosia vogelii* were added on the seeds and manually agitated, labeled accordingly and left to air dry. The seeds were afterwards placed into jute bags and replicated 3 times. The seeds in the control were treated with ethanol. Thirty unsexed insects were released into each bag as well as that of the control. The bags were kept inside drums in the laboratory. Monthly readings were taken for 6 months in which 100 g of the treated and untreated cowpea were taken from each bag and assessed for insect damage according to Odeyemi and Daramola[17].

Insect damage in grains: At the end of the adult emergence treatment, one hundred seeds were randomly selected from each treatment and examined for feeding holes with the help of hand lens (Mag. = X10). Seeds containing 3 or more holes were considered as damaged seeds. Number of damaged and un-damaged seeds were counted and recorded for each replicate.

Statistical analysis: The data obtained was analyzed using analysis of variance (ANOVA) using SPSS (11.0 versions) with least significant difference used to compare the means.

RESULTS

The result for the toxicity of the ethanolic extracts to *C. maculatus* is presented in Table 1. The result showed that, the trend in the toxicity showed that, *T. vogelii* is the most toxic followed by *H. suaveolens* with 24 h LC_{50} values of 4.56 and 5.65 mg L^{-1}, respectively while *A. boonei* was the least toxic with 24 h LC_{50} value of 6.13 mg L^{-1}. Similarly, after 48 h of exposure, *T. vogelii* with LC_{50} value of 2.27 mg L^{-1} remained the most toxic against *C. maculatus* followed by *H. suaveolens* with 3.38 mg L^{-1} while *A. boonei* remained the least toxic with LC_{50} of 3.98 mg L^{-1}. Toxicity factor can be calculated as following Eq:

$$\text{Toxicity factor (TF)} = \frac{48\text{ h } LC_{50} \text{ value of the least toxic compound}}{48\text{ h } LC_{50} \text{ value of the more toxic compound}}$$

The result for the adult mortality of *C. maculatus* due to exposure to cowpea grains treated with the 3 plants extracts is presented in Table 2. The result indicated significant ($p \leq 0.05$) increase in the number of adult insects that died with the increase in concentrations of the extracts. *Tephrosia vogelii* ethanolic extracts were found to induced the highest mortality rate of 79.10% under 8.00 mg L^{-1} concentration while that of *A. boonei* induced the least (64.20%).

Similarly, the result for percentage adult emergence from the laid eggs in the cowpea seeds is shown in Table 3. The result revealed that. In the cowpea seeds treated with the extracts obtained from *Tephrosia vogelii*, 14-26% adults emerged from the laid eggs. The effect is concentration dependent, increased with increase in concentration. However, 36-70% of adult insects emerged from the laid eggs on cowpea seeds treated with plant ethanolic extracts obtained from *H. suaveolens.* In *Alstonia boonei* ethanolic extracts treated cowpea, 33-57% of the adults emerged.

DISCUSSION

Leaf ethanolic extracts of *Hyptis suaveolens* (Lamiaceae), *Alstonia boonei* (Apocynaceae) and *Tephrosia vogelii* (Fabaceae) were found useful in controlling the stored cowpea seeds from *C. maculatus* infestation. All concentrations of the plants extracts used in the present study

Table 1: Relative toxicity of various test plant ethanol extracts on *C. maculatus*

Ethanolic extracts	24 h LC_{50} (mg L^{-1})	95% CL	TF	48 h LC_{50}	95% CL	TF
Tephrosia vogelii	4.56	2.61-15.95	1.44	2.27	-	1.52
Hyptis suaveolens	5.65	3.79-11.94	1.78	3.38	0.46-877.97	2.27
Alstonia boonei	6.13	-	1.95	3.98	-	2.67

CL: Confidence limit, LC_{50} values with no overlap in 95% confidence limits are significantly different

Table 2: Mortality of *C. maculatus* adults during exposure to grains treated with test plants ethanol extracts

	0.00	0.5	1.00	2.00	4.00	8.00
Ethanolic extract	mg L^{-1}					
Tephrosia vogelii	0.00[a]	46.50[a]	60.00[a]	67.37[a]	72.35[a]	79.10[a]
Hyptis suaveolens	0.00[a]	33.50[b]	55.00[b]	59.21[b]	63.64[b]	69.58[b]
Alstonia boonei	0.00[a]	33.00[b]	48.50[c]	54.12[c]	59.78[c]	64.20[c]

Mean values bearing the same letters are not significantly different (p = 0.05)

Table 3: Oviposition and progeny development of *C. maculatus* on cowpea seeds

Plant extracts	Concentration (mg L^{-1})	Mean number of eggs laid (±SE)	Mean adult emergence (±SE)	Mean adult emergence (%)
Tephrosia vogelii	0.00	97.05±1.32[a]	73.00±6.21[a]	75.22
	1.0	68.32±0.95[b]	17.75±2.24[b]	25.98
	2.0	53.10±3.16[c]	8.00±1.85[c]	15.07
	4.0	40.72±2.08[d]	6.01±1.23[d]	14.76
	8.0	35.52±0.98[d]	4.79±1.40[e]	13.49
Hyptis suaveolens	0.00	96.12±0.95[a]	73.39±6.59[a]	76.35
	1.0	63.12±2.08[b]	43.93±5.59[b]	69.59
	2.0	49.81±3.21[c]	32.89±3.59[c]	66.03
	4.0	39.76±3.16[d]	15.30±2.02[d]	38.48
	8.0	28.38±2.02[d]	10.29±1.29[d]	36.25
Alstonia boonei	0.00	98.30±1.02[a*]	81.00±4.18[a]	82.40
	1.0	46.21±2.14[d]	26.56±1.23[c]	57.48
	2.0	34.31±0.91[e]	12.10±1.79[d]	35.27
	4.0	29.15±1.08[c]	10.54±1.12[b]	36.16
	8.0	21.05±0.98[b]	7.01±0.14[b]	33.25

*Means bearing the same superscripts down a column are not significantly different (p = 0.05)

affected the insects' pest significantly by inducing mortality and suppressing development of *C. maculatus* in comparison with control, which may probably be due to the presence of certain chemical constituents in the 3 plants. This finding is in conformity with that of Hole *et al*.[18], who reported significant insecticidal activity of some plant extracts against aphids, jassids, thrips and whiteflies. More so, Mkindi *et al*.[19] reported the use of pesticidal plants against insects' pest. However, Muhammad and Bashir[20] reported that, plants ethanolic extracts from Neem (*Azadirachta indica*), Garlic (*Allium sativum*), West African pepper (*Piper guinense*), drum stick (*Moringa oleifera*), African Basil (*Ocimum gratissimum*), Moss plant (*Funaria hygrometrica*), *Barbula indica* and *Clausena anisata* have been used in the control of *C. maculatus*. Similarly, the presence of significant difference between the ethanolic treatments and the control in inducing mortality of the test insect conforms to the finding of Lawal *et al*.[21], who reported significantly differences between plant products treatments and the synthetic treatment over the control in inducing mortality of *C. maculatus*.

The significant mortality rate of *C. maculatus* adults due to exposure to different concentrations of the test plants reported by this study is in agreement with the work of Mofunanya and Nta[22], who reported significant mortality in *C. maculatus* adults exposed to different plant extracts. The ethanolic extracts of the test plants showed significant insecticidal activity against the stored grain insect pests and suppressing emergence of progeny in treated grains. This finding is in agreement with the work of Gotyal *et al*.[23] and Rajashekar *et al*.[24], who individually reported similar activity in *Lantana camara* methanolic extracts. More so, Ivbijaro and Agbaje[25] reported that maize grains mixed with dry neem seeds were protected in storage from damage by *S. oryzae,* while Makanjuola[26] reported that neem extract protected cowpea seeds against *C. maculatus*. Both lower and higher doses of the extracts controlled the weevils but the effect is concentration dependent, increase with increase in concentration. This finding is in conformity with that of Omotoso[27] who reported that lower doses of plant extracts below 5 mL do not necessarily control the weevil but higher doses do. Similar finding was reported by Adedire and Lajide[28], Ashamo and Akinnawonu[29] and Omotoso[30]. The higher mortality of ethanolic extracts are in accordance with the findings of Aku *et al*.[31], who also reported a higher toxicity of extracts of *Annona senegalensis* on *C. maculatus*. The ovicidal properties of the three plant extracts reported by this study agrees with the work of Ofuya[32] and Lale[33], who individually reported that the mechanisms of action of plant extracts include their toxicity to adults, reduction of oviposition, ovicidal activity and toxicity to immature stages of the insect.

The inhibitory effect of the ethanolic extracts on oviposition showed that the number of eggs of *C. maculatus* was significantly high in the control compared to the remaining cowpea seeds treated with the test plants. This inferred that the insect's reproduction and development were impaired in all the botanicals pesticides. This finding is in conformity with the work of Bekele *et al*.[34], who reported that the toxic properties of plants products compiled within the growth and development of eggs decreased the number of progeny that emerged in treated cowpea grains. Similarly, the finding of this study that higher doses of the extracts inhibit oviposition and progeny development of *C. maculatus* agrees with the findings of Rajapakse *et al*.[35] and Swella and Mushobozy[36] who individually reported high insecticidal activity against oviposition and progeny development by higher doses of *Annona senegalensis* seed powder thereby protecting cowpea seeds against the weevil's infestation.

Thus, the present study establishes the insecticidal potency of *Hyptis suaveolens*, *Alstonia boonei* and *Tephrosia vogelii* in the control of cowpea weevil's infestation of stored cowpea seeds. However, 8.0 mg L^{-1} of *Tephrosia vogelii* is recommended for use as it shows high efficacy against the weevil. The study is limited to the use of 6 different ethanolic concentrations on cowpea weevil.

CONCLUSION

From the result of the present study it was concluded that, ethanolic extracts from the three test plants play a significant role in protecting cowpea seeds under storage from the weevil's infestation. It was recommended that, 8.0 mg L^{-1} of the extract obtained from *Tephrosia vogelii* be used in the control of the weevil.

SIGNIFICANCE STATEMENTS

This study discovered that, ethanolic extracts obtained from the three test plants are effective in protecting cowpea seeds from *C. maculatus* infestation. As such, the extracts can be beneficial in the biological control of the weevil using natural insecticides that are eco-friendly and non-toxic to man and other non-target populations. It adds to the existing list of plants with insecticidal potency. This study will also help the researcher to uncover the critical areas of using bio-pesticides that many researchers were not able to explore. Thus a new theory on the Bio-efficacy of natural botanical pesticides in the biological control of insect pests of stored products may be arrived at.

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