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Efficacy of Three Plants Ethanolic Extracts as Protectants Against Cowpea Weevil (*Callosobruchus maculatus* Fabricius [Coleoptera: Chrysomelidae]) Infestation in Hermetic Storage Condition

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Abstract
This research was conducted to determine the effect of ethanolic extracts from three plants on cowpea weevil (Callosobrus maculatus) that causes significant damage to stored cowpea seeds thereby imparting significant economic losses. Different ethanolic extracts concentrations (0.5, 1.0, 2.0, 4.0 and 8.0 mg/l) and 0.0 mg/l as the control were prepared from three different plants: Jatropha curcas, Plumbago zeylanica and Uvaria chamae. Clean cowpea seeds were introduced with the adult test organisms, treated with the various concentrations of the ethanolic extracts and stored in jute bags. The set up was replicated three times under hermatic condition (air-tight) for a period of six months. The seeds in the control were treated with only ethanol. The mortality of the insects was determined and used to compute mean lethal concentration (LC_{50}) values by probit analysis. Observations were made on monthly basis for a period of six months. The result obtained revealed significant difference ($P \leq 0.05$) in the percentage mortality of C. maculatus among the various treatments compared to the controls. More so, the result indicated that, no infestation was recorded among U. chamae and J. curcas treatments due to C. maculatus for the first two months. The result implies that, all the concentrations tested showed appreciable toxicity against C. maculatus and hence gave protection to the insects' infestation. The effect of U. chamae extracts was found to be more effective compared to the remaining two extracts. It was found that the higher the concentration of the ethanolic extract, the higher the mortality of C. maculatus. It was therefore inferred that, the U. chamae, P. zeylanica and J. curcas ehanolic extracts have potentials for use during storage of cowpea seeds, ensuring food security, profit maximization and availability of seeds for the next planting season without being damaged by C. maculatus. The insecticidal effect is in the following trend: U. chamae>J. curcas>P. zeylanica.

Keywords: *Callosobruchus maculates,* Hermetic storage, Jute bags, *Sitophilus zeamais.*

INTRODUCTION
Cowpea is known scientifically as *Vigna unguiculata* (L.) Walp and is one of the most commonly important leguminous food crops grown in the sub-saharan Africa (Ndiaye, 2007; Adedire *et al.*, 2011). It is mostly grown for its seeds and foliage which are good sources of

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protein (23-35%), carbohydrate (60-68%), minerals (iron and calcium), vitamins and carotene (Adedire *et al*., 2011) and are used in preparing several dishes for man and livestock (Bressani, 1985). Cowpea is relatively cheap and supplements the protein requirements of many families in Africa (Nta *et al*., 2013) where meat and other sources of animal protein are very expensive. Nigeria is the largest producer and consumer of cowpea in the world (Prereia *et al*., 2001; Lowenberg-Deboer and Ibro, 2008) and it was estimated by FAO that 3.3 million tons of cowpea dry grains were produced in 2000 (IITA, 2009). Despite the several economic values of *Vigna unguiculata* and its relative importance to Nigeria's economic development, it fails to meet the qualitative and quantitative needs of the population. This is because its cultivation and storage are limited by pathogenic and pests infestations such as attack by field and storage pests. but only a small proportion enters international trade due to losses by insects pests during storage. Insects are the most serious pests of stored cowpea (Tripathy *et al*., 2001). Loss of yield and stored produce due to insects' infestation is said to be between 20-40% annually (Gosh and Durbey, 2003; Ngakou *et al*., 2008). These pests which have a worldwide distribution are numerous in species and number particularly in West Africa.

Callosobruchus maculatus, a major insect pest of stored cowpea seeds in Nigeria and has been reported to cause tremendous reduction in weight, viability and marketability of cowpea seeds (Adedire and Akinneye, 2004). The devastating effects of this insect pest (*Callosobruchus maculatus*) on cowpea have call for an urgent need for the application of effective and affordable control measures so as to increase food production thereby avoiding food crises (Ileke, 2015). Although synthetic insecticides are effective and quick in action, they are not eco-friendly and are mostly toxic if consumed. Safer and more environment-friendly alternative methods of controlling insect pests on stored grains are therefore needed. Hermetic (Air tight) storage which is commonly used in Africa prevents any insect from entering and also cause death due to lack of oxygen and excess carbon dioxide. It also stops water from getting into the stored grain from outside. However the airtight nature of the hermetic storage leads to moisture migration and condensation, which leads to caking of the grains. Hermetic storage is one of the oldest forms of food preservation in the world (De Lima, 1990), it provides an airtight, safe and pesticide free means of storing dry food commodity. In modern hermetic storage, constant moisture content can be maintained. Under these conditions, respiration by insects initially present in the grain as well as the grain itself releases CO_2 into the surrounding enclosed space thereby depleting the available oxygen. This biologically modified atmosphere arrest the development of pest populations. Some studies have shown that botanicals may serve as such alternatives (Denloye *et al*., 2007). Botanical insecticides remain important in insect pest management because they are believed to provide the most effective control against insect pests that have become resistant to other insecticides (Weinzierl, 2000). This study therefore aimed at investigating the insecticidal efficacy of three different plants ethanolic extracts in combination with hermetic storage condition as protectants against *Callosobruchus maculates* infestation.

MATERIALS AND METHOD

Test Plants

Fresh seeds of *Jatropha curcas,* bark of *Uvaria chamae* and stem of *Plumbago zeylanica* 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 larvae and adults of *C. maculatus* were derived from a laboratory mass rearing facility of the Department of Zoology, University of Lagos. Insects were supplied with fresh cowpea seeds and were reared in wood cages according to the technique described by Dabire *et al*. (2005). Toxicity tests were carried out on 1st, 3rd and 5th instars larvae and adults of *C. maculatus*.

Preparation of Plants Ethanolic Extracts
Fresh bark of *Uvaria chamae,* stem of *Plumbago zeylanica* and seeds of *Jatropha curcas* plants were air dried at room temperature for 48 hours, pounded to fine powder using pestle and mortar according to the protocols described by Dabire *et al*. (2008). 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 24 hours and then filtered through a gauze and then through 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. Different concentrations of 0.5, 1.00, 2.00, 4.00 and 8.00 mg/l 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 six hours to kill all life stages of insects within the seeds. The seeds were then left respectively for 24 hours 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 500g of disinfested cowpea seeds in 1L kilner jars respectively within 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 six 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 of the ethanolic extracts of *J. curcas*, *P. zeylanica* and *U. chamae* were applied on the seeds and manually agitated and left to air dry. The seeds were afterwards placed into jute bags and replicated three 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 six months in which 100g of the treated and untreated cowpea seeds were taken from each bag and assessed for insect damage according to Odeyemi and Daramola (2000).

Insect damage in grains
Monthly insect damage in each treatment and control was determined from 100g batches of grains in each jute bag as described by Odeyemi and Daramola (2000):

$$\text{Percentage weight loss} = \frac{(W_u \times N_d) - (W_d \times N_u)}{W_u (N_d + N_u)} \times 100$$

Where:

W_u =weight of undamaged grains

N_u = Number of undamaged grains

W_d = weight of damaged grains

N_d =Number of damaged grains

Data Analysis

The data obtained were analyzed using Analysis of variance (ANOVA) using SPSS (11.0 versions) with Least Significant Difference used to compare the means.

RESULTS

The results for the effect of the three plants ethanolic extracts on the seeds of cowpea from *C. maculatus* are presented in Fig 1. The result showed that, the three plants extracts offered protection to the cowpea seeds against the test insects for a period of two months. However, the protection against the weevil infestation is extended to three months by the extracts of *J. curcas* and *U. chamae*. Thus, the protection offered by the three extracts is in the order of *J. curcas*>*U. chamae*>*P. zeylanica*>control.

There was no grain damage on treated cowpea seeds due to the test's insect infestation on *J. curcas* for four months, while *P. zeylanica* and *U. chamae* gave two and three months' protection respectively (Fig 1).

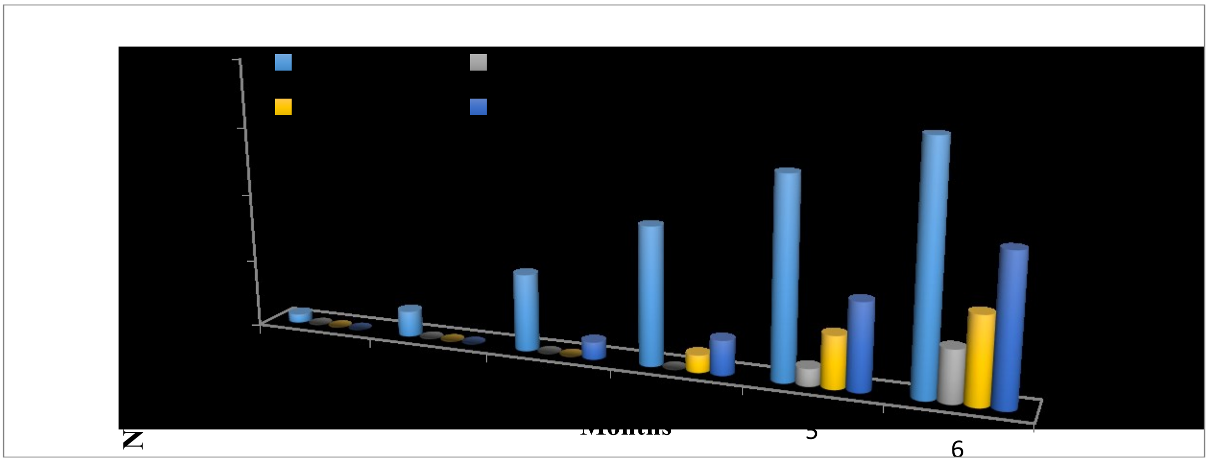

Figure 1: Damage of Cowpea Seeds Protected with Test Plant Extracts

More so, the result for the relative toxicity of the extracts to *C. maculatus* is presented in Table 1. The result revealed that, the trend in the toxicity showed that, *U. chamae* is the most toxic followed by *J. curcas* with 24h LC_{50} values of 3.163mg/l and 3.774mg/l respectively while *P. zeylanica* was the least toxic with 24h LC_{50} value of 4.524mg/l. Similarly, after 48h of exposure, *U. chamae* with LC_{50} value of 1.492mg/l still remained the most toxic against *C. maculatus* followed by *J. curcas* with 1.518 mg/l while *P. zeylanica* remained the least toxic with LC_{50} of 2.267mg/l.

Table 1: Toxicity of Three Plants Ethanolic Extracts on *C. maculatus*

Ethanolic extracts	24hr LC_{50}(mg/l)	95% CL	TF	48hr LC_{50}	95% CL	TF
Jatropha curcas	3.774	2.506-5.647	1.19	1.518	-2.027-7.309	1.02
Uvaria chamae	3.163	-	1.00	1.492	-1.409-5.687	1.00
Plumbago zeylanica	4.524	2.926-19.581	1.43	2.267	-0.547-9.575	1.2

N.B: CL = Confidence Limit **LC_{50}= Test of significance**

Values that do not overlap in 95% confidence limits are significantly different

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

Furthermore, the result for the percentage mortality induced by various concentrations of the three plant extracts is shown in Table 2. It showed the presence of significant difference (P≤0.05) in the percentage mortality induced by various concentrations of the three plants extracts on *C. maculatus*. The result signifies that, higher concentrations of the extracts (8.0 mg/l) exerted the highest mortality (78.9%, 77.5% and 73.5%) for *U. chamae, J. curcas* and *P. zeylanica* respectively. The effect of the extracts is concentration dependent. Increases with increase in concentration with the magnitude of activity in the order *U. chamae*>*J. curcas*>*P. zeylanica*.

Table 2: Mortality of *C. maculatus* **Adults during Exposure to Seeds Treated with Ethanolic Extracts**

Ethanolic extract	0.00mg/l	0.5mg/l	1.0 mg/l	2.0mg/l	4.0 mg/l	8.0mg/l
Jatropha curcas	0.00^{f*1}	52.5^{e}	59.7^{d}	72.1^{c}	74.9^{b}	77.5^{a}
Uvaria chamae	0.00^{e}	55.0^{d}	62.3^{c}	75.2^{b}	77.6^{a}	78.9^{a}
Plumbago zeylanica	0.00^{f}	48.5^{e}	54.2^{d}	61.9^{c}	65.8^{b}	73.5^{a}

Key: *1Mean values bearing the same letter(s) across a row are not significantly different (at P= 0.05)

Similarly, the result for the effects of the ethanolic extracts on the oviposition and progeny development of *C. maculatus* on cowpea treated with various test plant formulations is presented in Table 3. The result indicated that, the number of laid eggs decrease with increase in concentration of the various extracts. The lowest number of eggs was laid on the seeds treated with 8.0 mg/l of the *U. chamae*. The highest number of eggs was laid on the controls. However, the lowest mean number of eggs that emerged from each treatment showed that the number decreased with increase in concentrations of the extracts. Among the seeds treated with *J. curcas* extracts, the mean percentage number of adults that emerged from the eggs ranges between 42-60% with decrease in concentrations. However, among the seeds treated with *P. zeylanica* the mean percentage number of adults that emerged ranges between 56-66% with decrease in concentration. Similarly, among the seeds treated with U. chamae extracts, the percentage number of adults that emerged ranges between 24-40%.

Table 3: Oviposition and Progeny Development of *C. maculatus* on Cowpea Seeds

Plant Extract	Concentration (mg/L)	Mean number of eggs laid (±SE)	Mean adult emergence (±SE)	Mean percent adult emergence (%)
J. curcas	0.00	95.25±2.22[a*1]	70.00±6.11[a]	73.49
	1.0	26.14±1.08 [d]	15.79±1.14[c]	60.41
	2.0	19.25±0.96[e]	10.36±0.99[d]	53.82
	4.0	35.25±1.26[c]	18.93±1.92[b]	53.70
	8.0	47.00±1.83[b]	19.82±4.61[b]	42.17
P. zeylanica	0.00	95.75±0.96[a]	73.39±6.59[a]	76.65
	1.0	66.75±2.75[b]	43.93±5.59[b]	65.81
	2.0	42.75±5.32[c]	35.89±3.59[c]	83.99
	4.0	25.32±2.81[d]	15.3±2.02[d]	60.43
	8.0	25.50±2.52[d]	14.29±1.29[d]	56.03
U. chamae	0.00	96.00±0.82[a]	73.00±6.21[a]	76.04
	1.0	45.00±0.82[b]	17.75±2.24[b]	39.45
	2.0	29.25±1.26[c]	8.00±1.85[c]	27.35
	4.0	19.89±1.06[d]	6.01±1.23[d]	30.17
	8.0	19.75±1.71[d]	4.79±1.40[e]	24.25

Key: *1 Means bearing the same superscript(s) down a column are not significantly different (P=0.05)

DISCUSSION

Different plants species exhibit different magnitude of insecticidal potency against different insects. The differences in protection capacity of the different test plants against *C. maculatus* can probably be related to the proportion of the active chemicals in the extracts and their differential solubility in the ethanol solvent. This finding is in conformity with that of Ito and Ighere (2017) who reported differences in the insecticidal activities of five plant extracts (*Allium sativum* [Garlic], *Cordia millenii* [Manjack], *Monodora myristica* [Nutmeg], *Xylopia aethiopica* [Negro pepper] and *Zingiber officinale* [Ginger]) against *Callosobruchus maculatus* infesting cowpea seeds. This study therefore confirmed the previous findings of Emeasor *et al*. (2005), Adebowale and Adedire (2006), Alyelaagbe *et al*. (2007) and Rahuman *et al*. (2008) on pesticidal properties of *J. curcas* against *C. maculatus*. Also Adebowale and Adedire (2006) found that, *J. curcas* extract was effective as a grain protectants as no bruchids developed in grain legumes stored for 12 weeks. The present study therefore revealed that, extracts from both species of plants used caused a reduction in *C. maculatus* emergence and infestation levels with increase in concentrations. This can be attributed to the fact that, higher concentrations of the extracts probably exert repellency and higher toxicity leading to damaging of most of the eggs or drastically change the environmental conditions needed for the emergence of adult pests. This can also be confirmed from the fact that all the tested plants extracts were toxic and could be used as protectant against *C. maculatus* infestation on stored cowpea seeds. This finding is in agreement with that of Opareke and Dike (2005), Mukanga *et al*. (2010), Ileke and Oni (2011) who individually reported that, certain botanicals are effectively toxic against storage insect pests including *C. maculatus*. The three test plants extracts are therefore very effective protectants against *C. maculatus* as they caused more than 50% mortality of the test insect. This is in line with the work of Detheir *et al*. (1996) and Grøntved and Pittler (2000) who reported the presence of certain phytochemicals which believed to be toxic or act as insects' repellants.

The toxicity of the three plant extracts on the test organism signifies the relative importance of the extracts in preventing the damage induced by *C. maculatus* on stored cowpea seeds. This may probably increase the longevity of the seeds and their market qualities without disrupting the nutritional qualities. This finding is in conformity with the work of Adeniyi *et al*. (2010) who reported that plant extracts from *Vernonia amygdalina* (leaf) at 4.0% concentration resulted in higher toxicity (measured as percentage mortality) to *Acanthoscelides obtectus*. This finding therefore adds to the existing data on the efficacy of plant extracts as biopesticides of stored food as highlighted by Adeniyi *et al*. (2010) that extracts from leaves of *Oscimum gratissimum*, *Sida acuta*, *Telfaria occidentalis* and *V. amygdalina* possess good insecticidal potential because of their phytochemical constituents and the order of toxicity at different concentrations was 4.00 > 3.00>2.00>1.00%. This is similar to the finding of the present study. The toxicity of the ethanolic extracts of the test plants used in this study is in agreement with that reported by Alabi *et al*. (2005) and Ijeh and Ejike (2011) who individually reported that, aqueous extract of the leaves of *Vernonia sp*. has high phytotoxic properties. The active ingredients in the extracts caused different magnitude of toxicity to *C. maculatus* either by contact, stomach or through respiratory poison as reported by Adebiyi and Tedela (2012) and Adenakan *et al*. (2013).

CONCLUSION

It was concluded that, ethanolic extracts obtained from *U. chamae*, *J. curcas* and *P. zeylanica* are very effective botanical insecticides that are toxic to *C. maculatus* thereby served as protectants against the weevil's infestation on stored cowpea seeds. They have potentials for use during storage of cowpea seeds, ensuring food security, profit maximization and availability of seeds for the next planting season without being damaged by *C. maculatus*. Their efficacy increased with increase in concentrations and their insecticidal effect is in the following trend: *U. chamae*>*J. curcas*>*P. zeylanica*.

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