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Insecticidal Activity of Stem Bark Extract of *Lophira Alata* (Ekki) Against Cowpea Bruchid (*Callosobruchus Maculatus*)

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ABSTRACT
Methanolic extract of *Lophira alata* was evaluated for its efficacy as contact and fumigant insecticides on cowpea bruchid, *Callosobruchus maculatus* in the laboratory at ambient tropical conditions of temperature and relative humidity. The plant powder tested was applied at rates 0.0 (control), 2.0 g and 3.0 g / 20 g of cowpea seeds either directly for contact with the insect pest or in plastic containers to assess its fumigant toxicity. Results of contact toxicity assay showed that powders of *L. alata* was effective against the adult *C. maculatus* causing 90 % mortality (4.00 ± 0.57) within 2 days of application at 3.0 g / 20 g of cowpea seeds as compared with 90 % mortality (5.38 ± 0.50) recorded on day 4 of 2.0 g concentration application. The results of fumigant assays showed that *L. alata* had the highest insecticidal activity causing 95 % mortality of *C. maculatus* within 4 days of application at rate 3.0 g / 20g of cowpea seeds in contrast to 80 % mortality recorded in 96 hrs of 2.0g concentration application. The phytochemical screening of the plant revealed alkaloids, saponins, glycosides, phytosterols, tannis, flavonoids and terpenoids while reducing sugar was absent. This study showed that the tested plant product is toxic to cowpea bruchid and the powders can be mixed with cowpea seeds to prevent hatching of the eggs thereby helping in their management.

KEYWORDS: *Callosobruchus maculatus, Lophira alata, Fumigant, Cowpea, Insecticidal*

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INTRODUCTION

Cowpea is one of the most significant pulse crops local to Africa. Cowpea is called vegetable meat because of the high measure of protein in grain with better organic incentive on a dry weight premise. On a dry weight premise, cowpea grain contains 23.4 percent protein, 1.8 percent fat, and 60.3 percent sugars and it is rich in calcium and iron. Aside from this, cowpea structures brilliant scrounge and it gives a substantial vegetative development and spreads the ground so well that it checkmates the soil erosion disintegration. As a leguminous crop, it fixes around 70 – 240 kg for each ha of nitrogen every year [1]. Cowpea is chiefly cultivated in tropical and sub-tropical areas for its vegetable, seed and to lesser degree as a fodder crop. It is an adaptable crop in view of its covering nature, drought resistant nature, soil reestablishing properties and multi-consumption methods. As a pulse, cowpea fits well into a large portion of the trimming frameworks of cropping [1]. The current day cultivars display lower productivity, non-simultaneous blooming and fruiting, non-reaction to high portions of sources of organics like composts and fertilizers, water system, culturing, resistance to major insect pest and diseases which cause considerable damage and very poor harvest [1]. The cowpea bruchid is planted and found everywhere, with its underlying invasion beginning in the field not long before harvest and are conveyed into the store where it quickly increases [2,3]. Ripe and matured dry cowpea seeds are used for preparing delicasies for consumption purposes. In Nigeria, cowpea is associated to pestivorus attack during pod and dry seed stages in the field during cultivation and in storage after harvesting. C. maculatus has caused huge weight reduction and reduced market value of cowpea seeds [4]. It has been accounted for that both quantitative and subjective loses emerging from physical, chemical and biological factors happen during stocking of grains in different forms of facilities and stores [4].

The control of insect pests of stored grains and pulses are achieved through use of synthetic chemical insecticides, such as methyl bromide. Several nations have however banned the usage due to environmental concerns and health hazards associated to human consumption of these grains [5]. The adverse effects of chemical insecticides have led researchers to try to find new avenue of insect control, which has led to the discovery of plant products as an alternative way of controlling insects [6,7]. Moreover, tropical regions are believed to be endowed with many plant species with insecticidal properties and some of them are with medicinal properties [1]. Therefore, this research investigates the insecticidal activity of oil extracts Ekki (*Lophira alata*) against *C. maculatus* which is an important insect pests of cowpea in storage.

Materials and methods

Study area

The study was conducted in the Zoology Departments Research Laboratory, University of Lagos and Pharmacology Department Laboratory, Lagos University Teaching Hospital Lagos.

Insect culture

The insects used to establish a laboratory colony of *C. maculatus* came from a batch of infested cowpea seeds, *Vigna unguiculata* (L.) Walp variety Ife brown collected from Nigeria Stored Products Research Institute Lagos, Nigeria. Beetles were reared subsequently by replacement of devoured and infested cowpea seeds with fresh un-infested cowpea seeds in 2-L kilner jars covered with muslin cloth to allow air circulation. Insect rearing and the experiments were carried out at ambient temperature of 28 + 2 ^{o}C and 75 + 5 % relative humidity.

Plant collection

The plant evaluated in this work was *Lophira alata* (Stem bark). It was obtained in fresh form, free of insecticides from LUFASI Nature Park, Eti-Osa, Lagos State, Nigeria and authenticated by the Botany Department, University of Lagos, Lagos State. The plant materials were rinsed in clean water to remove sand and other impurities, cut into smaller pieces before air dried in a well-ventilated laboratory for four weeks and ground into very fine powder using a mechanical blender. The powder was packed in plastic containers with tight lids and stored in a refrigerator at 4 ^{o}C prior to use.

Collection of cowpea seeds

Cowpea seeds used for this study were obtained from a newly stocked seeds free of insecticides at Nigeria Stored Products Research Institute Lagos State, Nigeria. Firstly, the seeds were cleaned and disinfested by keeping at -5 ^{o}C for 7 days to kill all hidden infestations. This is because all the life stages, particularly the eggs are very sensitive to cold [8]. The disinfested cowpea seeds were then placed inside a Gallenkamp oven (model 250) at 40 ^{o}C for 4 hours [9] and later air dried in the laboratory to prevent mouldiness [10] before they were stored in plastic containers with tight lids.

Extract preparation

Hydroalchoholic extracts of *L. alata* was carried out using cold extraction method. 730 g of the powder was weighed using an analytical balance (OHAUS, model number: **PA124**), and soaked separately in an extraction bottle containing 5 liters of absolute methanol and water in the ratio 8:2. The mixture was stirred occasionally with a glass rod and extraction was terminated after 72 hrs. The resulting mixture was filtered using a double layer muslin blanket and the solvent was evaporated using a rotary evaporator (EYELA, model number **SB-1300**) at 30 to 40 ^{o}C with rotary speed of 3 to 6 rpm for 8 hrs. The resultant moisture solid was transferred into a desiccator for 24hrs to remove any residual moisture, until a dry weight was achieved. The solid extract was ground into a fine powder using a mortar and pestle and the powder was stored at 4^{0}C in the refrigerator prior to further use. [11].

Phytochemical screening

Phytochemical analysis and screening was carried out using simple chemical tests to detect the presence of secondary plant constituents such as alkaloids, tannins, flavonoids, saponins, triterpenes, sterols, phenols, glycoside, reducing sugar and soluble carbohydrate in the sample was carried out as described by the general method of [12].

Test of contact toxicity of plant extract on adult mortality, oviposition and progeny development of *Callosobruchus maculatus*

Plant extract of *L. alata* was admixed with cowpea seeds at the rates of 2.0g / 10g of cowpea seeds in 250ml plastic containers. Ten pairs of adult *C. maculatus* (2 to 3 days old) sexed according to the methods described by [13] were introduced into the treated. Untreated cowpea seeds were similarly infested. Four replicates of the treated and untreated controls were laid out in Complete Randomized Block Design in insect cage. Insect mortality was assessed every 24 hours for four days. Adults were assumed dead when probed with sharp objects and no response is made. At the end of day 4, all insects, both dead and alive were removed from each container.

Test of plant extract as fumigant insecticide on adult mortality, oviposition and progeny development of *C. maculatus*

Ten grams of the cowpea seeds were weighed into 50ml transparent plastic tubes that had been cut opened at the bottom and sealed with muslin cloth. Plant extract of *L. alata* weighing 2 and 3g concentrations were put into another half-cut 25ml plastic tubes. The 50ml tube and 25ml tube were then joined together with the aid of gum [14]. Ten pairs of adult *C. maculatus* (2 to 3 days old) sexed according to the methods described above were introduced to the tube containing 10g of cowpea seeds and tightly sealed [14]. Untreated cowpea seeds were similarly infested. Four replicates of the treated and untreated controls were laid out in Complete Randomized Block Design in insect cage. Bruchid mortality was assessed every 24 hours for four days. Adults were assumed dead when probed with sharp objects and made no responses. The experiment was kept inside the insect cage for another 30 days to allow for the emergence of the first filial (F_1) generation. The number of adults that emerged from each replicate was counted with an aspirator and recorded. The percentage adult emergence was then calculated using the method described by [13].

$$\% \text{ Progeny development} = \frac{\text{No of adult emerged}}{\text{No of eggs laid}} \times \frac{100}{1}$$

Statistical Analysis

Statistical Package for Social Science (SPSS, version 23) was used in the analyses of the quantitative data. Data were subjected to one-way analysis of variance (ANOVA). Where significant differences existed, treatment means were separated using the Tukey test and considered significant at $P \leq 0.05$.

Results

Phytochemical screening

The phytochemical analysis of the methanolic extract of *Lophira alata* stem bark reveals that the plant has alkaloids, saponins, glycosides, phytosterols, tannis, flavonoids and terpenoids while reducing sugar was absent as shown in Table 1 below.

Table 1: Result of phytochemical screening of the methanolic extract of *Lophira alata* stem bark

Phytoconstituents	*Lophira alata*
Alkaloids	+
Reducing sugar	-
Saponins	+
Glycosides	+
Phytosterols	+
Tannis	+
Flavonoids	+
Terpenoids	+
Cyanide -	
"+" represent class of phytochemicals present	
"-" represent class of phytochemicals not present	

Table 2: shows the percentage mortality, standard error of mean of adult *Callosobrochus maculatus* treated with *Lophira alata* powder at rate 2.0 g / 20 g and 3.0 g / 20 g of cowpea seeds for contact toxicity.

Treatment	Mortality % ± SEM after			
	24hrs	48hrs	72hrs	96hrs
2g/20g	0.75 ± 0.25^{b}	1.38 ± 0.18^{b}	2.50 ± 0.19^{b}	5.38 ± 0.50^{c}
3g/20g	3.38 ± 0.46^{c}	4.00 ± 0.57^{c}	2.00 ± 0.68^{b}	0.63 ± 0.38^{b}
t-value	5.01	4.41	0.71	7.62
p-value	0.02*	0.07*	0.02*	0.34*
Control	0.00 ± 0.00^{a}	0.00 ± 0.00^{a}	0.00 ± 0.00^{a}	0.00 ± 0.00^{a}

Each value is a mean ± standard error of four replicates. Means within the same column, with different letters are significantly different at $P < 0.05$ using Tukey's test.

Effectiveness of plant powder as contact insecticide at rate 2.0 g / 20 g of cowpea seeds

Figure 1 presents the effect of plant powder on mortality of *C. maculatus.* Within 24 - 48 hrs of application. The plant powder was less potent on the insects, causing 25 % mortality and at 72 hrs, about 35 % insect mortality was also recorded. The plant powder had a significantly high mortality effect on the insects at 96 hrs (Day 4), killing 90 % of the tested insects.

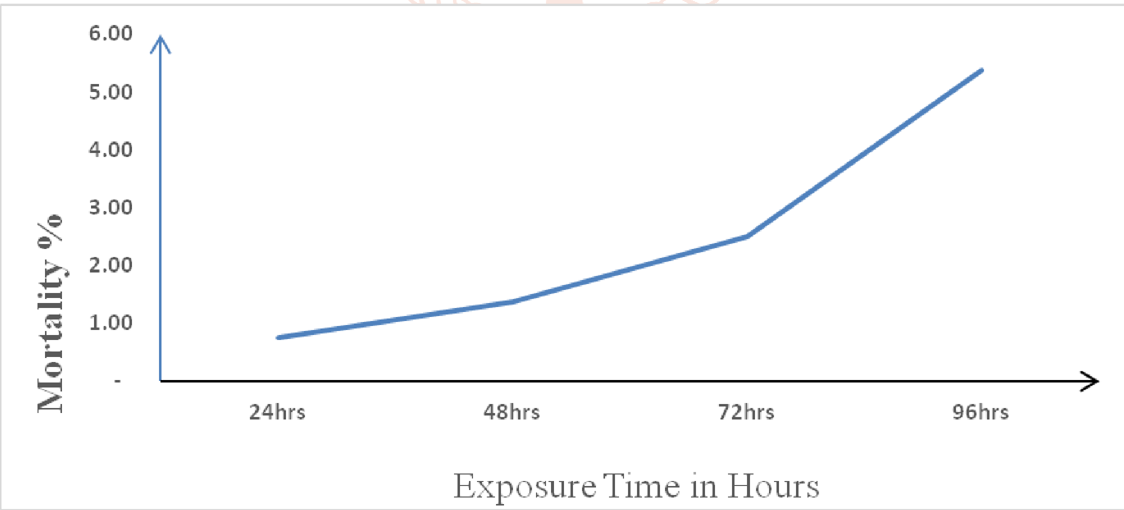

Figure 1: Percentage mortality of adult *C. maculatus* treated with powder of *L. alata* at 2.0 g / 20 g cowpea seeds for contact toxicity

Effectiveness of plant powder as contact insecticide at rate 3.0 g / 20 g of cowpea seeds

Figure 2 presents the effect of plant powder on mortality of *C. maculatus.* The plant powder had a significantly high mortality effect on the tested stored product insects at 24 - 48 hrs (Days 1-2), killing 90 % of the insects. However, the insect biomass reduced as shown in the graph (Figure 2).

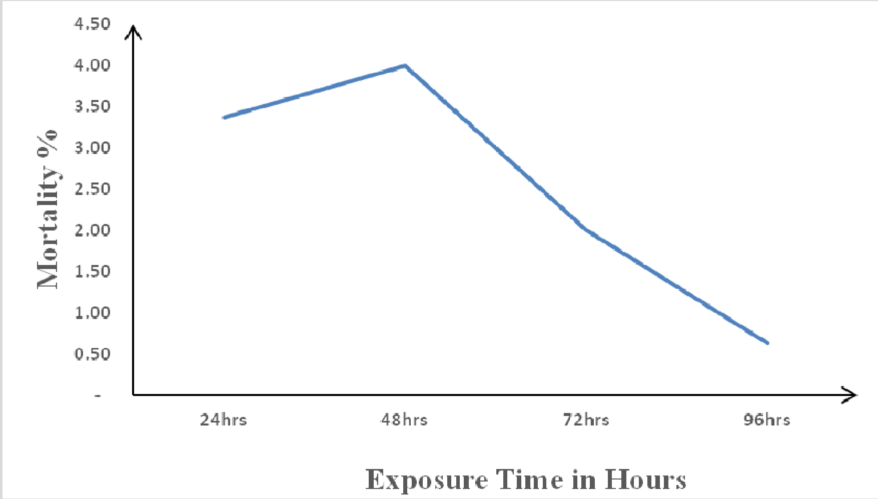

Figure 2: Percentage mortality of adult *C. maculatus* treated with powder of *L. alata* at 3.0 g / 20 g cowpea seeds for contact toxicity

Fumigant effect of plant powder of *Lophira alata* on adult *Callosobrochus maculatus* at rate of 2.0 g / 20 g and 3.0 g / 20 g cowpea seeds

At day 4 after post treatment, *L. alata* was able to cause 80 % of mortality of adult *C. maculatus* applied at 2.0 g / 20 g, days 1, 2, and 3 had less mortality ranging from 15 %, 20 %, and 25 % respectively. The toxicities of the plant powder to cowpea bruchid increased with an increase in concentration and period of exposure to the plant powder as shown in figure 3 below.

Bruchid mortality was rapid and highest at early expose rate of 3.0 g / 20 g on cowpea with about 95 % mortality recorded between 48hrs exposure time. While the powder remains very active and potent due to its increased concentration as shown in figure 4. The insect biomass had reduced before the day 4 (96 hrs) limit.

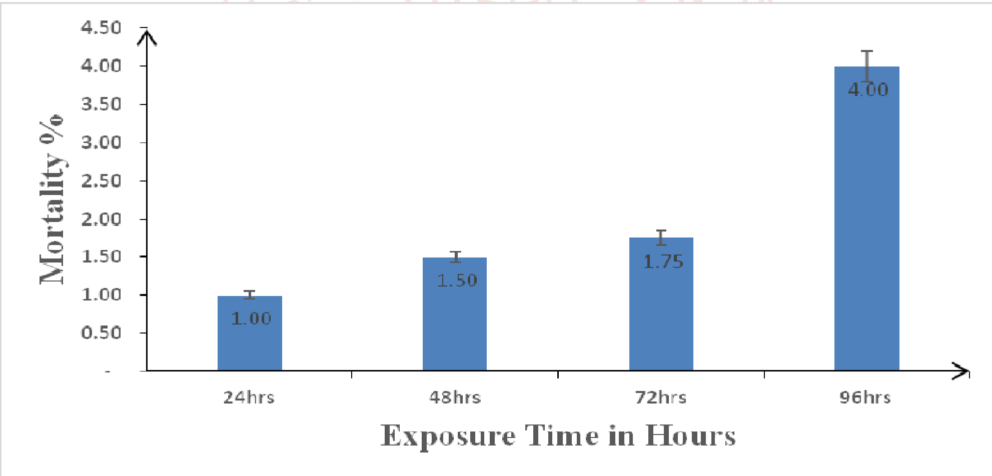

Figure 3: Percentage mortality of adult *C. maculatus* treated with powder of *L. alata* at 2.0 g / 20 g cowpea seeds as fumigant insecticides

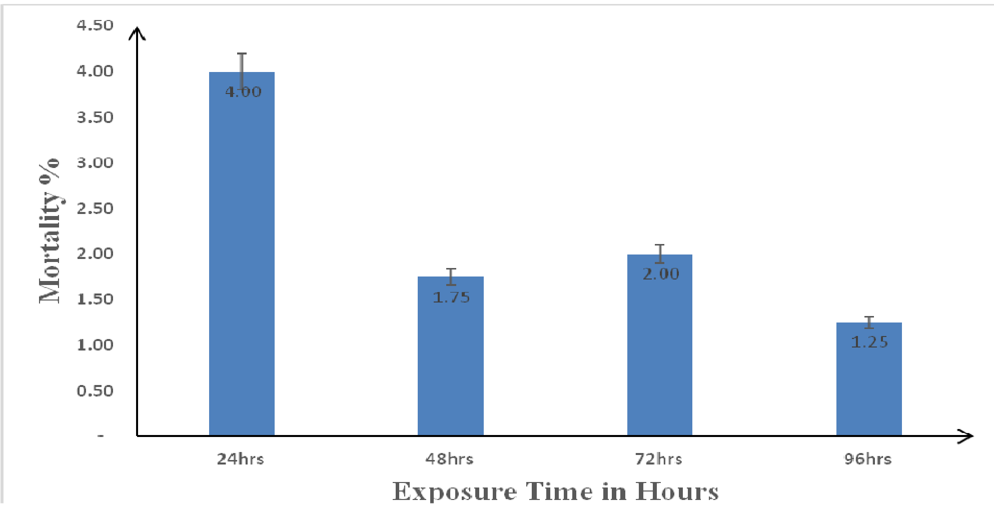

Figure 4: Percentage mortality of adult *C. maculatus* treated with powder of *L. alata* at 3.0g / 20g cowpea seeds as fumigant insecticides.

Discussion

Botanically derived insecticides are good alternatives to popularly used synthetic chemical insecticides as many of them have often times yielded positive results when been used against insect pests of stored products, belonging to, but not limited to Coleoptera and Lepidoptera [15]. They are known to be easily biodegradable and not toxic to none target organisms which oftentimes are species of flora and fauna. The discovery of organochlorine and organophosphate chemical insecticides in the late 1930s and early 1940s, botanical insecticides have remained an important weapon in the farmers armory in managing insect pests of their farm produce and consumables [16]. Many Nigerian plant species are medicinal and are proven to be effective against a wide range of insect pests [17-19].

In this current study, the methanolic extract of *Lophira alata* stem bark show a high effectiveness, it caused 100% mortality of *C. maculatus* within 48 hrs and 96 hrs of application at all concentrations of 2.0g / 20g and 3.0g / 20g of cowpea seeds. The powder may have also blocked the spiracles of these insects which resulted in suffocation. This results agree with the previous studies in which powders and oils of *A. indica, Z. zanthoxyloides, A. occidentale* and *M. oleifera* have been used as botanicals against different life stages of storage insects [20, 1].

The preliminary phytochemical screening of methanolic extract of *Lophira alata* stem barks revealed the presence of alkaloids, tannins, saponins, phytosterols, flavonoids, triterpenoids and glycosides. Alkaloids, flavonoids, saponins, triterpenes and tannins have been reported to possess central nervous system modifying activity such as depression, sedation, anxiolysis, psychotropic, analgesic and anti-convulsant activity [21-23]. The toxicity of *L. alata* to the insect could be attributed to the presence of many chemical ingredients such as triterpenoids, which includes azadirachtin, salanin and meliantriol. [1]. The toxic effect of *L. alata* could be related to the presence of secondary phenolic compound known as phytosterols and this had been reported to have mortality and ovicidal effect on stored product insect pests [11, 19]. Also, the high mortality evoked by the powder of *L. alata*, could be linked to the occurrence of anacardic acid, cardinal, quercetin and kaempferol glycosides as suggested by [24].

The inability of these insects to emerge may be due to the death of the insect larvae which may occur due to inability of the larvae to fully cast off their exoskeleton which remained attached to the posterior part of their abdomen. This is in agreement with the observation made by [25] who worked on insecticidal properties of an alkaloid from *Alstonia boonei*. Also, different chemical compositions of these plants as mentioned earlier could be responsible for the in ability of the adult insects to emerge as they are found to disrupt growth and reduced larval survival as well as disruption of life cycle of insects [26, 27].

This result agrees with the work of various researchers in which the extracts and powders of *A. indica, Z. zanthoxyliodes, A. occidentale* and *M. oleifera* were used to prevent the emergence of adult insects as well as the inhibition of their development [28, 11].

The high mortality and low progeny development caused by the powder of *L. alata* can be attributed to strong choky odour disrupting respiratory activity of the beetles. The results obtained from this study agree with those reported by [29] in studies with six Citrus species peel oils against *C. maculatus, S. zeamais and Dermestes maculatus*.

In this study, the lethal effect of plant powder on the cowpea bruchid could be as a result of contact toxicity. Insects breathe by means of trachea which usually opens at the surface of the body through spiracles [10]. These spiracles might have been blocked by the powder thereby leading to suffocation. The powder also prevented oviposition and progeny development when applied as contact insecticides. The choky effect of these powder also disrupt mating activities, sexual communication and inhibit locomotion an effect that have been reported by many researchers [30-32].

Conclusion

This research has proven that methanolic powder extract of *L. alata* is a good and cheap means to control insect pests of stored products, especially those insects in the order Coleoptera which have characteristic biting and chewing mouthparts and are clumsy fliers. Therefore, can be integrated into other insect pest management systems for effective pest control.

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