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PESTICIDAL EFFICACY OF *GMELINA ARBOREA* ROXB POWDERS AND ETHANOL EXTRACTS ON BEAN BRUCHIDS, *CALLOSOBRUCHUS MACULATUS* (COLEOPTERA: CHRYSOMELIDAE)

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AEA & JT designed the study and carried out the experiment, OOA & HAA collected the data & reviewed the article.

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ABSTRACT

Cowpea has become an easy source of protein worldwide and especially in Africa, yet most of the yields are lost as a result of infestation by pest especially *Callosobruchus maculatus*. The pesticidal efficacy of *Gmelina arborea* was tested on *C. maculatus*. The experiment was conducted using a Complete Randomized Block Design. *G. arborea* leaf powder showed a mean mortality of 4.25±1.35, 2.75±1.29 and 1.92±1.38 when 10g 5g and 2.50g of the powder was applied and a percentage mortality of 85%, 55% and 38.33% respectively. Stem bark powder showed a mean mortality of 1.33±1.16, 2.08±1.31 and 2.92±1.38 when 2.50g, 5.00g and 10.00g was applied and a percentage mortality of 26.67%, 41.67% and 58.33% respectively. The leaf ethanol extract showed a mean mortality of 4.67±2.27, 3.67±1.72 and 2.83±2.17 at 500mg, 250mg and 125mg concentrations and a mortality percentage of 93.33%, 73.33% and 56.67% respectively while the *G. arborea* stem bark ethanol extract revealed a mean mortality of 2.75±1.29, 3.58±1.83 and 4.17±1.95 at 125mg, 258mg and 500mg concentrations with percentage mortality of 55%, 71.67% and 83% respectively. The pesticidal efficacy between the leaf and stem bark ethanol extracts showed that $p \geq 0.05$ for all concentrations used and between the powders, $p \geq 0.05$ at 5.00g and $p \leq 0.05$ at 2.50g and 10.00g. In view of these findings both *G. arborea* leaf and stem bark extracts and powders can be used to effectively control infestation of cowpea by *C. maculatus.*

1. INTRODUCTION

Crop loss due to pest and disease is about 35% on the field and 14% in storage [1]. Post-harvest losses of grains are higher in developing countries compared to developed countries. Pest infestation (insects, birds and rodents), microbial infection, change in moisture content, excessive temperature, poor handling and grain respiration have been implicated as responsible for grain losses in most developing countries [2].

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However, insect pest infestation has been reported as the major cause of food grain losses in most developing countries [3]. As in field crops, a wide range of insect pests attacks stored products, the commonest being beetles and moths [2]. In Nigeria, control of stored product insect population is primarily dependent on continued application of synthetic insecticides. Although effective, their repeated use for several decades has led to outbreaks of insect pests, wide spread of development of

resistance, undesirable effects on non-target organisms, environmental and human health concerns [2, 4]. The highlighted shortcomings of synthetic insecticides needed to explore and develop new sources of chemical compounds from plant origin that constitute a rich source of bioactive chemicals and eco-friendly [5] which are non-toxic, safe, biodegradable and of broad activity spectrum. Fortunately, Nigeria has a wide range of herbal plants spread across the various ecological zones which are largely unexplored. Some of these plants species have been reported to have insecticidal properties [6], against some stored and field pest of crops. Such plants include (Neem) *Azadirachta indica* [7], (Lemon grass) *Cymbopogon citratus*, (Martle) *Myrtus communis*, (Mint) *Mentha longifolia, Pulicaria gnapholodes* (sunflower family plant) and *Achiilea wihelmsii* [8, 9], *Eucalyptus globulus and Eucalyptus camaldulensis,* [3], (Water hyacinth) *Eichhornia crassipes*and (pawpaw plant)*Carica papaya* [10].The search for a natural, and eco-friendly substitute to synthetic pesticides motivated this study which was aimed at investigating the pesticidal efficacy of *G. arborea* ethanol extracts and powders on *C. maculatus*.

2. MATERIALS AND METHODS

2.1 Collection of *C. maculatus* insect pest

C. maculatus were collected from infested cowpea grains purchased at the Terminus market, Jos, Plateau State, Nigeria. The weevils were identified as described by [11].

2.2 Identification of *C. maculatus* sexes

Male and female bean weevils were identified by general appearance. The most distinguishing characteristic is the coloration on the plate (elytron) covering the end of the abdomen. In the female, the plate is enlarged and is darkly coloured on both sides. In the male, the plate is smaller and lacks stripes. Generally, females are larger in size than males, but there is much variation. In some strains, females are black in coloration and males are brown, but in others both sexes are brown.

2.3 Insect culture

Parent stocks of *C. maculatus* were collected from infested cowpea grains. The insects were cultured in the Entomology and parasitology Laboratory Department of Zoology, University of Jos, under ambient temperature of 28±2^{o}C. Three kilograms (3kg) of cowpea was weighed and packed into three (3) plastic containers. Thirty adult (fifteen males and fifteen females) obtained from the already infested cowpea grains were introduced into each culturing medium and covered with muslin cloth held tightly in place by rubber bands. The plastic containers were then kept in wooden cages. The setup was left for six to eight (6 – 8) weeks for adult emergence.

2.4 Drying of plant materials

The leaves were obtained by direct plucking from trees and stem bark using machete. Both plant parts were dusted to remove dirt, quickly rinse in clean tap water and air-dried in the laboratory under shade to avoid photo-degradation of active ingredient by ultra-violet ray of light in line with recommendation of [12]. The dried materials were kept in separate polythene bags, labeled and stored until needed.

2.5 Preparation of plant powder

Plant powders were prepared by pulverizing the dried plant materials separately in the laboratory using a pestle and a mortar. A 0.25mm sized muslin cloth was used to sieve the plant material to obtain fine powders. Fine powders of leaf and stem bark obtained were stored in covered air-tight containers under laboratory conditions until required. Each plastic container containing the powder was labeled.

2.6 Preparation of plant extracts

Two hundred grams (200g) each of the plant powders were weighed into 500ml conical flasks and were soaked in 70% ethanol. These were left to stand for twenty (24) hours and shaken for three (3) hours on a mechanical shaker. The content was filtered using a non-absorbent cotton wool on a Buchner funnel-flask using a vacuum pump. The residues were subjected to several rinsing and filtration with fresh solvents to attain some level of exhaustive maceration. The collected filtrates were evaporated to dryness using a water bath in a drying cabinet. The percentage yield of the extract was determined and

the extract transferred into a stirrer sample container and preserved in a refrigerator. Its phytochemical screening and identification of some basic compounds were also carried out.

2.7 Preservation of Cowpea Grains Used for the Experiment

The Cowpea grains were cleared of broken seeds and debris. Three kilograms (3kg) of cowpea grains were packed into three (3) four litres (4ltrs) containers and covered. To guaranty un-infestation, the method as described by [13] was used to disrupt the life cycle of the pest.

2.8 Experimental design

Each treatment was replicated three times. Three replicates of the treatment and control were laid out in Complete Randomized Design (CRD).

2.9 Treatment of Bean Seeds Using Leaf and Stem Bark Powders

Three dosages of 2.5g, 5.0g, 10.0g and 0.0g (control)per powder was applied into three separate (3) plastic jars (measured 15cm by 8.5cm)containing 50g of non-infected cowpea grains. These were mechanically shaken until the powder spread on all the cowpea grains. Twenty (20) freshly emerged adult (10 males and 10 females) of *C. maculatus* of about 24 hours old were introduced into each plastic jars containing treated grains and the control. Plastic jars were covered with 0.25mm muslin cloth and held tightly in place with rubber bands to prevent suffocation and escape of insects.

2.10 Treatment of Bean Seeds Using Leaf and Stem Bark Ethanol Extracts

Three different concentrations of extracts; 125mg, 250mg, and 500mg were used to coat 50g of cowpea grains except the control. The coated grains were then spread on different clean plastics trays and air-dried for an hour and half. The dried coated cowpea grains were poured into plastic jars measuring 15cm in length and 8.5 cm in diameter then twenty (20) freshly emerged adult *C. maculatus* (10 males and 10 females) of about 24 hours old were introduced into each treatment and control. The plastics containers were then labeled according to treatment and concentrations. Plastic jars were covered with 0.25mm muslin cloth and held tightly in place with rubber bands to prevent suffocation and escape of insects.

2.11 Experimental Duration

Bio-assay of the set-up was carried out for a time period of ninety-six hours (4-days) and the observation made at regular interval of twenty-four (24) hours.

2.12 Observation of treatments

Twenty four (24) hours after the treatment, each replicate and control were poured out carefully on a white plain board and weevils were sorted out of the grains and the dead counted and recorded. The insects were considered dead when they did not respond to stimulus after probing their abdomen with a blunt pin [14].

2.13 Statistical Analysis

The data collected were analyzed using SPSS version 21. Percentage and mean mortality of adult *C. maculatus* were obtained and the plant parts and concentration were compared using Paired Sample T-test.

3. RESULTS

3.1 Comparison of the effects between leaf and stem bark extracts of *G. arborea* on the mortality rate of *C. maculatus.*

Paired sample T-test was used to compare the effect of *G. arborea* on *C. maculates*, and $p \leq 0.05$ were considered to be significant. From table 6, the leaf and stem extracts showed no significant difference in their effect on *C. maculatus* across the concentrations respectively.

3.2 Comparison of the effects between leaf and stem bark powders of *G. arborea* on the mortality rate of *C. maculatus.*

Paired sample T-test was used to compare the effect of *G. arborea* on *C. maculatus,* and p-values less than or equal to 0.05 were considered to be significant. From table 7, the leaf and stem extracts showed that there is a significant difference at 2.50g of *G. arborea* (leaf powder has a higher effect of 1.92 ± 1.38 than the stem powder 1.33 ± 1.16). There is

also a significant difference at 10.00g of *G. arborea* (leaf powder has a higher effect of 4.25 ± 1.35 than the stem powder 2.92 ± 1.37). At 5.00g of *G. arborea*, there is no significant difference in the effect of both leaf powder and stem powder on *C. maculatus.*

4. DISCUSSION

Although the leaves and stem bark are of the same plant, the results demonstrate that they showed different potencies against adult *C. maculatus* as shown in the Tables 2, 3, 4 and 5. *G. arborea* plant showed sufficient protection of cowpea grains from damage by *C. maculatus* when applied at 500mg of leaf extract per 50g of grain. This was revealed by the highest mean mortality of 4.67 ± 2.27 and percentage mortality 93.33%. The least effect was seen in the stem bark powder with a mean mortality 1.33 ± 1.16 and a percentage mortality of 26.67% at its lowest concentration of 2.5g. The efficacy of the leaf extract could be attributed to the presence of terpenes and tannins which are highly present in the plants leaf.

The mortality of *C. maculatus* by the plant parts was observed to increase with increase in concentration and increase in time of exposure. This is possible due to the fact that active ingredients of *G. arborea* require higher concentration and longer period of time to bio-magnify in *C. maculatus.* This is in agreement with [15] who worked on the bioefficacy of three plant products as post-harvest grain protectants against *Sitophilus oryzea* L (Coleoptera: Lurculionidae) on stored wheat (*Triticum aestivum*) and reported that mortality of *S. oryzea* is dependent on the exposure time and concentration of the plants used.

Data on the phytochemical composition of *G. arborea* provides an insight into the mechanism of action of *G. arborea*. Phytochemicals such as tannins have been reported to possess strong activities against several plant pathogens and pest [16]. [17] reported that tannin exerts its action by a combination of mechanisms that include iron chelation and enzyme inhibition. Terpenes are known to have a pungent odour and act as a deterrent to the insect [18]. Though the exact mechanism behind the observed actions of *G. arborea* is not yet known, the preponderance of tannin in its leaf may suggest a role in pesticidal potencies. Saponins and Cardiac glycosides are also present in *G. arborea* plant parts. [19] extensively reviewed the insecticidal effects of Saponins, linking the insecticidal interaction with Cholesterol which results in impaired ecdysteriod synthesis. [20] on the other hand reported evidence for the insecticidal effects of purified cardiac glycosides from *Digitalis purpurea* against camel tick (*Hyalomma dromedarii*)

Due to the fact that adult *C. maculatus* do not feed, *G. arborea* could be seen to demonstrate the ability of acting as suffocating material with the possibility of preventing respiration. This is in line with the findings by [2] who stated that plant oils can act as a suffocating agent to *C. maculatus*.

It was also observed that the ethanol extracts (leaf and stem bark) were more active as compared to the powders. Leaf ethanol extract showed more pesticidal potential compared to the leaf powder and also, the stem bark ethanol extract showed more pesticidal potential compared to the stem bark powder. This may be attributed to the fact that, the extraction was able to release more of the active ingredients of *G. arborea*.

5. CONCLUSION

Based on the results of this work, *G. arborea* is effective against *C. maculatus.* However, efficacy depends on the dose/concentration and the exposure interval. This finding is of great value to further research on the use of this plant for the control of *C. maculatus.* Extract and powder of *G. arborea* leaf and stem bark are lethal to adult survival of *C. maculatus,* though there are variations in adult mortalities. The extract and powder of the leaf and stem bark do not have equal potency on *C. maculatus* due to the active phytochemical constituents variations according to plant parts.

6. RECOMMENDATIONS

This study revealed that the pesticidal efficacy of leaf and stem bark of *G. arborea* are concentration dependent, therefore, a standardized method of concentration formulation should be established. Further studies should also be conducted with higher concentration of the extracts.

Other parts of *G. arborea* could be tested in various forms for example the fruit, root and stem wood. Different parts of *G. arborea* could be mixed to carry out such test to find out if better result could be obtained. Local farmers can use the leaf powder in preserving their stored grain since it does not require the knowledge of an expert. More research should be done to establish more of the active ingredients that are insecticidal and can be used in formulation of plant based insecticides. From the study, *G. arborea* leaf and stem bark are readily available and poor resource farmers could use these parts to protect cowpea grains meant for short duration storage and mostly for consumption. This will reduce the use of synthetic insecticides thus curtailing the risks associated with contact by attendant upon usage.

However, considering the fact that peeling the bark of any tree is tantamount to killing the tree, it becomes reasonable to restrict the use of *Gmelina arborea* plant part to the leaves as agreed by [21] who used *G. arborea* plant in controlling bean pod borer (*Maruca vitrata).*In line with the findings of this study, ethanol extracts of leaves and stem bark of *G. arborea* have higher pesticidal potentials against *C. maculatus* as compared to the powders, we therefore, strongly recommend that the extracts of these plant parts be used as against the powders in the control of *C. maculatus*.

7. CONFLICT OF INTEREST

The authors wish to state that there is no conflict of interest.

8. ACKNOWLEDGMENT

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Table 1: Phytochemical analysis of leaf and stem bark ethanol extracts of *G. arborea*.

Constituents	Leaf Extract	Stem Bark Extract
Alkaloids	++	++
Saponins	_	++
Tannins	+++	++
Flavonoids	+++	+++
Carbohydrate	++	+++
Steroids	_	+
Anthraquinones	_	_
Cardiac glycosides	++	++
Terpenes	+++	_

- Absent + Present ++More Present +++ Highly Present

Table 2. Effect of *G. arborea* Leaf Powder on Mortality of *C. maculatus.*

Conc. (g)	Number of Insects	24Hrs.	48Hrs.	72Hrs.	96Hrs.	Total Mortality	Mean Mortality	% Mortality
0.00	60	00	00	00	00	00	0.00±0.00	00
2.50	60	01	04	07	11	23	1.92±1.38	38.33
5.00	60	05	06	09	13	33	2.75±1.29	55.00
10.00	60	09	12	15	15	51	4.25±1.60	85.00

Mortality rate value is mean of twelve replicates.

Table 3. Effect of *G. arborea* Stem Bark Powder on Mortality of *C. maculatus.*

Conc. (g)	Number of Insects	24Hrs.	48Hrs.	72Hrs.	96Hrs.	Total Mortality	Mean Mortality	% Mortality
0.00	60	00	00	00	00	00	0.00±0.00	00
2.50	60	00	03	06	07	16	1.33±1.16	26.67
5.00	60	02	05	07	11	25	2.08±1.31	41.67
10.00	60	04	07	10	14	35	2.92±1.38	58.33

Mortality rate value is mean of twelve replicates.

Table 4. Effect of *G. arborea* Leaf Ethanol Extract on Mortality of *C. maculatus.*

Conc. (mg)	Number of Insects	24Hrs.	48Hrs.	72Hrs.	96Hrs.	Total Mortality	Mean Mortality	% Mortality
0	60	-	-	-	-	-	0.00±0.00	-
125	60	04	05	08	17	34	2.83±2.17	56.67
250	60	07	09	11	17	44	3.67±1.72	73.33
500	60	09	10	13	24	56	4.67±2.27	93.33

Mortality rate value is mean of twelve replicates.

Table 5. Effect of *Gmelina arborea* Stem Bark Ethanol Extract on Mortality of *Callosobruchus Maculatus.*

Conc. (mg)	Number of Insects	24Hrs.	48Hrs.	72Hrs.	96Hrs.	Total Mortality	Mean Mortality	% Mortality
0.00	60	00	00	00	00	00	0.00±0.00	00
125	60	03	07	11	12	33	2.75±1.29	55.00
250	60	04	10	15	14	43	3.58±1.83	71.67
500	60	05	15	17	13	50	4.17±1.95	83.33

Mortality rate value is mean of twelve replicates.

Table 6: Level of Significant for Leaf and Stem Bark Ethanol Extracts

Conc. (mg)	Leaf Extract	Stem Extract	P- values	Remarks
125	2.93 ± 2.17	2.75 ± 1.28	0.866	$p \geq 0.05$
250	3.67 ± 1.72	3.58 ± 1.83	0.866	$p \geq 0.05$
500	4.67 ± 2.27	4.17 ± 1.95	0.509	$p \geq 0.05$

Table 7: Level of Significant for Stem Bark and Leaf Powder

Conc. (g)	Leaf powder	Stem Bark powder	P- values	Remarks
2.50	1.92 ± 1.38	1.33 ± 1.16	0.046	$p \leq 0.05$
5.00	2.75 ± 1.29	2.08 ± 1.42	0.111	$p \geq 0.05$
10.00	4.25 ± 1.60	2.92 ± 1.37	0.012	$p \leq 0.05$