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Entomotoxicity of Six Indegenous Plants Extracts in Controlling Callosobrochus Maculatus (Fabricius) (Coleoptera: Chrysomelidae) Infestation in Stored Vigna Unguiculata L.Walp.

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***Abstract**: Biological investigations were conducted to assess the insecticidal activities of n-hexane leaf extract of Solanum tuberosum, Annona muricata, Cymbopogon citrates, Vernonia amygddalina, Caesalpinia pulcherima and Lantana camara individually against the cowpea weevil Callosobruchus maculatus. The six plants leaf extracts showed significant ($p \leq 0.05$) insecticidal activities relative to control by increasing repellant and mortality rate, inhibiting oviposition and first filial generation progeny emergence and reducing seed damage. 100% mortality was accomplished by Lantana camara (at 5%) and Annona muricata (at 5%) in 72 hours, and both plant extracts at (3%) in 96 hours. Lowest significant ($p \leq 0.05$) oviposition first filial progeny emergence and seed damage were all obtained in Lantana camara extract at 5% concentration while the control gave the highest value for the three parameters (oviposition = 355.0, F_1 progeny emergence = 88, seed damage = 100). Repellant class varied between II and V; with Lantana camara and A. muricata having a comparable and significant ($P \leq 0.05$) value of 84.42 and 80.88 respectively at 5% concentration. All test plant extracts at high concentrations of (3 and 5%) caused significant increase in repellant and mortality but a significant reduction ($P \leq 0.05$) in oviposition, fecundity, progeny emergence and seed damage relative to control.*

***Key words**: Callosobruchus maculatus, Entomotoxicity, Plant extracts, Vigna unguiculata,*

I. Introduction

Cowpea, *Vigna unguiculata (L. Walp)* is a commonly grown and important food resource in communities of sub-saharan Africa [1], [2]. The seeds and foliage are good sources of protein (23-35%), carbohydrate (60-68%), minerals (iron and calcium), vitamins and carotene [2], and are used in preparing several dishes for man and livestock [3]. Cowpea is relatively cheap and supplements the protein requirements of many families in Africa [4] where meat and other sources of animal protein are very expensive. In spite of the several economic values of *Vigna unguiculata*, 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. Insects are the most serious pests of stored cowpea [5]. Loss of yield and stored produce due to insects infestation is said to be between 20-40% annually [6], [7]. These pests which have a worldwide distribution are numerous in species and number in West Africa. *Callosobruchus maculatus*, a major insect pest of *Vigna unguiculata* in Nigeria, has been reported to cause tremendous reduction in weight, viability and marketability of cowpea seeds [8]. Because of the devastating effects of this insect pest (*Callosobruchus maculatus*) on this economic crop, there is therefore an urgent need for the application of an effective and affordable control measures so as to increase food production thereby avoiding food crises [9].

To protect cowpea seeds from insect attack in the store, most farmers and traders apply synthetic insecticides in form of sprays and dust to minimize loss in quality and quantity. These synthetics have often been misused resulting in adverse effects on the environment and non-target organisms [10], [11], [12], development of resistant variety and resurgence of pests [13]. Presently crop protection strategies involve alternative methods of insect control employing plant products and their secondary metabolites which are cheap, affordable, readily available and environmentally friendly [14].Extracts and powders of different plants, such as *Piper guinense, Aframomum melegneta*; and plant parts such as leaves, stem, bark, roots and flowers have demonstrated anti-feedant, larvicidal repellant and toxic effects on insects of stored grains [15], [16], [17]. In view of the importance of *V. unguiculata, C. maculatus* (responsible for crop losses) the environmental and health hazards associated with the use of synthetic insecticides to minimize the effects of insect pests, it is therefore necessary to evaluate the biological effectiveness of six plants *C. citratus, S. tuberosum, C. pulcherima, A. mauricata, V. amygdalina and L. camara* against the cowpea weevil (*Callosobruchus maculatus*) in stored cowpea (beans).

II. Materials And Methods

2.1. Experimental site

The experiments were conducted in the Department of Botany, University of Calabar, ($5^0$45'N; $8^0$30'E), Nigeria between January and December 2015 at prevailing environmental temperature of 28±4^0C, pressure of 75±5% of Hg and 12 Hours photoperiod.

2.2. Procurement of plants materials

Vigna unguiculata seeds and the test plants leaves were obtained from the grain stores and herbal section respectively in Watt Market located in Calabar South Local Government Area, Cross River State, Nigeria.

2.3. Rearing of *Callosobrochus maculatus*

Vigna unguiculata (500 g) were screened for damaged and infested grains. The un-infested grains were sterilized by storing in deep-freezer (Thermocool model) at 18^0C for 24 hours. The sterile *V. unguiculata* was left to attain normal temperature and pressure (28±4^0C and 75±5% of Hg) before mass rearing of the insect (*C. maculatus*).Ten pairs of *C. maculatus* (ten males and ten females) separated from the infested grains were introduced into a clean glass bottle containing 200 g of Cowpea. The caps of the bottles were drilled with tiny holes (2 mm each) for proper ventilation and the mouth tied with muslin cloth to maintain humidity for the insects to remain alive, and reproduce. The stock was maintained till after experimentation.

2.4. Preparation of test plant materials

The procured plants leaves were separately shade-dried after washing with distilled water. The dried leaves of each plant were powdered using an electric miller (Super Master, model SMB 2977, Japan) then sieved through a 0.25mm mesh cloth to have a fine homogenous powder. The extraction of essential oil from each plant leaves was done using Soxhlet apparatus and n-hexene as solvent. The extracted oils were stored in air-tight bottles and used within 24 hours.

2.5. Contact Toxicity Test

Contact toxicity test of the test plants extracts on Cowpea weevil, *C. maculatus* were done according to the method of [18]. The insects were cooled in a freezer for 15 minutes to immobilize them. The immobilized insects were taken one after the other and one milliliter solution of each treatment (0, 1, 3 and 5%) was applied or placed dorsally on the thorax of each insect using a micro-capillary tube, thereafter the insects were placed in a 9cm diameter Petri-dish. Treatments were applied in a completely randomized design with each treatment replicated thrice. Each replicate consisted of ten insects. Insects mortality rate were assessed at 24, 48, 72 and 96Hours after treatment. Insect was considered dead, if it did not show any sign of movement when touched. Percentage mortality was calculated using the formula;

$$\text{Percentage mortality} = 100 \text{ X } \frac{\text{Total number of dead insects in each treatment}}{\text{Total number of Callosobruchus realized in each treatment}}$$

2.6. Residual Toxicity Test

The extracts of the six test plants were separately admixed with 100 g of *V. unguiculata* seed at the rate 0, 1.0%, 3.0% and 5.0% ($^{w}/_{v}$) for each extract. The treated *V. unguiculata* were air-dried for 30 minutes and then placed in separate plastic container (7 cm X 7 cm).Ten newly emerged weevils (one-day old) five females and five males were introduced into the plastic containers containing seeds treated with various dosages of each test plant extract. A control experiment in which no plant extract was added was also set up side by side. The experiment was laid out in a completely randomized design with each treatment replicated thrice. Data on oviposition and fecundity were taken one week after introducing the weevils. Egg laid were counted with the aid of hand lens (Mag. = X10).

2.6.1. Adult emergence Test

Thirty days after oviposition adult *C. maculatus* started emerging. The emerged adults were counted and removed daily from each container from the date of first emergence to two weeks after emergence. The emergence rate inhibition rate was computed using the following formulae

$$IR(\%) = \frac{C_n - T_n \ x \ 100}{C_n}$$

Where C_n = number of insect in control

T_n = number of insect in each treatment

2.6.2. Seed Damage Test

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 three or more holes were considered as damaged seeds. Number of damaged and un-damaged seeds were counted and recorded for each replicate.

2.6.3. Repellent Test

This test was carried out using the method of [19]. Data were expressed as percentage repellent (PR) using the formula below

PR(%) = (NC – 50) X 2.

Where NC = Percentage of insects present in control

Positive (+) values = repellent
Negative (-) values = attraction

2.7. Data Analysis

Data obtained were subjected to analysis of variance (ANOVA) and means were grouped according to [20]

III. Results

Table 1: Effects of six test plant extracts on mortality of *Callosobruchus maculatus* in stored cowpea.

		Percentage (%) Mortality			
Treatments	Conc. (%)	24HRS	48HRS	72HRS	96HRS
Annona muricata	1	26.67^{d}	46.33^{b}	46.67^{c}	66.33^{de}
	3	40.63^{f}	83.00^{d}	93.33^{f}	100.00^{a}
	5	53.67^{g}	93.67^{e}	100.00^{e}	100.00^{a}
Solanum tuberosum	1	0.00^{a}	0.00^{a}	6.67^{a}	6.67^{i}
	3	6.67^{b}	20.00^{a}	23.33^{b}	30.00^{g}
	5	13.33^{c}	36.67^{b}	40.00^{b}	56.67^{f}
Cymbopogon citratus	1	13.33^{c}	13.33^{a}	20.67^{b}	20.67^{he}
	3	30.67^{e}	56.33^{c}	80.33^{e}	86.33^{ac}
	5	43.00^{f}	66.33^{c}	96.33^{f}	96.67^{ab}
Vernonia amygdalina	1	6.33^{b}	16.67^{a}	33.67^{b}	43.33^{fg}
	3	13.67^{c}	36.33^{b}	50.00^{c}	60.00^{de}
	5	23.33^{d}	43.67^{b}	66.67^{d}	83.33^{ac}
Lantana camara	1	40.00^{f}	64.33^{c}	83.33^{e}	90.67^{ab}
	3	56.33^{g}	90.33^{e}	93.67^{f}	100.00^{a}
	5	76.33^{h}	96.67^{e}	100.0^{f}	100.00^{a}
Caesalpinia pulcherima	1	0.00^{a}	3.33^{a}	6.67^{a}	08.67^{i}
	3	3.33^{a}	3.33^{a}	13.33^{a}	30.00^{h}
	5	6.67^{b}	13.67^{a}	26.67^{b}	40.00^{fg}
Control	0	0.00^{a}	0.00^{a}	0.00^{a}	0.00^{hi}

Values are mean values of three replicates. Values in each column having similar superscript are not significantly different based on Waller Duncan Test.

Direct toxicity effect of n-hexene leaves extracts of *A. muricata*, *S. tuberosum*, *C. citratus*, *V. amygdalina*, *L. camara* and *C. pulcherima* against the *C. maculatus on V. unguiculata* indicated that four treatments *A. muricata* and *L. camara* at 3 and 5% concentration each, had the highest and similar mortality value of 100% at 96 HRS after treatment. The performance was significantly ($P \leq 0.05$) different from values obtained from all other treatments and control. Closely following these four treatments were *C. citratus* leaves extracts at 5% and *L. camara* at 1% concentrations which had a comparable but slightly lower mortality (96.80% and 90.67% respectively) of *C. maculatus*. *V. amygdalina* at 5% have similar mortality effects with *C. citratus* at 3%. The treatments that demonstrated medium mortality effect were S. *tuberosum* (at 3% and 5%), *V. amygdalina* at (1 and 3%) and *C. pulcherima* at 5% concentration. All plant treatments however, recorded significant difference ($P \leq 0.05$) and higher mortality than the control (Table 1)

Table 2: Effects of six plants leaves extracts on oviposition, progeny emergence of *Callosobruchus maculatus* and seed damage.

Treatments	Conc. (%) of plant extract	No. of Eggs	% F1 Adult emergence	% seed damage
Annona mauricata	1	198.98^{d}	79.79^{e}	46.86^{a}
	3	116.86^{c}	63.45^{d}	07.02^{a}
	5	79.67^{b}	35.75^{a}	06.54^{a}
Solanum tuberosum	1	201.10^{e}	80.05^{f}	78.3^{f}
	3	198.90^{d}	62.89^{d}	61.25^{de}
	5	175.00^{d}	49.56^{b}	23.68^{c}
Cymbopogon citrates	1	215.00^{e}	82.86^{f}	55.65^{e}
	3	190.45^{d}	63.60^{d}	40.24^{d}
	5	166.50^{d}	49.98^{b}	21.68^{c}
Vannonia amygdalina	1	153.00^{c}	82.00^{f}	26.6^{c}
	3	120.00^{c}	63.02^{d}	10.33^{b}
	5	90.s65^{b}	46.20^{b}	6.00^{a}
Lantana camara	1	68.00^{b}	51.45^{c}	12.38^{b}
	3	43.12^{a}	38.35^{a}	8.00^{a}
	5	18.23^{a}	30.56^{a}	5.78^{a}
Caesalpinia sp.	1	311.00^{g}	90.95^{g}	98.21^{g}
	3	282.00^{f}	87.33^{f}	99.6^{g}
	5	243.00^{e}	85.10^{f}	94.00^{g}
Control		355.8^{g}	95.33^{g}	100.00^{g}

Values are mean of three replicates. Values in each column with similar superscript are not significantly different based on Waller-duncan post-hoc test Effect of treatments (plant leaves extracts) on percentage of seeds damaged indicated that five treatments; *A. muricata* at 3 and 5% treatment concentrations, *V. amygdalina* at 5%, and *L. camara* at 3 and 5% had the lowest percentages of damaged seeds (7.02, 6.54, 8.00 and 5.78% respectively). These percentages were comparable and significantly ($P \leq 0.05$) different from that obtained from all the other treatments including control (100% seed damaged). *V. amygdalina* at 3% treatment dose and *L. camara* at 1% treatment dose had similar percentage of seed damaged. There were no significant differences ($P \geq 0.05$) in percentage of seed damaged by 1, 3 and 5% *Caesalpinia pulcherima* and the control (100%). All other treatments except *C. pulcherima* significantly reduced the percentage of damage caused by *C. maculatus* to *V. unguiculata* (Table 2). The least number of eggs were laid on seeds treated with 3 and 5% of *Lantana camara* while the largest number were laid on *C. pulcherima* treated seeds and untreated control. Number of adults emerged was significantly ($P \leq 0.05$) reduced in *L. camara* treated seeds (Table2), but highest in control and *Caesalpinia pulcherima*.The highest percentage of repellent was observed in seeds treated with 5% *A muricata* (80.90%) and 5% *Lantan camara* (84.42%). When compared with control which had a percentage repellent of 35.23, all other treatments except *Ceasalpinia pulcherima* (at 1%) were characterized between repellent class III and V (Table III).

Table 3: Effects of six plant leaves extracts on repellent of *Callosobruchus maculatus* adults

Name of plant	Conc. of plant extract	% rate repellency rate at 5HRS post treatment	Repellency class
Annona mauricata	1	68.87	IV
	3	72.68	IV
	5	80.88	V
Solanum tuberosum	1	61.12	IV
	3	62.79	IV
	5	68.45	IV
Cymbopogon sp	1	58.87	III
	3	62.75	IV
	5	75.05	IV
Vernonia amygdalina	1	60.56	IV
	3	62.22	IV
	5	67.23	IV
Lantana camara	1	66.66	IV
	3	67.65	IV
	5	84.42	V
Caesalpinia sp.	1	38.89	II
	3	42.76	III
	5	51.68	III
Control		35.23	II

IV. Discussion

The use of plant substances to protect grains from insect pest degradation is a long time practice [21]. Essential oils extracts and the chemical ingredients from different plants have been used greatly in grain protection in laboratories and field trials in many parts of the world especially Africa, China and India [22]. In this investigation, the insecticidal properties of leave extracts of *L. camara, A. muricata, C. pulcherima, V. amygdalina, S. tuberosum* and *C. citratus* were assessed for the control of *C. maculatus* on *V. unguiculata* in 2015. Results showed that the extracts of the six plant species investigated exhibited insecticidal activities by repelling, killing and suppressing development of *C. maculatus* and confirm significant differences from the control. Volatile compounds of plants extracts contain many bioactive molecules, which have contact and fumigant properties. Extract of *L. camara* has been used to protect stored grain against almond moth in India [23]. Methanol extract of *L. camara* has also been reported as being insecticidal against all developmental stages of stored grain insect pests and suppressing of emergence of progeny in treated grains [24]. The result of the present investigation is in consonance with the reports of [23] and [24]. These insecticidal properties exhibited by leaf of *Lantana camara* is attributed to the presence of glycoalkaloids, some of which are 4H-1-Benzo Pyran-4-one, Coumaran earlier reported as fumigant molecules [25] and Lantoniside, Linaroside and carmarinic acid as contact poisons and active toxic groups [26]. Leaves extract of *A. muricata* demonstrated a significant level of insecticidal action comparable with that of *Lantana camara* (100%). This also agrees with the findings of [27] who reported that extracts of *A. muricata* caused 100% mortality of *C. chineense* within 24 hours of exposure. [28] reported that acetogenins (Solanin) alkaloids, and are active components of *A. muricata* responsible for the insecticidal action.Leave extracts of *C. citratus* had moderate insecticidal activity (66.3% mortality) during the first two days, and high insecticidal activities (96% mortality) at the third and fourth days after treatment. This shows that it is highly effective in controlling beetles infestations, due to the presence of bioactive compounds including phenols, flavonoids, saponins and alkaloids. The mortality effect of *V.*

amygdalina and *C. citratus* were however comparable.*Caesalpinia pulcherima* and *S. tuberosum* have similar effects on mortality, seed damage, progeny emergence and repellent. This corresponds with the report of [29].

V. Conclusion

All test plant substances exhibited insecticidal properties by increasing percentage of repellent and mortality, but decreasing egg laying, progeny emergence and seed damage. *L. camara*, *C. citratus, C. pulcherima, V. amygdalina, S. tuberosum* and *A. muricata* all have bioactive compounds which confer insecticidal action on them. Therefore these plants parts (leaf) should be incorporated in modern pest management strategies.

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