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INSECTICIDAL AND REPELLENT ACTIVITIES OF THE LEAF EXTRACTS OF *MORINDA LUCIDA* AGAINST COWPEA BEETLE, *CALLOSOBRUCHUS MACULATUS* (COLEOPTERA: CHRYSOMELIDAE)

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ABSTRACT

Toxicity and repellent potential of acetone, *n*-hexane and methanol extracts of the leaves of the medicinal plant, *Morinda lucida* (Benth.) against *Callosobruchus maculatus* (F.) was evaluated at 10, 15, 20 and 25% treatment levels. Adult mortality and repellent tests were monitored daily for 4 days while result of ovicidal test was recorded at 35 days after treatment. The results obtained showed that toxicity was concentration and exposure period-dependent, *n*-hexane extract being the most toxic to adult *C. maculatus* by causing 100% adult bruchid mortality at 25% treatment level within 3 day exposure period. All concentrations of the plant extracts applied were able to cause significant ($p<0.05$) adult mortality of the bruchid beetle. All the treatment levels evaluated were able to repel adult *C. maculatus* from the cowpea seed. All concentrations of *n*-hexane extracts caused 100% egg mortality while acetone and methanol extracts caused 100% mortality of eggs at 15, 20 and 25% concentrations. The results obtained in this study indicate that extracts of the leaves of *M. lucida* would be effective in protecting cowpea seed against *C. maculatus*.

Keywords: Toxicity**,** ovicidal potential, repellent potential, *Callosobruchus maculatus*, *Morinda lucida***,** mortality

INTRODUCTION

Cowpea, *Vigna unguiculata* (L.) Walp. is an important food crop in tropical countries especially in West Africa where it is a cheap source of dietary protein (Labeyrie, 1981). The dry seed consists of about 25% protein and 67% carbohydrate. It is also a good source of calcium, iron, vitamins and carotene. Initial infestation of cowpea seeds by the beetle *Callosobruchus maculatus* (F.) occurs in the field just before harvest and the insects are carried into the store where their population build up rapidly (Huignard, 1985). Caswell (1973) estimated that in Nigeria alone, the dry weight loss due to *C maculatus* exceeded 2900 tonnes each year. In some cases damage in terms of holed seed can increase to 99% after 6 months of storage (Caswell, 1973).

To prevent the loss of crops on field and during storage, farmers usually rely on chemical insecticides. These tools used frequently cause environmental pollution. Their residues on treated crops or seeds also have adverse effects on human health. All these necessitated build-up of alternative means of pest control that are user and ecologically–friendly. Many tropical medicinal plants and spices have been used as insect pest control agents (Lale, 1992; Singh *et al*., 1978). Peasant farmers and researchers often claim successful use of plant materials in stored products insect pest control including powdered parts of plants (Adedire and Ajayi, 2003; Ashamo and Akinneye 2004, Akinkurolere *et* *al*., 2006), plant extracts (Ashamo, 2005); ash (Ofuya and Dawodu, 2002; Ajayi *et al*., 1987) vegetable oil (Lale, 1992) and spices (Ajayi *et al*., 1987; Lajide *et al*., 1998). Jackai and Daoust (1986) reported that the use of plant materials and local traditional methods in the control of storage insect pests are much safer than chemical insecticides and suggested that their exploitation.

Morinda lucida, is a medicinal plant that abound in the tropics (Soladoye, 2005). Crude extracts of the leaf have been recommended in the treatment of hypertension and cerebral complication showing distinct diuretic and tranquilizing effects (Debray *et al*., 1974). The plant has been used as a grand medicament of West African traditional medicine, valued for its antipyretic and anti-malaria properties and in the treatment of ulcers, leprosy and gonorrhea (Durodola, 1974). A bio-friendly natural dye had been previously extracted from *M. lucida* for the staining of collagen fiber and muscle fiber (Avwioro *et al*., 2005). It is against this background that this study sought to screen the leaf extracts of *M. lucida* for protection of stored cowpea seeds against infestation by *C. maculatus*.

MATERIALS AND METHODS

Callosobruchus maculatus culture

Adult *C. maculatus* were obtained from naturally infested cowpea seeds at Oba market, Akure, Southwestern Nigeria located between latitude $7^{*}\ 15^{'}$ N and longitude $5^{*}\ 12^{'}$ E. The insects were reared in two Kilner jars containing 200g of Ife-brown variety of cowpea seed. The jars were capped with muslin cloth and kept at ambient temperature (28 ± 2^{o}C) and relative humidity of 75 ± 5%. The muslin cloth allowed for ventilation but precluded the exit of bruchid and entry of other insects. From this stock, new generations of *C. maculatus* were raised and the culture was maintained by continually replacing infested cowpea seeds with fresh cowpea seeds.

Preparation of plant materials

The leaves of *M. lucida* were collected from the premises of the Federal University of Technology, Akure; Southwestern, Ondo State, Nigeria. The leaves were washed with clean water and air-dried in the laboratory. The leaves were later pulverized into fine powder using hammer milling machine (MODEL SLC 500, (KW) 7.5, (KG) 1500). The powder was kept inside black polyethylene bag tied with rubber band until needed.

Preparation of plant extracts

Methanol, *n*-hexane and acetone extracts were obtained from equal quantity (500g) of the pulverized leaves of *M. lucida*. The plant powder was soaked in methanol and acetone separately for 72 hrs and the mixture was filtered with muslin cloth. The filtrate was then transferred to rotary evaporator to separate the solvent from the extract. Each concentrated extracts was freeze-dried using freeze dryer to obtain methanol and acetone extracts. The extracts were wrapped in aluminum foil placed in moisture-proof container and kept in freezer until required. The *n*-hexane extract was obtained using Soxhlet extractor as described by Harbone (1984).

Toxicity test

Substrate cowpea seeds were disinfested for 72 h in freezer at -20^{o}C. Twenty grammes of disinfested cowpea seeds were put in plastic container (8cm diameter and 3.5cm depth). Four concentration levels of 10%, 15%, 20%, and 25% w/v of methanol and acetone extracts were prepared by dissolving 2, 3, 4 and 5g of the extracts in 20 ml of methanol and acetone respectively. For *n*-hexane extract, 2, 3, 4 and 5 ml was dissolved in 20 ml of hexane to obtain 10%, 15%, 20%, and 25% v/v solution of the extract. The cowpea seeds and 1ml of each extract concentration level were thoroughly mixed to ensure uniform coating of the seed with extract. Untreated (0.00%) was set-up. Solvent treated experiment was also set-up by applying 1ml of the solvents to verify effect of solvent on potency of the extracts. The experiments were left for 1 hr to air dry before introduction of ten (0-24 h old) adult *C. maculatus*. All treatments were arranged in completely randomized design in an insect cage and replicated three times. Percent mortality of the adult *C. maculatus* was recorded daily for 5 days after treatment as follows:

$$\% \text{ Mortality} = \frac{\text{Number of Dead Insects}}{\text{Total Number of Insects}} \times \frac{100}{1}$$

The percent mortality obtained was corrected for mortality in control using Abbot's (1925) formula:

$$P_T = \frac{P_o - P_c}{100 - P_c} \times \frac{100}{1}$$

Where: P_T = Corrected mortality (%)
P_o = Observed mortality (%) and
P_c = Control mortality (%)

Ovicidal activity test

One hundred (0-24 h old) eggs of *C. maculatus* with average of two eggs per seed were put in plastic container and replicated three times. More seeds were added to make up to 20g in plates which contain seeds having a total of 100 eggs but do not weigh up to 20g. To each replicate, 1ml of 10%, 15%, 20% and 25% v/w of each extract was added and gently mixed. Solvent treated and untreated experiments were set-up as controls. The set up was left inside the cage for 35 days for the eggs to develop to adult. The number of adults that emerged from the treated eggs were taken as percent adult emergence since 100 eggs were put in each replicate. This was used as index of ovicidal potential of the extracts.

Repellency Test

Melted wax was poured inside 14cm diameter Petri-dishes and allowed to solidify. Six holes were carefully made on the solidified wax in the dishes. Five sets of 20g cowpea seed were treated with 10%, 15%, 20%, 25% v/w of the different extracts. Untreated (0.00%) cowpea was set-up as control. Five seeds from each treatment level were placed in the hole made on the solidified wax inside the dish. Experiments were replicated three times. Twenty unsexed 0-24 h old adult *C. maculatus* were placed at the centre of the Petri dishes. The set-up was examined daily for 4 days. The number of *C. maculatus* found on the treated and untreated seeds was recorded.

Data Analysis

Data obtained in percentage from mortality and ovicidal tests were arcsine transformed. Square root transformation was performed on the count data of repellent test. Transformed data were analyzed using analysis of variance (ANOVA) and the mean separated by Tukey's test at 5% level of significance. Back-transformed data was presented in the tables.

RESULTS

Effect of leaf extracts of *M. lucida* on mortality of adult *C. maculatus*

All the extracts from the leaves of *M. lucida* tested caused significant adult mortality of *C. maculatus* at high concentrations. Effectiveness of the extracts was concentration and exposure-period dependent (Tables 1). The highest mortality obtained at 25% concentration of acetone, *n*-hexane and methanol were 53.33%, 100.00% and 30.33% respectively at 3 days of observation. The *n*-hexane extract was the most effective against *C. maculatus*. There were significant differences ($p<0.05$) between the mortality caused by the three extracts at the various concentration applied at all the periods of observation: day1 (df = 17, F = 34.176, *Pvalue* = 0.000, α = <0.050), day 2 (df = 17, F =

37.130, *Pvalue* = 0.000, α = <0.050); day 3 (df = 17, F = 91.003, *Pvalue* = 0.000, α = <0.050) and day 4 (df = 17, F = 155.548, *Pvalue* = 0.000, α = <0.050). Solvent treated and untreated experiments had the lowest mortality which ranged between 0 to 13 and 0 to 10 respectively.

Ovicidal activity of *M. lucida* extracts on eggs of *C. maculatus*

The result of ovicidal activity of acetone, *n*-hexane and methanol extracts of *M. lucida* is shown on Table 2. Adults that emerged from the 10% (w/v) treatment of all the extracts ranged between 5.67% and 6.33%. No adult emerged from seeds treated with 15, 20 and 25% extract concentration for all the different extracts. Adult emergence in the solvent treated and untreated experiments ranged between 94.33% and 95.67%; and 93.00% and 94.67 respectively. There was no significant difference (p>0.05) between adults that emerged from the solvent-treated (df = 2, F = 0.613, *Pvalue* = 0.573, α = <0.050) and the controls (df = 2, F = 0.131, *Pvalue* = 0.879, α = <0.050) of the three extracts. There were significant differences between the number of adults that emerged from eggs treated with 10% concentrations of the three extracts (df = 2, F = 163.500, *Pvalue* = 0.00, α = <0.050). Adult emergence in 10% *n*-hexane treated experiment was 0.0% while adult emergence recorded in 10% methanol and acetone treatments were 5.67 and 6.33% respectively. Zero emergences were recorded in 15, 20, and 25% treated experiment of all the extracts.

Repellent activity of *M. lucida* extracts against *C. maculatus*

Table 3 shows the result of the repellent test of *M. lucida* extracts against adult *C. maculatus*. All the extracts of *M. lucida* obtained (acetone, *n*-hexane and methanol) repelled the insect from the treated cowpea seeds. The highest numbers of insect (9 and 10) was found on untreated experiments.

Table 1: Mortality of adult *Callosobruchus maculatus* exposed to leaf extracts of *Morinda lucida*

	Treatment	Mortality at days post-treatment (Mean±SE)			
Extract	**Conc (% v/w)**	**1**	**2**	**3**	**4**
	Untreated	0.00^{a}±0.00	0.00^{a}±0.00	0.00^{a}±0.00	6.67^{a}±0.00
	Solvent	0.00^{a}±0.00	0.00^{a}±0.00	0.00^{a}±0.00	13.33^{abc}±3.33
	10	16.67^{cdef}±3.33	26.67^{bcd}±3.33	33.33^{bc}±3.33	43.33^{ef}±3.33
Acetone	15	20.00^{defg}±0.00	30.00^{bcde}±2.57	43.33^{cd}±3.33	56.67^{fg}±3.33
	20	23.33^{efgh}±3.33	33.00^{cde}±3.33	46.67^{cd}±4.41	60.00^{gh}±5.77
	25	26.67^{fgh}±3.33	40.00^{def}±3.33	53.33^{d}±3.33	73.33^{hi}±3.33
	Untreated	0.00^{a}±0.00	0.00^{a}±0.00	0.00^{a}±0.00	3.33^{a}±3.33
	Solvent	0.00^{a}±0.00	0.00^{a}±0.00	0.00^{a}±0.00	10.00^{abc}±0.00
	10	23.00^{efgh}±3.33	43.33^{def}±3.33	53.33^{d}±3.33	83.33^{i}±3.33
n-hexane	15	33.33^{hi}±3.33	46.67^{efg}±3.33	86.67^{e}±3.33	100.00^{j}±0.00
	20	30.00^{gh}±0.00	53.33^{fg}±3.33	93.33^{e}±3.33	100.00^{j}±0.00
	25	43.33^{i}±3.33	63.33^{g}±3.33	100.00^{e}±0.00	100.00^{j}±0.00
	Untreated	0.00^{a}±0.00	0.00^{a}±0.00	0.00^{a}±0.00	10.00^{a}±0.00
	Solvent	0.00^{a}±0.00	0.00^{a}±0.00	0.00^{a}±0.00	6.67^{a}±3.33
	10	3.33^{ab}±3.33	16.67^{abc}±3.33	20.00^{b}±0.00	20.00^{bcd}±0.00
Methanol	15	6.67^{abc}±3.33	13.33^{ab}±2.57	20.00^{b}±3.33	23.33^{cd}±3.33
	20	10.00^{abcd}±0.00	13.33^{ab}±3.33	23.33^{b}±4.41	30.00^{de}±0.00
	25	13.33^{bcde}±3.33	16.67^{abc}±3.33	30.00^{bc}±0.00	33.33^{de}±3.33

Means followed by the same letter (s) within the column are not significantly different (p>0.05) using Tukey's Test.

Table 2: Percent adult emergence from *Callosobruchus maculatus* eggs treated with extract of *M. lucida* (Mean ± SE)

	Extract Concentration (%v/w)					
Extract	**Untreated**	**Solvent**	**10**	**15**	**20**	**25**
Acetone	93.00^a±0.57	95.67^a±0.88	6.33^b±0.33	0.00^a±0.00	0.00^a±0.00	0.00^a±0.00
n-hexane	94.67^a±0.88	94.33^a±1.45	0.00^a±0.00	0.00^a±0.00	0.00^a±0.00	0.00^a±0.00
Methanol	93.89^a±1.52	94.89^a±2.84	5.67^b±5.80	0.00^a±0.00	0.00^a±0.00	0.00^a±0.00

Means followed by the same letter (s) within the column are not significantly different (p>0.05) using Tukey's Test.

Table 3: Number of adults of *C. maculatus* in seed treated with *M. lucida* extracts

	Extract Concentration (%v/w)				
Extract	**Untreated**	**10**	**15**	**20**	**25**
Acetone	9.00±0.00	1.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00
***n*-hexane**	10.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00
Methanol	9.00±0.00	1.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00

Each value is a mean ± SE of three replicates.

DISCUSSION

The efficacy of plant extracts against *Callosobruchus maculatus* in causing adult mortality, oviposition inhibition, progeny reduction and repellency have been reported (Kim *et al*. 2003; Ahmed and Gazzy, 2011; Okonkwo and Okoye, 1996).

Extract of *n*-hexane was the most effective followed by acetone extract while methanol gave the least mortality at all concentrations. Extract of *n*-hexane of *M. lucida* leaves was able to cause 100% adult mortality of *C. maculatus* at 25% concentration at day 3 after treatment. This finding agrees with the report of Ahmed *et al*. (1999) which shows that 100% mortality of *Callosobruchus chinensis* adult was obtained at 3 day post-treatment of the bean seed with neem oil extract. Rahman and Talukder, (2006) reported total mortality and F_1 progeny reduction of *C. maculatus* when 20g of cowpea was treated with 3% oil of *Vitex negundo* (L.). The highest mortality obtained in cowpeas treated with acetone and methanol extracts (73.33% and 33.33% respectively) at 25% extract concentration was at 4 day post-treatment. Acetone is less polar compared to methanol; this enhances its affinity for no-non polar plant constituents which are known to be toxic (Harbone, 1985). Certain unsaturated fatty acids such as oleic acid have been reported to be toxic to insects (Dadd, 1973). Since most insects breathe through the spiracles, the high adult mortality recorded in *n*-hexane treatment could be as a result of blockage of the spiracles or air chamber of the bruchid causing death by suffocation. Plant oils have been reported to block a key neurotransmitter receptor site called octopamine which is found in all invertebrates. Octopamine regulates an insect hearts' rate, movement, behaviour and metabolism. These modes of action result in a total breakdown of the insects' nervous system. (Industrial Research and Development Organization (IDRC, 1997).

Some plant extracts especially oil, have been reported to have inhibitory effect on development of eggs of C. maculatus (Pathak *et al*., 1997; Dwivedi and Kumari, 2000). Ovicidal effect of oils had been attributed to its ability to penetrate the chorion of the eggs which creates hypercarbic condition within the eggs (Don-Pedro, 1989). High percentage ovicidal result obtained in this study might be as a result of activities of extracts components which may have disrupted blastokinesis and inhibits formation of instars.

According to Asawalam *et al*. (2007), insecticidal activity of any plant extract depends on the active constituents of the plant extract.

The major constituents of M. lucida as reported by Nweze *et al*. (2004) and Akinyemi *et al*. (2005) are the alkaloids-anthraquinones, tannins, flavonoids and anthraquinols. Insecticidal activity of the ripe fruit of this plant against Drosophila had been reported (Ajayeoba *et al*., 2006). Effectiveness of the plant was attributed to its alkaloids, tannins, flavonoids and glycosides components (Kemabonta and Okogbue, 2000; Ajayeoba *et al*., 2006). Alkaloids are toxic nitrogenous substances distributed in plants. They are mostly crystalline in nature, colorless and optically active substances (Trease and Evans, 1985). Tannins are phenolic compounds, soluble in water and alcohol, amorphous, and dark coloured; characterized by astringent taste. They are organic acids, the product of sugar metabolism in plant tissues. It precipitates protein and has strong binding ability to toxins. These are natural defense mechanisms in plants against pathogens, herbivores and hostile environmental conditions (Asquith and Butler, 1986; Reed, 1995). This could account for the level of repellency observed in this study. The observed adult mortality, ovicidal and repellent potential of the extracts might be as result of the combined activities of these components and extraction power of the solvents.

CONCLUSION

The extracts of *M. lucida* obtained through different solvents were effective against *C. maculatus*, by causing adult and egg mortality. They also have repellent properties against the adult bruchid. The *n*-hexane extract of the leaves of *M. lucida* was the most effective of the extracts tested in this study.

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