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Bioactivity of the Leaf Extracts of *Morinda Lucida* (Benth.) Against Cowpea Bruchid, *Callosobruchus Maculatus* (F.) (Coleoptera : Chrysomelidae)

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Abstract

Toxicity, ovicidal and repellent potential of acetone, *n*-hexane and methanol extracts of the leaves of a medicinal plant, *Morinda lucida* (Benth) against *Callosobruchus maculatus* (F.) was evaluated at 10, 15, 20 and 25% treatment levels in triplicates. Adult mortality and repellent test was 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% extract concentration 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. All the extract concentrations evaluated were able to repel adult bruchid 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 indicated that extracts of the leaf of *M. lucida* would be effective in protecting cowpea seed against the destructive activities of *C. maculatus*.

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

1. Introduction

The aim of agriculture is high yield in order to provide food for the populace Ngamo *et al*. (2007) but one of the most important constraints of having every day sufficient food is the post–harvests preservation of its quality and quantity (Ngamo *et al.,* 2001).

During storage, foods are destroyed by insects and other pests. In Nigeria, these insects of stored grains are mostly of the Orders Coleoptera and Lepidoptera (Ngamo *et al.,* 2001). Small scale holders could lose 80% of their stock because of insect after 6 to 8 months of storage (Nitoukam and Kitch, 1998).

Cowpea, *Vigna unguiculata* (Walp) is an important food crop in tropical countries especially in West Africa where it is a cheap source of dietary protein (Adedire and Ajayi, 2003). 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 occurs in the field just before harvest and the insect are carried into the store where population builds up rapidly (Ajayi *et al*., 2000). In Nigeria alone, the dry weight loss due to *Callosobruchus maculatus* (F) exceeded 2900 tonnes each year. In some cases damage in terms of holed seed can increase to 99% after 6 months of storage (Ngamo *et al*., 2001).

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

Morinda lucida, is a medicinal plant (Soladoye, 2005). Crude extracts of the leaf have been recommended in the treatment of hypertension and cerebral complication showing distinct diuretic and tranquilizing effects. *M. lucida* 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 (Kemabonta and Okogbue, 2000). The bio-friendly natural dye had been previously extracted from *M. lucida* for the staining of collagen fibre and muscle fibre (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.*

2. Materials and Methods

2.1 Culture of Callosobruchus Maculatus

Adult *C. maculatus* was obtained from naturally infested cowpea seeds at Oba market, Akure Nigeria located between latitude 7.25^{o}N and Longitude 5.2^{o}E. The bruchid was reared on a clean Ife-brown variety of cowpea seeds in two Kilner jars containing 200g of cowpea seeds 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 entry or exit of bruchids and other insects. From this stock, new generations of *C. maculatus* were raised and the culture was maintained by continually replacing the devoured and infested cowpea seeds with fresh, disinfested cowpea seeds.

2.2 Collection of Plant Materials

The leaves of *M. lucida* were collected along the Federal University of Technology road, Akure, Ondo State, Nigeria. The leaves were washed with clean water and air-dried in the laboratory. The leaf was pulverized into fine powder using hammer milling machine. The powder was kept inside black polyethylene bag tied with rubber band until needed.

2.3 Preparation of Plant Extracts

Equal quantity, 500g of the pulverized plant material was extracted with three different solvents: methanol, *n*-hexane and acetone. 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 aluminium foil in moisture-proof container and kept in freezer until required. The *n*-haxane extract was obtained using soxhlet extractor as described by Harbone (1984).

2.4 Leaf Extracts Toxicity Test Against C. Maculatus

Substrate cowpea seed was disinfested for 72 hrs in Thermo cool freezer at -20^{o}C. Twenty grammes of disinfested cowpea seeds was put in plastic container (8cm diameter and 3.5cm depth). Four concentrations levels of 10%, 15%, 20%, and 25% v/v using the respective solvent of each extract as diluents were tested. Untreated (0.00%) and solvent treated by applying 1ml of the solvents were also set up for the treatment with each of the extract as controls. The cowpea seeds and 1ml of each extract concentration level were thoroughly mixed to ensure uniform coating of the extract on the seeds and left for about 1 hr to air dry before introduction of ten (0-24 hrs old) adult *C. maculatus.* All treatments were arranged in completely randomized design in an insect cage and replicated three times. Percentage mortality of the adult bruchid was recorded at day 1, 2, 3, 4 and 5 after treatment and corrected for mortality in control using Abbott's formula (Lale, 2006):

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

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

2.5 Evaluation of Leaf Extracts Ovicidal Potential Against C. Maculatus Egg

One hundred 0-24 hrs old eggs of *C. maculatus* with average of two eggs per seed were put in plastic container and replicated three times. More seed was added to make up to 20g and to each replicate, 1ml of 10%, 15%, 20% and 25% 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. First filia generation was recorded in percentage.

2.6 Extracts Repellent Activity Test Against Adult C. Maculatus

Melted wax was poured inside six Petri-dishes and allowed to solidify. Six holes were carefully made on the solidified wax in the dish. Five different sets of undamaged cowpea seed were treated with 10%, 15%, 20% and 25%, of the different extracts. Untreated (0.00%) was set-up as control. Five seeds of each treatment were placed in the hole made in the solidified wax inside the dish. Treatments were replicated three times. Twenty unsexed 0-24 hrs old of 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.

2.7 Data Analysis

Data obtained were analyzed using analysis of variance and the mean separated by Tukey's Test at 5% level of significance.

3. Results

3.1 Effect of Extracts of M. Lucida Leaf on Mortality of Adult C. Maculatus

All the extracts of the leaf 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 was obtained at 25%w/v of acetone, *n*-hexane and methanol was 53.33%, 100.00% and 30.33% respectively after 3 days of treatment. Extract from *n*-hexane were the most effective against *C. maculatus*. There was no significant difference (P>0.05) between mortality recorded in solvent treated and untreated experiments. Mortalities recorded in *n*-hexane treated experiments was significantly (P<0.05) higher than in acetone and methanol. There was no significant difference (P>0.05) between the effect of 15, 20 and 25% treatment levels of n-hexane at day 3 and day 4 after treatment.

3.2 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, which was significantly different (P<0.005) from others.

Table 1. Mortality of adult *C. maculatus* exposed to leaf extracts of *Morinda lucida*

Extract	Treatment Conc (%)	Mortality at days post-treatment (mean±SE). 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^{a-c}±3.33
	10	16.67^{c-f}±3.33	26.67^{b-d}±3.33	33.33^{bc}±3.33	43.33^{ef}±3.33
Acetone	15	20.00^{d-g}±0.00	30.00^{b-e}±2.57	43.33^{cd}±3.33	56.67^{fg}±3.33
	20	23.33^{e-h}±3.33	33.00^{c-e}±3.33	46.67^{cd}±4.41	60.00^{gh}±5.77
	25	26.67^{f-h}±3.33	40.00^{d-f}±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^{a-c}±0.00
	10	23.00^{e-h}±3.33	43.33^{d-f}±3.33	53.33^{d}±3.33	83.33^{i}±3.33
n-hexane	15	33.33^{hi}±3.33	46.67^{e-g}±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^{a-c}±3.33	20.00^{b}±0.00	20.00^{b-d}±0.00
Methanol	15	6.67^{a-c}±3.33	13.33^{ab}±2.57	20.00^{b}±3.33	23.33^{cd}±3.33
	20	10.00^{a-d}±0.00	13.33^{ab}±3.33	23.33^{b}±4.41	30.00^{de}±0.00
	25	13.33^{b-e}±3.33	16.67^{a-c}±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: Adult emergence from *C. maculatus* eggs treated with extract of *M. lucida* (Mean % ± SE)

Extract	Extract Concentrations: 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.

3.3 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) were able to repel the insect from the treated cowpea seeds. The highest number of insect (9-10) was found on the untreated experiments.

Table 3: Number of adult of *C. maculatus* found on seed treated with *M. lucida* extracts

	Extract Concentrations				
Extract	**Untreated**	**10**	**15**	**20**	**25**
Acetone	$9.00^{a}\pm0.00$	$1.00^{a}\pm0.00$	$0.00^{a}\pm0.00$	$0.00^{a}\pm0.00$	$0.00^{a}\pm0.00$
n-hexane	$10.00^{a}\pm0.00$	$0.00^{a}\pm0.00$	$0.00^{a}\pm0.00$	$0.00^{a}\pm0.00$	$0.00^{a}\pm0.00$
Methanol	$9.00^{a}\pm0.00$	$1.00^{a}\pm0.00$	$0.00^{a}\pm0.00$	$0.00^{a}\pm0.00$	$0.00^{a}\pm0.00$

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

4. Discussion

The use of plant extracts to control stored products insects is an ancient practice (Akinyemi *et al*., 2005). Insecticidal properties of a number of plant extract have been evaluated against stored product insects (Adedire and Ajayi, 2003). Essential oils from some medicinal and aromatic plants are known to possess bioactive compounds that are either toxic to a number of insects at various stages of life or elicit anti-feedant properties (Huang *et al.*, 2000). Kemabonta and Okogbue (2000) reported efficacy of *M. lucida* in causing mortality of adult *C. maculatus* and reduction of oviposition and first filia generation emergence. Significant differences between mortality recorded in the extract treated and controls (solvent and untreated experiments) shows that the active compounds responsible for the mortality of the insects are embedded in plant extracts. According to Asawalam *et al.* (2007), insecticidal activity of any plant extract depends on the active constituents of the plant extract. This study confirms the theory as mortality obtained was both extract and concentration dependent. *n*-hexane extract was the most effective followed by acetone extract while methanol gave the least mortality at all concentrations. *n*-hexane extract of *M. lucida* leaf 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) that 100% mortality of *C. chinensis* adult was obtained at 3 day post-treatment of the bean seed with neem oil extract. The highest mortality obtained in acetone and methanol extracts experiments, 73.33% and 33.33% at 25% extract concentration was at 4 day post-treatment. Certain unsaturated fatty acids such as oleic acid have been reported to be toxic to insects (Ahmed, 1999). Since most insects breathe through the use of spiracles, the high adult mortality recorded in n-hexane treatment could be as a result blockage of spiracles or air chamber of the bruchid causing death by suffocation and its ovicidal effect could be due to its ability to penetrate the chorion of the eggs (Don Pedro, 1989). The major constituents of *M. lucida* as reported by Nweze, *et al.* (2004) and Akinyemi *et al.* (2005) are anthraquinones and anthraquinols. Insecticidal activity of the ripe fruit of the plant against *Drosophila* had been reported and the effectiveness of the plant was attributed to its tannins, alkaloids, flavonoids and glycosides components (Ajayeoba *et al*., 2006). The observed adult mortality, ovicidal and repellent potential of the extracts might be as result of the combined activities of these components and extraction ability of the solvents.

5. Conclusion

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

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