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Full Length Research Paper

Biopesticidal potential of hexane extracts from three plant species against bean weevil and maize weevil

O. O. Ogunsina[1]*, M. O. Oladimeji[2] and L. Lajide[1]

[1]Department of Chemistry, Federal University of Technology, Akure, Ondo State, Nigeria.
[2]Joseph Ayo Babalola University, Ikeji-Arakeji, Osun State, Nigeria.

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A study was conducted to evaluate the insecticidal action of hexane extracts of three locally available plants namely: *Lantana camara* (Verbenaceae), African nutmeg [*Monodora myristica* (Gaerth) Dunal] and Enuopiri [*Euphorbia lateriflora*, Schum and Thonner] against bean weevil *Callosobruchus maculatus* (F.) *and* maize weevil, *Sitophilus zeamais)* Motsch with response to the insect mortality. Results revealed that all test materials exhibited mortality action against bean weevil and maize weevil. African nutmeg extracts had greater mortality action against both the beans weevil (100%) and maize weevil (96%) after 24 h of treatment. *Lantana* extracts were observed to have moderate mortality effect; bean weevil (93%) and maize weevil (73.3%) while Enuopiri had average mortality effect; bean weevil (46.7%) and maize weevil (50%) all with extract concentration of 10 g/100 ml (extract/solvent). The overall results showed that bean weevil was much more susceptible to all the extracts than maize weevil, having recorded the height mortality rate.

Key words: Bean weevil, maize weevil**,** *Lantana camara*, African nutmeg, Enuopiri, mortality.

INTRODUCTION

Corn, *Zea mays* L., is a cereal grass related to wheat, rice, oat and barley; ranking second after wheat in order of world grain. This plant is regarded as versatile and has many uses since it can thrive in diverse climates; hence, it is grown in many countries. Aside from being one of the major sources of food for both human and animals, it is also processed into various food and industrial products including starches, sweeteners, oil, beverages, industrial alcohol and fuel ethanol. Moreover, thousands of foods and other everyday items such as toothpaste, cosmetics, adhesives, shoe polish, ceramics, explosives, construction materials, metal molds, paints, paper goods and textiles contain -corn components. In addition, corn products are rapidly replacing petroleum in many industrial applications. Polylactide (PLA), a biodegradable polymer made from corn is being used successfully in the manufacture of a wide variety of everyday items such as clothing, packaging, carpeting, recreational equipment and food utensils of renewable resource (Garcia, 1990).

Cowpea (*Vigna unguiculata* (L.) Walp.) is one of the most widely adapted, versatile, and nutritious grain legumes (Ehlers and Halla, 1997). It had been consumed by humans since the earliest practice of agriculture in developing countries of Africa, Asia and Latin America, where it is especially valuable as a source of dietary proteins as well as vitamins and minerals (Singh et al., 2003). Though substantial quantities of cowpeas are produced, before harvest or during storage the seeds are vulnerable to infestation by many insect pests, constituting the major constraint on the food availability. Over 90% of the insect damage to cowpea seeds is caused by the "cowpea weevil" *Callosobruchus maculatus* F. (Coeloptera: Bruchidae), a pest to several pulses including chickpeas (*Cicer arietinum* L.), lentils (*Lens culinars* Medik.), soybeans (*Glycine max* Mer.) and common beans (*Phaseolusvulgaris* L.). Indeed *C. maculatus*

*Corresponding author. E-mail: oluwasinagbenga@yahoo.com.

infestation on stored legumes may reach 50% within 3 to 4 months of storage (Pascual-Villalobos and Ballesta-Acosta, 2003). Maize weevil (*Sitophilus zeamais* Motschulsky), may infest the corn grain during storage and transport, attack may start in the mature crop when the moisture content (MC) of the grain had fallen to 18 to 20%. Subsequent infestations in store result from the transfer of infested grain into store or from the pest flying into storage facilities, probably attracted by the odor of the stored grain. In stored maize, heavy infestation of this pest may cause weight losses of as much as 30 to 40%, although losses are commonly 4 to 5% (Casey, 1994).

The chewing damage caused by the weevil (bean and maize), brings about increased respiration in the seeds (hot spots), which promotes evolution of heat and moisture and in turn provides favorable living condition for molds leading to production of aflatoxin. Subsequently, at very high moisture levels, bacterial growth is favored which ultimately gives rise to depreciation and finally total loss (Dahiya, 1999).

Controlling stored pests is not an easy job although synthetic chemicals are available for use. Effective pest control is no longer a matter of heavy application of pesticides (because many rural area farmers resulted to the use of large quantity insecticides because of lack of application knowledge), partly because of rising cost of petroleum – derived products but largely because excessive use of pesticide promotes faster evolution of resistant form of pests, destroys natural enemies, turns formerly innocuous species into pests, harms other non-target species and contaminates food (Busungu and Mushobozy, 1991).

There is, thus, an urgent need for control agents, which are less toxic to man and more readily degradable. Among which is the use of botanical pesticides with low mammalian toxicity and can effectively prevent and/or suppress insect pests especially in storage (Golob and Webley, 1980) . It had been well reported that extracts from a variety of plants have potent insect pest-control properties, and they have being found to affect the biology of target insects in different modes such as ovicides, repellents, antifeedants, fumigants and contact toxicants, and insecticides (Watanabe et al., 1993; Hough-Goldstein, 1990; Karr and Coats, 1988; Rice and Coats, 1994; Tsao et al., 1995) . Recently, herbal extracts that are not harmful to the environment, have been shown to be effective natural preservatives (Grafius and Hayden, 1998; Sen, 2001). In addition, plant- based pesticides are renewable in nature and cheaper. Also, some plants have more than one chemical as an active principle responsible for their biological properties. These may be either for one particular biological effect or they may have diverse ecological effects. The chances of developing quick resistance to different chemicals are highly unlikely (Saxena et al., 1989). Plant-derived pesticides can be transferred into practical applications in natural crop protection, which can help the small-scale farmers (Binggeli, 1999). Three locally available plants, *Lantana camara* (Verbenaceae), African nutmeg [*Monodora myristica* (Gaerth) Dunal] and Enuopiri [*Euphorbia lateriflora*, Schum and Thonner] were evaluated to determine their nature as grain protectants against *Sitophilus zeamais* and *C. maculatus* (F.).

MATERIALS AND METHODS

This study focused on the effectiveness of hexane extracts of Enuopiri stems, *L. camara* leaves and the seeds of African nutmeg as protectants of stored maize and bean grains against attack by *S. zeamais and C. maculatus* (F.). Effectivity was based on the adult mortality tests under laboratory conditions. The experiment was conducted at the laboratory of Chemistry Department Federal University of Technology, Akure, Ondo State, Nigeria, from August 2006 to November 2006.

Collection and preparation of test materials

Fresh and matured leaves of *L. camara (*from police headquarter Akure) were gathered and brought immediately to the laboratory and stems of Enuopiri (*Euphorbia lateriflora* Shum and Thonner) were obtained from Anu-Olu Hospital, Ilobu, Osun, State), while African nutmeg seeds [*Monodora myristica* (Gaerth) Dunal] were bought at Akure Main Market (Oja Oba). Mr. Solomon O. Aduloju of Department of Crop Soil and Pest Management, FUTA, authenticates these materials. The leaves and the stem materials were air dried in the laboratory until crispy and African nutmeg seeds were dehulled. The materials were pulverized using a laboratory blender and sieve to obtain uniform particle size. The resulting powders were kept separately in glass containers with screw capa and stored at room temperature prior to use. 300 g of each of the materials were then exhaustively extracted with n-hexane using a soxhlet apparatus. These extracts were concentrated and kept separately in different labelled bottle containers and stored in a refrigerator.

Mass rearing of weevil

Adult maize weevil (*S. zeamais* Motschulsky) and bean weevil [*C. maculatus* (F.)] were isolated respectively from already infested maize grains (*Z. mays*) and cowpea [V. unguicalata (L.) Walpa] obtained from the Research Laboratory of the Department of Crop, Soil and Pest Management, FUTA, Ondo State, Nigeria. Materials such as corn seeds and cowpea used to culture the weevil were thoroughly cleaned and exposed in an oven to ensure the absence of insects, mites or disease-causing microorganisms. The treated corn seeds and cowpea seeds were then respectively put inside rubber containers previously washed, sterilized and dried. Into the containers containing corn seeds were added isolated maize weevil and to the container containing cowpea seeds were added bean weevil, respectively, from maize and bean weevil obtained from infested maize and bean grains. The plastic containers were covered with a net fastened tightly with rubber bands. The rearing of the insects was done in the laboratory to adapt them to the laboratory condition. The rearing was given enough time until new adult insects emerged; these were then used for the experiment.

Adult mortality test

Five different test concentrations (w/v of extract /hexane) were prepared: 10, 7.5, 5, 2.5 and 1 all in g/100 ml respectively. 0.5 ml

Table 1. Percentage yield of the extracts.

Plant	Solvent	Mass of extracts obtained (g)	% yield of extracts
African nutmeg	Hexane	58.35	19.45
Lantana camara		39.45	13.15
Enu opiri		43.52	14.51

prepared solution of varying concentration was applied inside different Petri-dishes that had been previously washed, sterilized and dried, while 0.5 ml hexane (without the extract) was poured inside separate Petri-dishes as control. All the Petri-dishes were left overnight for the solvent used for preparing the solution to evaporate before introducing the weevil. After 24 h, 10 untreated (uninfested) cowpea and corn seeds were respectively placed in the centre of different Petri-dishes, (including the control). Ten active adult bean weevil (*C. maculatus*) and maize weevil (*S. zeamais* Motschulsky) were placed in each of the Petri-dishes containing the cowpea and maize seeds, respectively. To the control Petri-dishes were also added ten active adult bean weevil (*C. maculatus*) and maize weevil (*S. zeamais* Motschulsky), respectively. The Petri-dishes were then loosely covered to allow passage of air. Weevil mortality was assessed and recorded after 1, 6, 12, 18 and 24 h. The experiment was carried out in triplicate. Percent adult mortality was determined by counting the number of dead insects divided by the total number of insects introduced multiplied by 100, (Lajide and Escoubas, 1990]

Statistical analysis

The data obtained were analysed by Probit analysis using Duncan's multiple range test (DMRT) and analysis of variance (ANOVA), while regression analysis model was used for the computation of LD_{50} and LD_{90}

RESULTS

The result of the percentage extract yields was presented in Table 1 below. African nutmeg hexane extract had the highest yield followed by Enu-opiri and *L. camara* respectively.

Table 2 presented the result of the percentage insect mortality of the extracts on bean weevil. Significant treatment effects were observed with the number of the dead insects after the treatment duration. Table 3 shows the lethal dose analysis of the percentage insect mortality. The result collaborated the effectiveness and the economic valuability of African nutmeg above other extracts

Table 4 presents the result of percentage insect mortality of extracts on maize weevil. The result showed that among the botanical extracts used, African nutmeg had the highest insect mortality and Enu-opiri had the lowest, after 24 h treatment. Table 5 presents the lethal dose activity of the extract, indicating that African nutmeg was superior protectant among all the extract, with small quantity of its extract achieving highest insect mortality

DISCUSSION

The result of the percentage extract yields from the various plants as presented in Table 1 showed that African nutmeg hexane- extract had the highest yield of 19.45%, while *L. camara* had 13.15% and Enuopiri had 14.51% yield. Thus African nutmeg had 4.91 and 6.30% yield higher than Enu-opiri and *L.* camara extracts, respectively. Enu-opiri had 1.36% extract yield higher than Lantana camara, which had the lowest extract yield among all the extract:

$$Extract\ Yield\ (\%) = \frac{mass\ of\ extract\ obtained}{\%\ mass\ of\ sample} x100$$

The result of the percentage insect mortality of the extracts on bean weevil as presented in Table 2 indicated that African nutmeg hexane extract had superior effectiveness among all the botanical extracts. Having 76.70% insect mortality with 1 g/ 100 ml of extract treatment after 24 h, while *L. camara* had 20.00% and Enu-opiri had 6.7%. The effectives of African nutmeg above other extracts were confirmed after treatment at various hour(s). African nutmeg extract was able to record 100% insect mortality with 10 g/100 ml of its extract at 1, 6, 12, 18 and 24 h of insect treatment, while *L. camara* had 93.3% and Enu-opirih had 46.70% all with 10 g/ 100 ml, only after 24 h of treatment. The result from Table 3 showed that it would be economical to embark on mass production of African nutmeg hexane extract for use as protectant of grains, because small quantity of .65 g/ 100 ml (LD_{50}) was able to achieve 50% insect mortality and 2.4 g/100 ml (LD_{90}) was able to kill 90% of the insect within 24 h of the treatment, as against the use of 5.30 g/ 100 ml (LD_{50}) and 9.51 g/100 ml (LD_{90}) for *L. camara* extract, while Enu-opiri (LD_{50}) and (LD_{90}) was undetectable within the test period and with the extract mass used.The result of percentage insect mortality of the extracts against maize weevil presented in Table 4 showed that insect mortality increased with increase in the mass of the extract used and treatment duration, though none of the extract was able to achieve 100% mortality after 24 h treatment. African nutmeg still had the highest mortality of 96.70% after 24 h treatment with 10 g/ 100 ml extract, *L.* camara had 73.3% and Enu-opiri had 50.00%.

Table 5 presented lethal dose result of the plant extracts.

Table 2. Percentage mortality of hexane extracts on bean weevil.

Plant	Extract	Conc (g/ 100 ml)	Exposure duration (h)				
			1	6	12	18	24
A. nutmeg	Hexane	1	20.0±0.0^{b}	33.3±3.3^{b}	50.0±5.8^{b}	66.7±3.3^{b}	76.7±3.3^{b}
		2.5	36.7±3.3^{c}	46.7±6.7^{c}	60.0±5.8^{b}	73.3±3.3^{c}	90.0±5.8^{c}
		5	53.3±3.3^{d}	70.0±5.8^{d}	86.7±6.7^{c}	93.3±3.3^{d}	100.0±0.0^{d}
		7.5	73.3±3.3^{e}	86.7±6.7^{e}	93.3±3.3^{c,d}	100.0±0.0^{d}	100.0±0.0^{d}
		10	100.0±0.0^{f}	100.0±0.0^{f}	100.0±0.0^{d}	100.0±0.0^{d}	100.0±0.0^{d}
L. camara	Hexane	1	3.3±3.3^{a}	6.7±3.3^{a,b}	13.3±3.3^{b}	16.7±3.3^{b}	20.0±0.0^{b}
		2.5	10.0±0.0^{b}	13.3±3.3^{b,c}	20.0±0.0^{b}	26.7±3.3^{c}	26.7±3.3^{b}
		5	16.7±3.3^{b}	23.3±3.3^{c,d}	33.3±3.3^{c}	40.0±0.0^{d}	46.7±3.3^{c}
		7.5	16.7±3.3^{b}	30.0±5.8^{d}	43.3±3.3^{d}	53.3±3.3^{e}	60.0±5.8^{d}
		10	30.0±0.0^{c}	43.3±3.3^{e}	56.7±3.3^{e}	66.7±3.3^{f}	93.3±3.3^{e}
E. opiri	Hexane	1	0.0±0.0^{a}	0.0±0.0^{a}	0.0±0.0^{a}	3.3±3.3^{a,b}	6.7±3.3^{a,b}
		2.5	0.0±0.0^{a}	3.3±3.3^{a}	6.7±3.3^{a,b}	10.0±0.0^{b}	10.0±0.0^{b}
		5	6.7±3.3^{a,b}	13.3±3.3^{b}	16.7±3.3^{b,c}	20.0±0.0^{c}	26.7±3.3^{c}
		7.5	10.0±5.8^{b}	16.7±3.3^{b}	23.3±3.3^{c,d}	33.3±3.3^{d}	40.0±0.0^{d}
		10	13.3±3.3^{b}	26.7±3.3^{c}	33.3±3.3^{d}	40.0±0.0^{d}	46.7±3.3^{e}
Control		0	0.0±0.0^{a}	0.0±0.0^{a}	0.0±0.0^{a}	0.0±0.0^{a}	0.0±0.0^{a}

Values are means of three replicate ± Standard error. Column means followed by different letters are significantly different at $P < 0.05$. A.nutmeg represents African nutmeg, E.opiri represents Enu opiri.

Table 3. The LD$_{50}$ (g/ 100 ml) and LD$_{90}$ (g/ 100 ml) of extracts on Bean Weevil.

Duration (h)	African nutmeg		*L. camara*		Enuopiri	
	Hexane LD$_{50}$	Extract LD$_{90}$	Hexane LD$_{50}$	Extract LD$_{90}$	Hexane LD$_{50}$	Extract LD$_{90}$
12	0.74	6.74	9.08	*	*	*
18	0.71	4.39	6.01	*	*	*
24	0.65	2.49	5.30	9.51	*	*

Represents cases where LD can not be calculated from result.

African nutmeg is the best extract for mass production, since it was able to achieve 50 and 90% insect mortality with the least extract mass of 1.48 g/100 ml (LD$_{50}$) and 8.97 g/100 ml among all the extracts, against 6.96 g/ 100 ml (LD$_{50}$) for *L. camara* and 9.62 g/100 ml (LD$_{50}$) for Enu-opiri. The LD$_{90}$ of *L. camara* and Enu-opiri was undetectable

In conclusion, results of the study revealed that all tested plant extracts exhibited mortality action against maize weevil and bean weevil, with bean weevil been the most susceptible to the plant extracts. African nutmeg hexane extract had 100% bean weevil insect mortality with 5 g/ 100 ml after 24 h of treatment, whereas 73.3% mortality was recorded for maize weevil. With 10 g/ 100 ml extract mass applied after 24 h African nutmeg, *L. camara* and Enu-opiri respectively were able to record 100, 9.30 and 46.7% for bean weevil, but had 96.7, 73.3 and 50% respectively for maize weevil.

The efficacies of different treatments varied, depending on the source of the active ingredient. *Lantadene* A, *Lantadene* B and high flavonoid content, which is mostly associated with deterrence against insects are present in *L. camara* (Ghisalberti, 2000), African nutmeg contained among other charicol, thymol, a – pinene and myristicin a poisonous constituent (Dales, 1996; Cobley, 1976) and Enuopiri contained high quantity of secondary metabolites (Segler, 1994). This result finds support from other studies in which plant extracts were effective in controlling bean weevil and maize weevil, and that they have been effectively used as stored product protectant against insect pests (Bunner, 1993; Ofuya et al., 1992; Aku et al., 1998; Singh et al., 1978; Pandey et al., 1981

Table 4. Percentage mortality hexane extracts on maize weevils.

Plant	Extract	Conc	Exposure duration (h)				
		(g/ 100 ml)	1	6	12	18	24
A. nutmeg	Hexane	1	16.7±3.3^{a,b}	26.7±3.3^{b}	3 3.3±3.3^{b}	46.7±3.3^{b}	46.7±3.3^{b}
		2.5	26.7±3.3^{b}	36.7±3.3^{b,c}	43.3±3.3^{b,c}	53.3±3.3^{b,c}	56.7±3.3^{b}
		5	43.3±8.8^{c}	43.3±3.3^{c}	53.3±3.3^{c}	63.3±3.3^{c,d}	73.3±6.7^{c}
		7.5	53.3±8.8^{c}	56.7±3.3^{d}	6 6.7±3.3^{d}	73.3±6.7^{d}	76.7±8.8^{c}
		10	60.0±5.8^{c}	70.0±0.0^{e}	83.3±8.8^{e}	90.0±5.8^{e}	9 6.7±3.3^{d}
L. camara	Hexane	1	3.3±3.3^{a}	6.7±3.3^{a,b}	10.0±0.0^{b}	10.0±0.0^{b}	10.0±0.0^{b}
		2.5	3.3±3.3^{a}	13.3±3.3^{b,c}	16.7±3.3^{b}	20.0±0.0^{c}	20.0±0.0^{c}
		5	1 3.3±3.3^{b}	20.0±0.0^{c,d}	30.0±0.0^{c}	36.7±3.3^{d}	40.0±0.0^{d}
		7.5	16.7±3.3^{b}	26.7±3.3^{d}	33.3±3.3^{c}	43.3±3.3^{d}	53.3±3.3^{e}
		10	2 6.7±3.3^{c}	40.0±5.8^{e}	53.3±3.3^{d}	63.3±3.3^{e}	73.3±3.3^{f}
E.opiri	Hexane	1	0.0±0.0^{a}	3.3±3.3^{a}	6.7±3.3^{a}	10.0±0.0^{b}	10.0±0.0^{b}
		2.5	3.3±3.3^{a}	10.0±0.0^{a}	16.7±3.3^{b}	16.7±3.3^{c}	20.0±0.0^{c}
		5	16.7±3.3^{b}	23.3±3.3^{b}	23.3±3.3^{b}	23.3±3.3^{d}	30.0±0.0^{d}
		7.5	16.7±3.3^{b}	26.7±3.3^{b}	33.3±3.3^{c}	33.3±3.3^{e}	36.7±3.3^{e}
		10	20.0±5.8^{b}	30.0±5.8^{b}	36.7±3.3^{c}	40.0±0.0^{f}	50.0±0.0^{f}
	Control	**0**	0.0±0.0^{a}	0.0±0.0^{a}	0.0±0.0^{a}	0.0±0.0^{a}	0.0±0.0^{a}

Values are means of three replicate ± Standard error. Column means followed by different letters are significantly different at $P < 0.05$. A.nutmeg represents African nutmeg, E.opiri represents Enu-opiri.

Table 5. The LD_{50} (g/100 ml) and LD_{90} (g/ 100 ml) of extracts on maize weevil**.**

Duration (h)	African nutmeg		*L. camara*		Enu Opiri	
	Hexane LD_{50}	Extract LD_{90}	Hexane LD_{50}	Extract LD $_{90}$	Hexane LD_{50}	Extract LD_{90}
12	4.09	∗	9.75	∗	∗	∗
18	1.78	9.60	8.02	∗	∗	∗
24	1.48	8.97	6.96	∗	9.62	∗

∗ Represents cases where LD can not be calculated from results.

Therefore, the results findings revealed that African nutmeg, L. *camara and* Enuopiri hexane extracts could be used as protectant against maize weevil, *S. zeamais* Motsch and bean weevil, *C. maculatus* (F.). It is recommended therefore that a similar study be conducted by using separately the other parts of the test plants like roots, flowers or even the whole plant to further evaluate their efficacy against maize weevil, bean weevil and other important stored product pests. In addition, the use of other solvent to extract those plants is also recommended to further determine their potential as insecticide against storage seed pests

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