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RESEARCH ARTICLE

TOXICITY OF THREE PLANT EXTRACTS AGAINST BEAN WEEVIL, *Callosobruchus maculatus* (F.) AND MAIZE WEEVIL, *Sitophilus zeamais* Motsch

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ABSTRACT

A study was conducted to evaluate the insecticidal action of hexane extracts of three locally available plants namely: *Anisomeles malabarica*, *Vitex negundo* and *Murraya koenigii* against the 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. *Anisomeles malabarica* extracts had greater mortality action against both the beans weevil (100%) and maize weevil (96.2%) after 24 h of treatment. *Vitex negundo* extracts were observed to have moderate mortality effect; bean weevil (92.3%) and maize weevil (74.6%) while *Murraya koenigii* had average mortality effect; bean weevil (48.7%) and maize weevil (54.1%) 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 highest mortality rate.

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INTRODUCTION

The cowpea weevil, *Callosobruchus maculatus* (Fabricus) (Coleoptera: Bruchidae) is one of the cosmopolitan pests affecting cowpea, *Vigna unguiculata* (L). Infestation by this beetle is common in the field, but most damage is done during storage. Over 90% of the insect damage to cowpea seeds is caused by *Callosobruchus maculatus* and it is the main constraint to increased cowpea production. The stored products are seriously affected by the insect infestation. However, post harvest losses are a serious problem and in India, as much as 20-50% of grain is lost because of infestations from the pest. The 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.

Insect damage in stored grains and pulses may amount to 40% in countries where modern storage technologies have not been introduced. Currently, the measures to control pest infestation in grain and dry food products rely heavily upon the use of gaseous and liquid insecticides, which pose possible health hazards to warm-blooded animals and a risk of environmental contamination. One alternative to synthetic insecticides is insecticidal plants (Thiam and Ducommun, 1993). The use of plant products has assumed significance as an important component of insect pest management because of their economic viability and eco-friendly nature. They hold promise as alternatives to chemical insecticides to reduce pesticide load in the environment.

The use of plant products such as nicotine, pyrethrum and root extracts of rotenone date back to the 17th and 19th centuries respectively. Mixing dried neem (*Azadirachta indica*) leaves with grain in storage is a classic example of natural product use that has been practiced by farmers in many countries for many years. Neem is very effective against a wide range of insect pests (Atwal and Pajni, 1964; Sachan and Pal, 1974; Chellapa and Chelliah, 1976; Rao and Prakash Rao, 1979). Pandey and Singh (1995) found that seeds of black gram could be effectively protected from damage by *Callosobruchus chinensis*, by mixing the seed with dried powder of neem leaves at a rate of 100-400 mg/50gm seed. The botanical insecticides are of great interest to many, because they are "natural" insecticidal toxicants derived from plants. The tropical flora is a major source of plant-based insecticides (Arnason et al., 1989). Aromatic species, particularly those in

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the family Labiatae (or Lamiaceae), are among the most widely used plants in insect pest control (Lambert *et al.*, 1985; Morton, 1981; Shaaya *et al.*, 1997). The flora of India includes several plant species having insecticidal properties. *Anisomeles malabarica* R.Br ex Sins (Family: Lamiaceae) is an erect shrub about 1.8m in height with obtusely tetragonous and softly white stems and branches. The leaves are simple, opposite, very thick, and aromatic; Flowers are purple in dense whorls of more or less interrupted spikes. The herb is reported to possess antispasmodic, antipyretic, diaphoretic, anticancer, diuretic and spermicidal properties. The plant is also acrid, bitter, aromatic, intellect promoting, stomachic, antihelminthic, febrifuge and sudorific. (Prajapati and Kumar 2003). The strong aromatic properties of Magabeera (*Anisomeles malabarica*) attract bees and butterflies and acts as bee tranquilizers. Though India is very rich in flora of medicinal and other indigenous plants, yet not much work has been done to explore the possibilities of using them as grain protectant under Indian conditions, except a few significant contribution of Pandey *et al.*, 1976) and Ketkar (1986) on the insecticidal and grain protectant properties of different plants. The bioactive compounds obtained from these plants kill, repel, inhibit oviposition, and reduce larval development, fecundity and adult fertility (Oparaeke 2007).

Vitex negundo, commonly known as the five-leaved chaste tree, is a large aromatic shrub with quadrangular, densely whitish, tomentose branchlets. It is widely used in folk medicine, particularly in South and Southeast Asia. The leaves are astringent, febrifuge, sedative, tonic and vermifuge. They are useful in dispersing swellings of the joints from acute rheumatism and of the testes from suppressed gonorrhoea. The juice of the leaves is used for removing foetid discharges and worms from ulcers, whilst an oil prepared with the leaf juice is applied to sinuses and scrofulous sores. A decoction of the stems is used in the treatment of burns and scalds. The dried fruit is vermifuge and is also used in the treatment of angina, colds, coughs, rheumatic difficulties etc. The fresh berries are pounded to a pulp and used in the form of a tincture for the relief of paralysis, pains in the limbs, weakness etc. The root is expectorant, febrifuge and tonic. It is used in the treatment of colds and rheumatic ailments. The plant is said to be a malarial preventative and is also used in the treatment of bacterial dysentery - extracts of the leaves have shown bactericidal and antitumor activity. The leaves are used to repel insects in grain stores. Extracts of the leaves have insecticidal activity. The fresh leaves are burnt with grass as a fumigant against mosquitoes. A decoction of the leaves and the vapours are employed in baths for treatment of febrile, catarrhal and rheumatic affections. The flowers are astringent and are used in fever, diarrhoea and liver complaints.

The Curry Tree (*Murraya koenigii*; is a tropical to sub-tropical small tree in the family Rutaceae, which is native to India. The leaves of *Murraya koenigii* are also used as a herb in Ayurvedic medicine. Their properties include much value as an antidiabetic, antioxidant, antimicrobial, anti-inflammatory, hepatoprotective, anti-hypercholesterolemic etc.

The present study was made to assess the insecticidal effect of three locally available plants, *Anisomeles malabarica*, *Vitex*

negundo and *Murraya koenigii* against the bean weevil *Callosobruchus maculatus* (F.) and maize weevil, *Sitophilus zeamais* Motsch with response to the insect mortality.

MATERIALS AND METHODS

Insect Culture

Adult maize weevil (*S. zeamais* Motschulsky) and bean weevil (*C. maculatus* (F.)) were reared and bred under laboratory conditions on the seeds of cowpea (*Vigna unguiculata*) and maize grains (*Z. mays*) inside a growth chamber at $30 \pm 2^{\circ}\text{C}$, 12:12 L: D and with 70% RH. Initially, 50 pairs of 1-2 day old adults were placed in a jar and sealed and a maximum of 7 days were allowed for mating and oviposition. Then parent stocks were removed and seeds containing eggs was transferred to fresh seeds in the breeding jars that were covered with pieces of cloth fastened with rubber band to prevent the contamination and escape of beetles. The subsequent progenies of the weevils were used for all experiments.

Collection and preparation of test materials

Fresh and matured leaves of *Anisomeles malabarica*, *Vitex negundo* and *Murraya koenigii* were collected at their respective places and brought to the laboratory. The leaves and the stem materials were air dried in the laboratory until crispy and 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 cap and stored at room temperature prior to use. 300 g of each of the materials were then exhaustively extracted with hexane using a Soxhlet apparatus. These extracts were concentrated and kept separately in different labeled bottle containers and stored in a refrigerator.

Bioassays

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 prepared solution of varying concentration was applied. 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 LD₅₀ and LD₉₀

RESULTS AND DISCUSSION

The result of the percentage extract yields was presented in Table 1 below. *Anisomeles malabarica* hexane extract had the highest yield followed by *Vitex negundo* and *Murraya koenigii* 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 *Anisomeles malabarica* 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, *Anisomeles malabarica* had the highest insect mortality and *Murraya koenigii* had the lowest, after 24 h treatment. Table 5 presents the lethal dose activity of the extract, indicating that *Anisomeles malabarica* was superior protectant among all the extract, with small quantity of its extract achieving highest insect mortality. The result of the percentage extract yields from the various plants as presented in Table 1 showed that

Table 1. Percentage yield of the extracts

Plant	Solvent	Mass of extracts obtained (g)	% yield of extracts
<i>Anisomeles malabarica</i>	hexane	57.25	19.08
<i>Vitex negundo</i>	hexane	38.35	12.78
<i>Murraya koenigii</i>	hexane	42.55	14.18

Table 2. Percentage mortality of hexane extracts on bean weevil

Plant	Extract	Conc (g/100 ml)	Exposure duration (h)				
			1	6	12	24	36
<i>A.malabarica</i>	Hexane	1	22.0±0.0 ^b	32.3±3.3 ^b	53.0±5.8 ^b	65.7±3.3 ^b	75.7±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	55.3±3.3 ^d	71.0±5.8 ^d	88.7±6.7 ^c	95.3±3.3 ^d	100.0±0.0 ^d
		7.5	71.3±3.3 ^e	87.7±6.7 ^e	93.1±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
<i>V.negundo</i>	Hexane	1	3.1±3.3 ^a	7.7±3.3 ^{a,b}	13.5±3.3 ^b	15.7±3.3 ^b	20.2±0.0 ^b
		2.5	10.0±0.0 ^b	14.3±3.3 ^{b,c}	21.0±0.0 ^b	26.2±3.3 ^c	27.7±3.3 ^b
		5	16.7±3.3 ^b	25.3±3.3 ^{c,d}	34.3±3.3 ^c	42.0±0.0 ^d	45.7±3.3 ^c
		7.5	17.7±3.3 ^b	33.0±5.8 ^d	45.3±3.3 ^d	54.3±3.3 ^e	61.0±5.8 ^d
		10	34.0±0.0 ^c	43.3±3.3 ^e	56.7±3.3 ^c	66.9±3.3 ^f	92.3±3.3 ^e
<i>M. koenigii</i>	Hexane	1	1.0±0.0 ^a	1.5±0.0 ^a	2.0±0.0 ^a	3.3±3.3 ^{a,b}	6.7±3.3 ^{a,b}
		2.5	2.6±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	14.3±3.3 ^b	28.7±3.3 ^c	35.3±3.3 ^d	42.0±0.0 ^d	48.7±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	

Values are means of three replicate ± Standard error. Column means followed by different letters are significantly different at P < 0.05.

Table 3. The LD50 (g/ 100 ml) and LD90 (g/ 100 ml) of extracts on Bean Weevil

Duration (h)	<i>A.malabarica</i>		<i>Vitex negundo</i>		<i>M. koenigii</i>	
	LD50	LD90	LD50	LD90	LD50	LD90
12	0.74	6.74	9.08	*	*	*
18	0.71	4.39	6.01	*	*	*
24	0.65	2.49	5.30	9.5	*	*

Represents cases where LD can not be calculated from result.

Table 4. Percentage mortality hexane extracts on maize weevils

Plant	Extract	Conc (g/100 ml)	Exposure duration (h)				
			1	6	12	18	24
<i>A.malabarica</i>	Hexane	1	16.4±3.3 ^{a,b}	22.6±3.3 ^b	31.3±3.3 ^b	47.2±3.3 ^b	47.7±3.3 ^b
		2.5	24.7±3.3 ^b	34.7±3.3 ^{b,c}	43.5±3.3 ^b	52.3±3.3 ^{b,c}	56.7±3.3 ^b
		5	41.3±8.8 ^c	43.2±3.3 ^c	53.9±3.3 ^c	65.4±3.3 ^{c,d}	73.1±6.7 ^c
		7.5	53.5±8.8 ^c	54.7±3.3 ^d	67.7±3.3 ^d	73.9±6.7 ^d	77.2±8.8 ^c
		10	61.5±5.8 ^c	70.8±0.0 ^e	83.9±8.8 ^e	91.1±5.8 ^e	96.2±3.3 ^d
<i>Vitex negundo</i>	Hexane	1	3.2±3.3 ^a	7.7±3.3 ^{a,b}	10.5±0.0 ^b	10.8±0.0 ^b	10.9±0.0 ^b
		2.5	3.3±3.3 ^a	13.6±3.3 ^{b,c}	16.5±3.3 ^b	22.0±0.0 ^c	24.0±0.0 ^c
		5	14.3±3.3 ^b	21.0±0.0 ^{c,d}	33.0±0.0 ^c	34.7±3.3 ^d	42.0±0.0 ^d
		7.5	16.4±3.3 ^b	26.6±3.3 ^d	33.5±3.3 ^c	43.6±3.3 ^d	52.3±3.3 ^e
		10	28.7±3.3 ^c	42.0±5.8 ^e	54.3±3.3 ^d	64.5±3.3 ^e	74.6±3.3 ^f
<i>M. koenigii</i>	Hexane	1	0.0±0.0 ^a	3.4±3.3 ^a	6.6±3.3 ^a	10.2±0.0 ^b	10.4±0.0 ^b
		2.5	3.2±3.3 ^a	10.4±0.0 ^a	16.4±3.3 ^b	16.6±3.3 ^b	20.5±0.0 ^c
		5	16.8±3.3 ^b	23.7±3.3 ^b	24.3±3.3 ^b	23.9±3.3 ^b	32.0±0.0 ^d
		7.5	17.7±3.3 ^b	28.6±3.3 ^b	33.5±3.3 ^c	37.3±3.3 ^e	39.7±3.3 ^e
		10	22.0±5.8 ^b	30.5±5.8 ^b	38.7±3.3 ^c	42.0±0.0 ^f	54.1±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	

Values are means of three replicate ± Standard error. Column means followed by different letters are significantly different at P < 0.05.

Table 5. The LD50 (g/100 ml) and LD90 (g/ 100 ml) of extracts on maize weevil

Duration (h)	<i>A.malabarica</i>		<i>Vitex negundo</i>		<i>M. koenigii</i>	
	LD50	LD90	LD50	LD90	LD50	LD90
12	4.04	*	9.72	*	*	*
18	1.72	9.63	8.01	*	*	*
24	1.44	8.95	6.90	*	9.64	*

* Represents cases where LD cannot be calculated from result

Anisomeles malabarica hexane- extract had the highest yield of 19.45%, while *Vitex negundo* had 13.15% and *Murraya koenigii* had 14.51% yield. Thus *Anisomeles malabarica* had 4.91 and 6.30% yield higher than *Vitex negundo* and *Murraya koenigii* extracts, respectively. *Murraya koenigii* had 1.36% extract yield higher than *Vitex negundo*, which had the lowest extract yield among all the extract:

$$\text{Extract Yield (\%)} = \frac{\text{mass of extract obtained}}{\text{mass of sample}} \times 100 \%$$

The result of the percentage insect mortality of the extracts on bean weevil as presented in Table 2 indicated that *Anisomeles malabarica* 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 *Vitex negundo* had 20.00% and *Murraya koenigii* had 6.7%. The effectiveness of *Anisomeles malabarica* above other extracts were confirmed after treatment at various hour(s). *Anisomeles malabarica* 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 *Vitex negundo* had 93.3% and *Murraya koenigii* 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 *Anisomeles malabarica* hexane extract for use as protectant of grains, because small quantity of .65 g/ 100 ml (LD₅₀) was able to achieve 50% insect mortality and 2.4 g/100 ml (LD₉₀) *Vitex negundo* was able to kill 90% of the insect within 24 h of the treatment, as against the use of 5.30 g/100 ml (LD₅₀) and 9.51 g/100 ml (LD₉₀) for extract, while *Murraya koenigii* (LD₅₀) and (LD₉₀) 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. *Anisomeles malabarica* still had the highest mortality of 96.70% after 24 h treatment with 10 g/ 100 ml extract, *Vitex negundo* had 73.3% and *Murraya koenigii* had 50.00%. Table 5 presented lethal dose result of the plant extracts. *Anisomeles malabarica* 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 (LD₅₀) and 8.97 g/100 ml among all the extracts, against 6.96 g/ 100 ml (LD₅₀) for *Vitex negundo* and 9.62 g/100 ml (LD₅₀) for *Murraya koenigii*. The LD₉₀ of *Vitex negundo* and *Murraya koenigii* 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. *Anisomeles malabarica* 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 *Anisomeles malabarica*, *Vitex negundo* and *Murraya koenigii* respectively were able to record 100, 93.30 and 46.7% for bean weevil, but had 96.7, 73.3 and 50% respectively for maize weevil. The principle constituents the leaf juice are casticin, isoorientin, chrysofenol D, luteolin, and p-hydroxybenzoic acid content, which is mostly associated with deterrence against insects are present in *Vitex negundo* (Ghisalberti, 2000), *Anisomeles malabarica* contained beta-sitosterol, letulinic acid, ovatodiolide and anisomelic acid. The essential oil from tops and flowers yield a terpene hydrocarbon, citral and geranic acid. (Dales, 1996; Cogley, 1976) and *Murraya koenigii* 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). Therefore, the results findings revealed that *Anisomeles malabarica*, *Vitex negundo* and *Murraya koenigii* 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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