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Leaves of *Lantana camara* Linn. (Verbenaceae) as a potential insecticide for the management of three species of stored grain insect pests

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Abstract Insects cause extensive damage to stored grains and their value added products. Among the stored grain pests *Sitophilus oryzae* (L.) *Callosobruchus chinensis* (Fab.) and *Tribolium castaneum* (Herbst.) are considered as destructive pests in India. Plants may provide alternatives to currently used insect control agents as they constitute rich source in bioactive molecules. *Lantana camara*, an erect shrub, which grows widely in the tropics, exhibits insecticidal activity against several insects. The methanol extract from leaves of *L. camara* has fumigant and contact toxicity against *S. oryzae*, *C. chinensis* and *T. castaneum*. In fumigant assays, The LC₅₀ for *S. oryzae* was 128 µl/L¹, *C. chinensis* 130.3 µl/L¹, and *T. castaneum* 178.7 µl/L¹. The LD₅₀ values for *S. oryzae*, *C. chinensis* and *T. castaneum* in contact toxicity were 0.158, 0.140 and 0.208 mg/cm², respectively. For grain treatment, a concentration of 500 mg/L¹ and 7 days exposure were needed to obtain 90 – 100 % population extinction in all three insects. Probit analysis showed that *C. chinensis* were more susceptible than *S. oryzae* and *T. castaneum*. Gaschromatography-Mass Spectrometer (GCMS) studies for extracts indicated the presence of potent fumigant molecules in *L. camara*. The prospect of utilizing *L. camara* as potent fumigant insecticide is discussed.

Keywords *Lantana camara* · Adult mortality · Fumigant and contact toxicity · *Sitophilus oryzae* · *Tribolium castaneum* · *Callosobruchus chinensis*

Introduction

Insects are serious pests of stored food grains and cause extensive damage in wheat, rice, pulses and other commodities. These pests are distributed worldwide and abundant, both, in species richness and numbers in India (Batish et al. 2008; Rajashekar et al. 2010). The overall damage by insects of stored grains has been estimated to be around 10–40 % annually (Matthews 1993). The common species of primary and secondary pests include moths such as the rice moth *Corcyra cephalonica* (Stainton), *Plodia interpunctella* (Hübner) and beetles/weevils such as *Tribolium castaneum* (Herbst), *Sitophilus oryzae* (L.), *Rhyzopertha dominica* (F.) etc. Synthetic insecticides, especially, fumigants such as methyl bromide and aluminium phosphide are commonly used to manage these pests. However, due to the possible role of methyl bromide in depletion of ozone layer, its use is being banned (Butler and Rodriguez 1996). Development of resistance to phosphine has been reported in several insect species in many countries (Subramanyam and Hagstrum 1995). As well, synthetic compounds have been predicted to undergo bioaccumulation and may cause environmental hazards and cause various biochemical changes in animals and humans (Markowitz 1992; Gupta et al. 2001). Thus, there is a need to develop cheaper and safer alternatives for insect pest control, including plant based products (Dayan et al. 2009).

Many products of botanical origin have been proved to show insecticidal activity against stored grain insects (Akhtar et al. 2008; Rajendran and Sriranjini 2008). Plant extracts have shown ovicidal, repellent, antifeedent and toxic effects in insects (Isman et al. 1990; Isman 2006; Devi and Devi 2011). Essential oils and their individual constituents have been known to play an important role as protectants of stored grains and proved to possess repellent and insecticidal properties (Weaver et al. 1991; Sim et al.

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2006; Yao et al. 2008). The organic extracts from root powder of *Decalepis hamiltonii* (Wight and Arn) showed potential to be used as grain protectant against grain insect pests (Rajashekar et al. 2010; Rajashekar and Shivanandappa 2010).

Lantana camara (Verbenaceae), grows widely throughout the tropical, sub tropical and temperate parts of World. Earlier work has shown that leaves of *L. camara* are a source of insecticidal activity (Ogendo et al. 2003; Dua et al. 2010) and preliminary studies indicated that the leaves of *L. camara* possess a rich source of bioactive molecules (Sharma et al. 1988). The leaves of *L. camara* seem to be promising as a source for a new biopesticide. In the present paper we report the insecticidal activity of the extract from leaves of *L. camara* against three storage pests: rice weevil, *Sitophilus oryzae* L. (Coleoptera: Curculionidae), rust red flour beetle, *Tribolium castaneum* Herbst. (Coleoptera: Tenebrionidae), and adzuki bean weevil, *Callosobruchus chinensis* Fab. (Coleoptera: Bruchidae). The results of the present study will be useful in promoting research aiming at the development of new biofumigant for pest control.

Materials and methods

Insects

Cultures of *S. oryzae* were maintained on whole wheat (*Triticum aestivum*), whereas *T. castaneum* were reared on the whole wheat flour with 2 % yeast powder. *C. chinensis* was reared on whole green gram (*Vigna radiata*). Cultures were maintained at 30 ± 1 °C and 70 % relative humidity. Adults of *S. oryzae* (3–5 days old) and other species (2–3 days old) were used in the experiments. Insect cultures were maintained at 27 ± 2 °C and 70 ± 5 % Relative humidity.

Sample preparation

The healthy mature leaves of *L. camara* were collected in and around Bangalore, Chittoor, Ooty and Pune, India. The leaves were cut into small pieces, dried at 40 °C and powdered. One hundred grams of leaf powder (100 g) was sequentially extracted with a series of solvents of increasing polarity viz., hexane, ethyl acetate, acetone and methanol in a Soxhlet apparatus. The solvent was evaporated *in vacuo* and the residue was dissolved in a known volume of methanol. This solution was screened for insecticidal activity through for contact/fumigant toxicity.

Insecticidal activity

Contact toxicity/residual toxicity The insecticidal activity of extract of *L. camara* against adults of *S. oryzae*, *C. chinensis* and *T. castaneum* was studied by direct contact application

(Rajashekar et al. 2010). Various concentrations (0.004, 0.02, 0.04, 0.08, 0.156 and 0.236 mg/cm^2) of extracts in 1 ml aliquots in methanol of each test compounds were sprayed on filter papers (Axiva Qualitative Disc Filters, 9 cm diameter) and control received 1 ml methanol. The solvent was allowed to evaporate for 20 min followed by release of known age of 20 unsexed adults of *S. oryzae*, *T. castaneum* and *C. chinensis* separately into each dishes and kept at 27 ± 2 °C and 70 ± 5 % relative humidity for 24 h. Each group treatment had four replicates. Insect mortality was recorded after 24 h exposure.

Fumigant toxicity

Filter paper discs of 1.5 – 2 cm diameter were impregnated with different extracts at doses calculated to give equivalent fumigant concentrations of 250– 350 $\mu\text{L/L}^1$ in air (Kim and Ahn 2001). The impregnated filter paper was then attached to the under surface of the screw cap of a glass bottle (320 ml). Known age of 20 adults of *S. oryzae*, *C. chinensis* and *T. castaneum* were released in each glass bottle before the caps were screwed tightly. Numbers of dead insects were determined at 3, 6, 12 and 24 h after exposure. Mortality rate was calculated using Abbott formula for natural mortality in untreated (Abbott 1925).

Comparison of insect toxicity with chemical fumigants

In order to compare the insect toxicity of methanol extract with that of chemical fumigants, the LC_{50} was determined for allyl acetate and ethylformate using the fumigant bioassay. Filter paper discs of 1.5 – 2 cm diameter were impregnated with different extracts/chemical fumigants at doses calculated to give equivalent fumigant concentrations of 250– 350 $\mu\text{L/L}^1$ in air (Kim and Ahn 2001). The impregnated filter paper was then attached to the under surface of the screw cap of a glass bottle (320 ml). Known age of 20 adults of *S. oryzae*, *C. chinensis* and *T. castaneum* were released in each glass bottle before the caps were screwed tightly. Numbers of dead insects were determined at 3, 6, 12 and 24 h after exposure. (Rajendran and Muralidharan 2005; Leelaja et al. 2007). The number of insects knocked down at the time points (3 – 24 h) and percentage mortality were determined.

Grain treatment

Preparation of mixed age culture From the above cultures, adults (1– 2 weeks old) were taken for preparation of mixed-age cultures. About 300 adults of *T. castaneum* were released into 1 kg of respective culture medium in 2 l glass jars. Similarly, 300 adults of *S. oryzae* and *C. chinensis* were allowed to breed separately in 1 kg of wheat and green gram

in 2 l glass jars. After 1 week, the adults were shifted from the cultures. A series of cultures of the respective species were thus maintained continuously. The insect culture jars were kept under the rearing temperature conditions i.e. 25 ± 1 °C for other insects. Insect cultures in six successive weeks (containing 5–6, 4–5, 3–4, 2–3, 1–2, and 0–1 week old insects) were pooled such that the pooled populations contained all developmental stages of the particular species. Rearing media containing mixed-age cultures of individual species was weighed separately in 50 g aliquots into cloth bags (20 cm×14 cm size). Cloth bags containing mixed-age cultures of individual species were placed individually in 850 ml screw cap glass bottles that served as the fumigation chambers. The impregnated filter paper was then attached to the under surface of the screw cap of a glass bottle (850 ml) (Rajendran and Muralidharan 2005).

Fumigation

The stored grain (mixed age culture) insects were exposed to a range of doses of methanol extracts (25 – 500 mg/ L) for 24 h and 7 days at 27 ± 2 °C. For each species, there were 4 replicates per dose with an equal number of untreated control replicates. At the end of exposure, the test insect bags were taken out of the desiccators (glass bottles). The contents of the bags were transferred to individual bottles (12 cm×5 cm size) and kept at rearing temperature and humidity conditions for 8 weeks. The insects, which emerged from wheat (*S. oryzae*) and green gram (*C. chinensis*) or wheat flour (*T. castaneum*) and survived as adults in their respective media were checked at weekly intervals for 8 weeks. Similarly, counts were made in untreated control batches every week. Percent kill was determined by taking the survival/ emergence in total controls as 100 % (Leelaja et al. 2007).

After counting mortality at 7 days of the treated grain the insects (dead and live) were removed and the grain kept under the same experimental conditions (for 28 days) till the emergence of F1 progeny. Based on the life cycle of the insect species, Percentage reduction in adult emergence of F1 progeny or inhibition rate (%IR) was calculated as

$$\text{Percent inhibition rate} = (C_n - T_n)100/C_n$$

Where C_n is the number of newly emerged insects in the untreated jar and T_n is the number of insects in the treated jar.

Gas chromatography–mass spectrometry

The active extract of the *L. camara* leaves was analyzed on a gas chromatograph (HP6890; Agilent Technologies UK Ltd., South Queensferry, West Lothian EH30 9TG) directly linked to a Agilent (model HP5973) mass selective detector operated in electron impact mode (source temperature

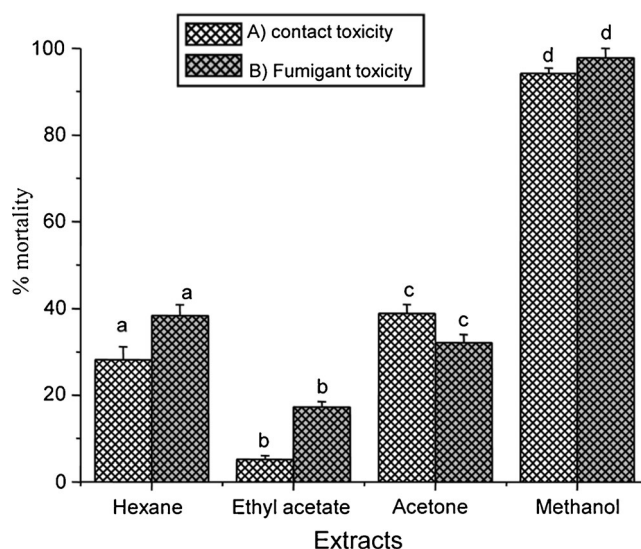


Fig. 1 Insecticidal activity of the solvent extracts of *L. camara* against *Sitophilus oryzae*. The extracts were applied for a) contact toxicity or residual toxicity at 0.27 mg/cm² and b) fumigant toxicity at 500 µl/L¹ ($n=4$, error bars, Standard deviation.) Asterisk values followed by different letters within the bar graphs are significantly different ($P < 0.005$) by One Way ANOVA

230 °C; transfer line 250 °C). The column used was HP-5MS, 5 % phenylmethylsiloxane capillary column (30 m×250 µm i.d. X 0.25 µm), 350 °C max (Agilent 19091S-433) and the carrier gas was helium of 99.999 % purity (1 ml min⁻¹). The column temperature was held at 70 °C for 2 min raised to 240 °C at a ramp of 4 °C per min, held for 10 min to 270 °C at ramp of 6 °C per min held for 12 min, to 290 °C at ramp of 8 °C per min held for 14 min, to 310 °C at ramp of 10 °C per min and held for 16 min. Mass spectra were compared with those in the Wiley 275 L mass spectral library.

Statistical analysis

The data was analysed using One-Way Anova ($p < 0.001$) and means were separated Duncan's multiple range test using the custom made computer programme and Statplus

Table 1 Insecticidal activity of methanol extracts from leaves powder of *Lantana camara* against *Sitophilus oryzae*, *Tribolium castaneum* and *Callosobruchus chinensis* in contact and fumigant toxicity assays

Insect species	Contact toxicity mg/cm ² LC ₅₀	Fumigant toxicity mg/L LC ₅₀
<i>Sitophilus oryzae</i>	0.158 (0.123–0.169) ^a	128.07 (115.2–139.2) ^a
<i>Tribolium castaneum</i>	0.208 (0.182–0.216) ^a	178.7 (160.5–196.2) ^a
<i>Callosobruchus chinensis</i>	0.140 (0.128–0.153) ^a	130.32 (119.0–139.2) ^a

Values are average of 4 replicates

^a Values in parenthesis represent Confidence limits

Table 2 Comparison of insecticidal activity of methanol extract from leaves of *Lantana camara* with the chemical fumigants

Fumigants	LC ₅₀ (mg/L ¹)		
	<i>Tribolium castaneum</i>	<i>Sitophilus oryzae</i>	<i>Callosobruchus chinensis</i>
Methanol extract	178.89	128.08	130.32
Allyl acetate	160.63	25.04	21.04
Ethyl formate	200.0	63.8	54.03

2007 software. The data was expressed as means $\pm$ Standard deviation (SD) ($n=4$). Probit analysis (Finney method) was performed for calculating LC_{50s} and LC₉₅ (Finney 1971).

Results and discussion

With limitations on the use of contact chemical insecticides and fumigants in stored products and increasing public demand for wholesome and pest-free food products, there is a need for developing biorational pest management technologies in stored products. The use of plant products to protect stored grain from insect pest depredation is an age-old practice (Ukeh 2008). Essential oils, extracts and the chemical ingredients have been used greatly in grain protection in many laboratory and field trials (Isman 2006; Ravindra et al. 2009). These natural insecticides are used for the control of stored product insect pests because of their relatively high efficacy against all stages of insects (Tunc et al. 2000; Akhtar et al. 2008).

Pyrethrin, extracted from flower of *Chrysanthemum cinerariaefolium* was considered an ideal insecticide. Today, its use is limited because of its high cost and photosensitive nature. Many studies have reported bioactive compounds from plant extracts with repellent/antifeedant/insecticidal activity against stored-product insect pests (Upasani et al.

Table 4 Mortality (%) of mixed-age cultures of stored-product insects exposed for 7 days to methanol extract of *Lantana camara*

Methanol extract of <i>Lantana camara</i> (mg/L ¹)	Mean mortality (% $\pm$ SD)		
	<i>Sitophilus oryzae</i>	<i>Tribolium castaneum</i>	<i>Callosobruchus chinensis</i>
25	27.7 $\pm$ 1.81 ^a	15.7 $\pm$ 4.01 ^a	25.3 $\pm$ 1.02 ^a
50	44.3 $\pm$ 4.61 ^b	38.3 $\pm$ 3.81 ^b	51.6 $\pm$ 7.89 ^b
100	58.3 $\pm$ 6.21 ^c	52.8 $\pm$ 4.42 ^c	75.8 $\pm$ 4.21 ^c
250	76.5 $\pm$ 4.01 ^d	63.4 $\pm$ 7.39 ^d	90.8 $\pm$ 2.82 ^d
500	93.8 $\pm$ 2.45 ^e	79.4 $\pm$ 1.48 ^e	100

* Values followed by different letters within the vertical columns are significantly different ($P<0.05$) by Duncan's multiple range test ($n=4$)

2003; Akhtar et al. 2008; Yao et al. 2008). The rhizomes of *Acorus calamus* and the active ingredient (β -asarone) were investigated for their insecticidal properties but, the effort to develop β -asarone as an insecticide received a severe setback with discovery of its mutagenic effect (Abel 1987). The seeds of *Azadirachta indica* were reported to show insecticidal activity against a variety of insect species and, azadirachtin, the active principle, exhibited insect antifeedant, moult inhibiting and anti-gonadotropic effects in insects (Schmutterer 1990; Morgan 2009). However, its bitter taste and lack of contact toxicity restricts its use and unsuited on stored products meant for human consumption.

Plant products and essential oils have been used for grain protection in small scale in different parts of the world, particularly India, China and Africa (Golob and Gudrups 1999). Tripathi et al. (2002) reported that 1–8 Cineole from *Curcuma longa* have grain protectant potential against *T. castaneum*. Essential oil of *Ocimum kilimandascharicum* at 1,200 ppm was used as grain protectant against *Rhyzopertha dominica* (Obeng-Ofori et al. 1998). The ethanol extracts of *Cassia tora* and *Clerodendrum inerme* (5 %) were used as grain protectants against *S. oryzae* (Yankanchi and Gadache

Table 3 Mortality (%) of mixed-age cultures of stored-product insects exposed for 48 h to methanol extract of *Lantana camara*

Methanol extract of <i>L. camara</i> (mg/L ¹)	Mean mortality (% $\pm$ SD) ^a		
	<i>Sitophilus oryzae</i>	<i>Tribolium castaneum</i>	<i>Callosobruchus chinensis</i>
25	7.7 $\pm$ 2.12 ^a	5.7 $\pm$ 2.41 ^a	17.7 $\pm$ 4.05 ^a
50	24.3 $\pm$ 2.82 ^b	18.3 $\pm$ 2.82 ^b	25.6 $\pm$ 8.02 ^b
100	38.3 $\pm$ 4.21 ^c	42.8 $\pm$ 2.01 ^c	54.5 $\pm$ 3.41 ^c
250	64.5 $\pm$ 5.01 ^d	56.4 $\pm$ 3.41 ^d	69.5 $\pm$ 9.61 ^d
500	73.8 $\pm$ 4.42 ^e	69.4 $\pm$ 3.42 ^e	89.5 $\pm$ 7.02 ^e

* Values followed by different letters within the vertical columns are significantly different ($P<0.05$) by Duncan's multiple range test ($n=4$)

^a Standard deviation

Table 5 Grain protection potential of methanol extract of *Lantana camara*: Adult emergence in F1 progeny of stored product insects from treated grain

Dosage (mg/L ¹)	% Reduction in F1 progeny (Mean $\pm$ SD)		
	<i>Sitophilus oryzae</i>	<i>Tribolium castaneum</i>	<i>Callosobruchus chinensis</i>
25	12.9 $\pm$ 2.81 ^a	10.2 $\pm$ 8.09 ^a	15.9 $\pm$ 2.04 ^a
50	24.1 $\pm$ 2.81 ^b	38.1 $\pm$ 3.85 ^b	27.4 $\pm$ 3.41 ^b
100	53.3 $\pm$ 3.02 ^c	67.6 $\pm$ 2.01 ^c	59.4 $\pm$ 1.21 ^c
250	77.3 $\pm$ 0.41 ^d	75.3 $\pm$ 1.23 ^d	84.3 $\pm$ 2.81 ^d
500	95.5 $\pm$ 0.81 ^e	88.5 $\pm$ 0.81 ^e	100 ^e

Values followed by different letters within the vertical columns are significantly different ($P<0.05$) by Duncan's multiple range test ($n=4$)

2010). Volatile compounds of many plant extracts are composed of many bioactive molecules, which exhibit fumigant/contact activity. The extracts of *L. camara* were used to protect grain against almond moth (Gotyal et al. 2010). Our preliminary screening showed that the methanol extract was most toxic against *S. oryzae*, followed by ethyl acetate, hexane and acetone (Fig. 1). There were no significant differences in methanol extract from leaves of *L. camara* of different geographical zones of India. Fumigant toxicity of the methanolic extract of *L. camara* to adults of *S. oryzae*, *C. chinensis* and *T. castaneum* is shown in Table 1. Based on LC_{50} values and their respective confidence intervals, the methanol extract was found to be significantly less effective in *T. castaneum* compared to *S. oryzae* and *C. chinensis* adults and this extract shows both fumigant and contact activity. The LC_{50} value for all the three species was variable and species-specific. The results clearly demonstrated that the extracts showed potent fumigant activity against stored grain insect pest and results indicated that the insecticidal mode of action of the compounds may be largely attributed to fumigant action. The fumigant toxicity of methanol extract of *L. camara* was comparable to that of chemical fumigants in *T. castaneum*, whereas in *S. oryzae* and *C. chinensis* it was significantly different (Table 2).

Further, the methanol extract was highly toxic to *S. oryzae*, *T. castaneum* and *C. chinensis* recorded 70–88 % mortality at dose of 500 mg/L¹ in 24 h exposure (Table 3), whereas 90–100 % mortality was recorded at dose of 500 mg/L¹ in 7 days exposure (Table 4), respectively. *T. castaneum* was, however, tolerant to the fumigant such that 80 % mortality of the insects was achieved only at the higher dose of 500 mg/L¹ in 7 day exposures. Generally, an extended exposure period of 7 days increased mortality in all three species (Tables 3 and 4). Results of grain protection showed that methanol extract caused significant reduction in F1 progeny of all the three species in the treated grain and the progeny was completely suppressed at 500 mg/L¹ (Table 5) In present study, for 80 % population extinction of *T. castaneum* the target concentration time (CT) product was high 500 mg/L¹ in 7 days exposures. This may be due to the exceptionally high tolerance of the eggs of *T. castaneum*. There is no previous report about the toxicity of methanol extract of *L. camara* to *T. castaneum*. Our results clearly demonstrate that *C. chinensis* was relatively susceptible probably due to less tolerance their eggs. Further our study shows that methanol extract of *L. camara* could be effective grain protectants, which act by killing various life stages of stored grain insect pests and total suppression of emergence of progeny in treated grain.

The methanol extract of *L. camara* showed a typical chromatogram of leaf volatiles using 5 % Phenylmethylsiloxane (HP-5MS) column (Supplementary Fig. 1). The substances identified by GC-MS in the leaf methanol extract are

presented in Supplementary Table 1. Analysis led to identification of 13 volatiles from the methanol extract of *L. camara* leaves. The main constituents were 4 H, - 1 – Benzo Pyran – 4 – one (54.2 %), Dihydro -1, 3- oxathiole (8.4 %), Benzoic acid (6.77 %), Coumaran (6.09 %), 3 methoxy 4,5,7 TriHydroxy Flavone (4.33 %), Proponoic acid (3.38 %), 5-Oxymethylfurfurole (3.38 %), 1, 2 – Benzenediol (2.71 %), 2, 6- Dimethoxy phenol (2.71 %), P-Hydroxy Benzaldehyde (2.57 %), 2-Hydrozinopyridine (2.03 %), 2- methoxy – 5 Vinyl Phenol (2.03 %) and Phytol (1.35 %). Among them the compounds such as 4 H, - 1 – Benzo Pyran – 4 – one, and Coumaran were earlier reported as fumigant molecules (Scharf et al. 2006; Barakat 2011). Further studies are necessary for the synthesis and bioassay of these compounds for confirming their potential as fumigant. However, technologies may be refined to use of *L. camara* as fumigant for managing *S. oryzae* and *T. castaneum*, as use of these plant volatiles may be environmentally safe with least toxicity to human.

Conclusion

The finding of the present study indicated that the methanol extract from leaves of *L. camara* was toxic to *S. oryzae*, *C. chinensis* and *T. castaneum*, showing promise of *L. camara* becoming a potential source of biopesticide for economical and environmentally friendly pest control strategies against stored grain pests during storage of grains or pulses.

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