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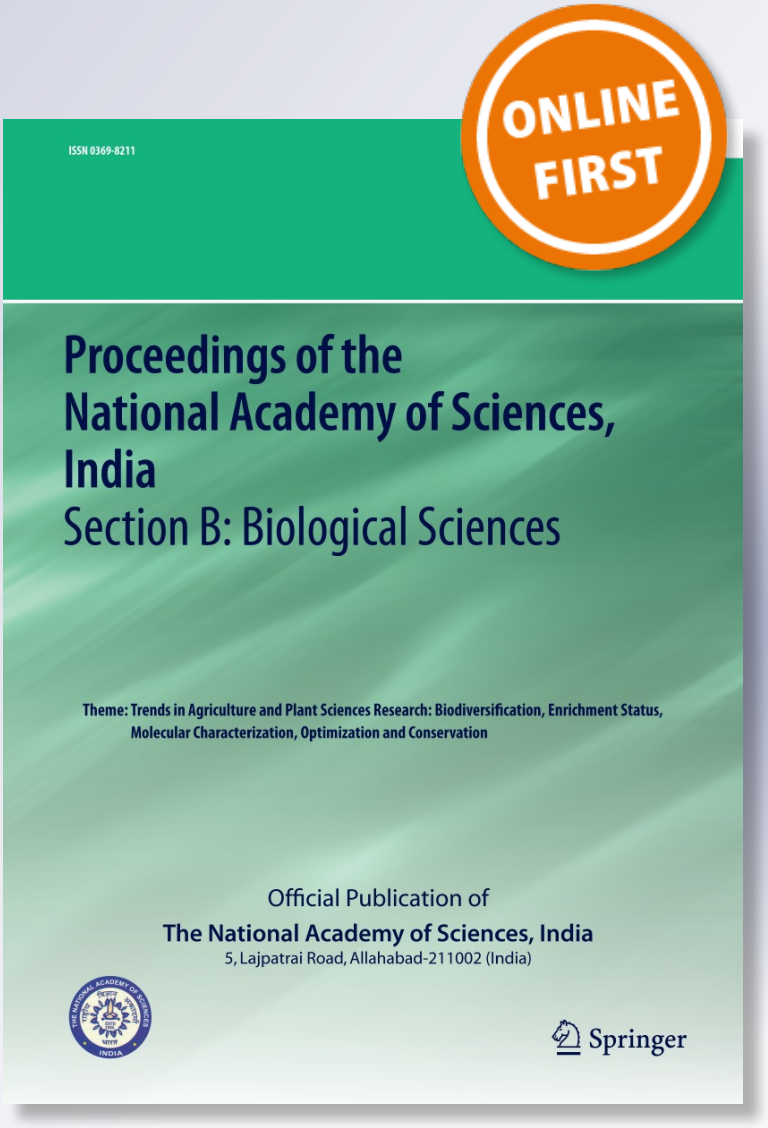

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RESEARCH ARTICLE

Toxicity of *Lantana camara* Leaf Extracts Against Almond Moth, *Cadra cautella* (Walker)

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Abstract The toxicity of various extracts of *Lantana camara* L. leaves was investigated against *Cadra cautella* (Walker) eggs, larvae and adults. For eggs and larvae film residue method and for adults specimen tube method was followed, then it was compared with azadirachtin technical (20 %). The toxicity of various extracts on egg, larval and adult mortality was compared. The egg mortality was based on their hatchability. It was observed that through film residue, most of the extracts of *L. camara* showed higher mortality as compared to azadirachtin. Based on LC_{50} values methanol fraction of hexane extract (LC-3) and hexane extract of hexane fraction (LC-4) of *L. camara* were found to be most effective against different stages of *C. cautella*. The mortality was dose dependent and it increased with increasing concentration. The leaf extracts of *L. camara* were positively toxic to insects. The effects of various extracts on different stages of *C. cautella* are discussed.

Keywords *Lantana camara* · Leaf extract · Toxicity · *Cadra cautella* · Bioassay · Mortality

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Introduction

Synthetic insecticides are widely used to control the insects. It causes serious hazards to both human beings and wild life. It also has an adverse effect on environment and the detrimental effects on the ecosystem. It is well demonstrated when beneficial insects, predators and parasitoids die because of the use of synthetic insecticides. Often pest surge occurs due to development of resistance to the chemicals used. Global concern with the health and environmental impacts of synthetic pesticides, from both consumer and government agencies has led to heightening of restrictions and limitations on the use of these products. The only solution to these problems is to replace synthetic chemicals by biodegradable natural compounds such as the phytochemicals having various biological activities like acute toxicity, feeding inhibition, growth regulation, oviposition deterrence, fecundity reducing and antifertility effects. Various plant byproducts have been tried with a good degree of success as protectants against a number of stored grain pests [1–8]. Being compounds of natural origin, problems with persistence in the environment are not anticipated [9]. The products based on plant extracts, phytooils and purified substances of plant origin can be an alternative to the conventional pesticides [10]. Botanicals like neem have a few advantages as they are cheap and easily available, apart from the fact they are eco-friendly in pest management. Azadirachtin, a complex tetranortri-terpenoid limnoid from the neem seeds, is the main component responsible for both antifeedant and toxic effects in insects. Antifeedancy varies markedly between species, with lepidoptera being particularly sensitive to azadirachtin.

Lantana camara commonly known as lantana or wild sage belongs to the family Verbenaceae. It is an evergreen hairy shrub whose height ranges from 1.22 to 3.00 m. The

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insecticidal, ovipositional deterrent and antifeedant activity of aerial parts of *L. camara* against *Callosobruchus chinensis* has been reported. Petroleum ether and methanol extracts of the plant showed 10–43 % mortality at 1–5 % concentrations and complete feeding deterrent action at 5 % concentrations. Loss of fecundity was noticed in both the extracts at higher doses. The antiovipositional values were 30 mg/100 g for petroleum ether extract and 40 mg/100 g of seed for methanol extract [11]. Several plant species have been noted for their insecticidal effects and one of them viz. *L. camara* is known to possess remarkable biological activity [12–14]. The almond moth, *Cadra cautella* (Walker) (Lepidoptera: Phycitidae) is one of the most economically important stored product pest. If not managed properly, this insect causes serious damage directly from larval feeding on a variety of dried fruits and stored vegetable matter. Additionally, frass and silk filled larval galleries cause significant contamination of the commodity [15]. After 70 days of release of *C. cautella* in different wheat cultivars about 9.8–64.8 % damage was observed [16]. Control of *C. cautella* population worldwide is primarily dependent on repeated application of contact insecticides as space and structural treatment or as protectants, and on fumigants such as methyl bromide and phosphine. Increasing levels of resistance to the most commonly used insecticides have caused multiple and over dosed treatments, fostering safety issues for human health by residue in stored products and serious environmental concerns. Toxic effects of *L. camara* were observed on storage pests, *i.e. Rhyzopertha dominica* and *Callosobruchus chinensis.* In view of these facts, the present study would be useful in promoting research aiming at the development of new agents for control of *C. cautella* based on bioactive chemical compounds from indigenous plant sources. Similarly in the present study toxicity of various extracts of *L. camara* leaves was investigated against different stages of *C. cautella.*

Material and Methods

Insect Culture

The nucleus culture of Almond moth, *C. cautella* was procured from storage laboratory of Entomology Division, Indian Agricultural Research Institute (IARI) New Delhi. The culture was developed in laboratory under controlled condition (26–28 °C and 65–75 % RH) on wheat flour for regular supply of test insect. Freshly emerged male and female adults were released in oviposition jar covered with wire guaze at the bottom to collect the eggs. The adults were supplemented with 10 % honey solution. The collected eggs were cleaned from the scales and used for further bioassay.

Plant Material

Lantana camara (Family: Verbenaceae) is procured from Forest Research Institute (FRI) campus, Dehradun of Uttaranchal State, India. Leaves were cut from the tender shoots and dried under shade and ground to make a fine powder. The powdered plant material was further used for extraction.

Extraction

The shade dried leaf powder of the plant material (8 kg) was placed in different flasks and was soaked overnight in methanol. The mixture was then stirred for 15 min with chemical stirrer and the solvent was filtered after 24 h in a soxhlet apparatus [17]. The filtrate was concentrated in vacuo under reduced temperature in rotary evaporator (Heidolph, Germany). The extraction process was repeated thrice to obtain greenish viscous mass. The plant material remained after extraction with methanol was re-extracted with hexane as above. Hexane extract was similarly concentrated to get a yellowish crude extract. This was kept in a refrigerator for 3–4 days to separate out liquid and solidified material from the crude. The viscous portion was decanted and partitioned between hexane and aqueous methanol solvent. Concentration of hexane extract yielded about 170 g of dark viscous material (LC-4) (Fig. 1). The remaining aqueous methanol fraction was concentrated under vacuo, diluted with water and partitioned with ethyl acetate. Concentration of the ethyl acetate extract yielded about 22 g of the reddish viscous material (LC-0). Due to very less quantity this extract could not be evaluated.

A greenish viscous material refrigerated for 3–4 days to separate out liquid and solid phase. The liquid phase was decanted and serially partitioned with hexane and aqueous methanol and extracts were obtained. The hexane fraction yielded 155 g of greenish dark viscous material (LC-3). The remaining aqueous methanol was partitioned with chloroform, and concentration of the chloroform extract yielded 102 g of solid (LC-2) mass. The same aqueous methanol was then partitioned with ethyl acetate, then concentrated in vacuo to obtain dark colored extract and 45 g of solid (LC-1) mass. The left over aqueous methanol was then partitioned with butanol. The butanol extract was concentrated under reduced pressure to obtain 98 g of solid dark coloured butanol extract, termed as LC-5.

Bioassay

Various extracts of *L. camara* and azadirachtin technical (20 %) were evaluated for their toxicity with seven concentrations (0.01, 0.05, 0.10, 0.50, 1.00, 1.50, and 2.00 %). Required quantity of extracts were weighed and dissolved in respective solvents. The activity of different extracts and

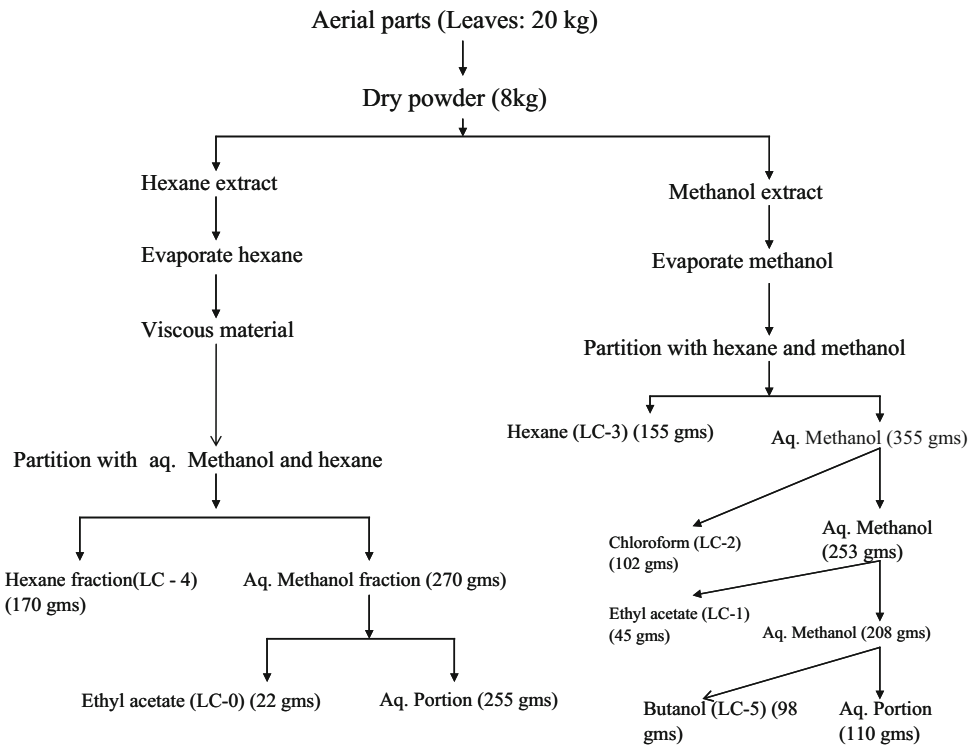

Fig. 1 Extraction of various fractions from leaves of *L. camara*

azadirachtin technical were assessed for their toxicity. The eggs, larvae and adults were introduced in different concentrations of extracts along with azadiractin. The bioassay of larval and egg stages were carried out through film residue method whereas for adults it was done through specimen tube method [18]. Further dilution was done in emulsifier (Tween-80) water by maintaining emulsifier level at 0.5 % to yield various doses. Concentrations were fixed after preliminary screening. Toxicity was evaluated against eggs (early: 0–24 h and late: 2–3 days), larvae (early: 5–7 days, mid: 10–12 days and late: 18–20 days), and adults (early: 0–24 h and late 2–3 days) of *C. cautella*. The different stages of *C. cautella* were released in concentrations of test solution in petri plate and were kept in laboratory at room temperature. Mortality was recorded after 24 h by counting the number of dead eggs, larvae and adults. For eggs, mortality was assessed based on their hatchability.

Statistical Analysis

The analysis was done in complete randomized design (CRD) and the significance was tested at 5 % level. The data was also subjected to probit analysis to obtain LC_{50} values [19].

Results and Discussion

The insecticidal activity of various solvent extracts of *L. camara* was studied on *C. cautella* by film residue and specimen tube method. It was found that different stages viz., egg, larva and adults are affected by various extracts (Tables 1, 2, 3).

Toxicity of *L. camara* Leaf Extracts on Eggs of *C. cautella*

Through film residue treatment of azadirachtin (20 %) and extracts, LC-3 and LC-4 caused maximum early eggs mortality and its LC_{50} values were 0.337, 0.186 and 0.032 % against azadirachin, LC-3 and LC-4 respectively after 24 h of treatment. The higher LC_{50} values were observed with late egg stage and it ranged from 0.525–0.598 % (Table 1) but clear effect of dose could not be observed. The extract LC-4 was found to be more toxic (LC_{50}, 0.032 %) as compared to LC-5 (LC_{50}, 2.04 %) against freshly laid eggs. The toxicity against late eggs and all the extracts are significantly similar as they are in the same fiducial limit. It ranged from 0.515 to 0.647 %. Similar study on ovicidal effect of seed extract of custard apple against *Corcyra cephalonica* and *Trogoderma granarium* was also studied. LC_{50} values for hexane, ethyl acetate and methanol extract against rice moth were reported as 0.218, 0.113 and 0.285 % [20]. The ovicidal activity of flower extract of *L. camara* against *C. cephalonica* eggs was studied in which it was observed that ovicidal effect was dose dependent which may be due to its easy penetration through delicate covering of vitelline and chorion membrane thereby increasing the mortality rate

Table 1 Toxicity of various extracts of *L. camara* against egg stage of *C. cautella*

Extracts	Regression equation	SE of b	LC_{50} (95 % FL)	Heterogeneity X^2 (df)
Early eggs				
LC-1	5.197 + 0.402x	0.062	0.324 (0.188–0.558)	5.315 (5)
LC-2	4.990 + 0.394x	0.095	1.055 (0.642–2.409)	2.653 (5)
LC-3	5.574 + 0.787x	0.067	0.186 (0.138–0.251)	4.510 (5)
LC-4	5.806 + 0.540x	0.083	0.032 (0.017–0.059)	0.147 (5)
LC-5	4.842 + 0.506x	0.067	2.044 (1.069–3.908)	3.593 (5)
Azadirachtin	5.155 + 0.328x	0.060	0.337 (0.173–0.653)	1.674 (5)
Late eggs				
LC-1	5.117 + 0.408x	0.080	0.515 (0.303–0.875)	9.297 (5)
LC-2	5.119 + 0.633x	0.068	0.647 (0.439–0.954)	6.889 (5)
LC-3	5.200 + 0.717x	0.068	0.525 (0.378–0.730)	8.624 (5)
LC-4	5.137 + 0.616x	0.102	0.598 (0.379–0.943)	5.040 (5)
LC-5	5.159 + 0.628x	0.066	0.557 (0.382–0.811)	8.667 (5)
Azadirachtin	60 % Mortality at lowest concentration of 0.01 %			

FL fiducial limit after 24 h of treatment

Table 2 Toxicity of different extracts of *L. camara* on larval stages of *C. cautella*

Extracts	Regression equation	SE of b	LC_{50} (95 % FL)	Heterogeneity X^2 (df)
Early larva				
LC-1	30 % Mortality at 2 % concentration			
LC-2	4.844 + 0.691x	5.465	1.679 (1.037–2.717)	5.465 (5)
LC-3	5.295 + 0.581x	0.064	0.310 (0.211–0.455)	4.262 (5)
LC-4	5.089 + 0.646x	0.090	0.726 (0.494–1.067)	2.194 (5)
LC-5	40 % Mortality at 2 % concentration			
Azadirachtin	5.371 + 1.033x	0.109	0.436 (0.335–0.568)	3.070 (5)
Mid larva				
LC-1	33 % Mortality at 2 % concentration			
LC-2	46 % Mortality at 2 % concentration			
LC-3	4.758 + 0.0995x	0.144	1.418 (0.939–2.140)	5.489 (5)
LC-4	33 % Mortality at 2 % concentration			
LC-5	40 % Mortality at 2 % concentration			
Azadirachtin	4.886 + 0.746x	0.131	1.393 (0.698–2.778)	5.621 (5)
Late larva				
LC-1	4.939 + 0.423x	0.063	1.393 (0.698 + 2.778)	1.272 (5)
LC-2	46 % Mortality at 2 % concentration			
LC-3	5.208 + 0.542x	0.063	0.412 (0.271–0.624)	6.158 (5)
LC-4	46 % Mortality at 2 % concentration			
LC-5	5.183 + 0.516x	0.063	0.441 (0.284–0.684)	10.094 (5)
Azadirachtin	4.839 + 0.426x	0.104	2.398 (0.875–6.574)	5.544

FL fiducial limit after 24 h of treatment

[21]. Hence, results obtained during the present study are in agreement with those of previous workers. The matured eggs were more tolerant to LC-1, LC-3 and LC-4 extracts as compared to early eggs. The LC_{50} of azadirachtin could not be calculated during statistical analysis with late eggs because of low mortality even at highest concentration.

Toxicity of *L. camara* Leaf Extracts on Larval Stages of *C. cautella*

The LC_{50} values in most of larval stages against different extracts could not be calculated because at the maximum concentration of 2 %, less than 50 % mortality was observed.

Table 3 Toxicity of different extracts of *L. camara* on adult stages of *C. cautella*

Extracts	Regression equation	SE of b	LC_{50} (95 % FL)	Heterogeneity X^2 (df)
Early adults				
LC-1	13 % Mortality at 2 % concentration			
LC-2	46 % Mortality at 2 % concentration			
LC-3	5.093 + 0.636x	0.067	0.711 (0.482–1.049)	5.600 (5)
LC-4	4.893 + 0.661x	0.094	1.447 (0.911–2.300)	0.758 (5)
LC-5	46 % Mortality at 2 % concentration			
Azadirachtin	5.264 + 0.386x	0.070	0.206 (0.112–0.381)	7.871 (5)
Late adults				
LC-1	30 % Mortality at 2 % concentration			
LC-2	5.293 + 0.803x	0.091	0.431 (0.319–0.582)	7.372 (5)
LC-3	5.201 + 0.602x	0.065	0.462 (0.316–0.677)	11.023 (5)
LC-4	5.375 + 0.608x	0.064	0.241 (0.166–0.348)	5.648 (5)
LC-5	4.804 + 0.483x	0.067	2.531 (1.229–5.214)	3.588 (5)
Azadirachtin	46 % Mortality at 2 % concentration			

FL fiducial limit after 24 h of treatment

Larval stage of *C. cautella* was more tolerant as compared to egg stage. The extract, LC-3 showed highest mortality against early and late stage of larvae with LC_{50} of 0.310 and 0.412 % respectively. The LC-5 extract was found to be more toxic (LC_{50} 0.441 %) against late larval stage, whereas the mortality of early and mid-larvae could not be calculated due to less than 50 % mortality at highest concentration. Similarly the earlier reports revealed that the lethal concentrations of *L. camara* leaf extracts were 203.49 ppm for the IV instar larvae of *Aedes aegypti* at 24 h of exposure period [22]. The extract LC-3 was found to be most toxic against early and late stages (LC_{50}, 0.310 and 0.418 % respectively) of larval life whereas middle aged larvae were less susceptible with LC_{50} value being 1.748 % (Table 2). The earlier studies showed that, among the five plants, *L. camara* showed best efficacy on larvicidal activity against *Culex quinquifasciatus* as compared with *Calotropis procera*, *Tagetus indica, Cassia fistula* and *Ipomea palmata* at different doses [23]. The activity of *L. camara* was also screened on *Dysdercus koengii* and morphological aberrations were observed because of interference of juvenile hormone (JH) like compounds which had an adverse effect on moulting and consequently resulted in irregular biometrical properties of different parts of the body. It was found that the resultant individuals were malformed and incapable of reproduction and ultimately died [24]. The early stage larvae were found to be susceptible (LC_{50} 0.436 %) with the toxicity of azadirachtin (20 %) as compared to mid and late larval stages and their LC_{50} values being, 1.418 and 2.398 % respectively.

Toxicity of *L. camara* Leaf Extracts on Adult Stage of *C. cautella*

The toxicity of LC-1 and LC-2 extracts was found to be less than 50 % against early stage of adults, even at highest concentration of 2 %, hence LC_{50} could not be calculated. Similar trend was also observed with azadirachtin against late adult stage. The extracts LC-2 and LC-5 were effective only against late stage adults but not to the early adults. The effect of extracts, LC-3 and LC-4 against late adult stage was more toxic with LC_{50} of 0.462 and 0.241 % respectively. The extract LC-5 showed highest LC_{50} value of 2.531 % against late adult stage (Table 3). The earlier researchers observed the insecticidal effect in petroleum ether extract of leaves and twigs of *Azadirachta indica, L. camara, Ageratum conyzoides* and other plant products against *R. dominica* and *C. chinensis* and also found that the contact toxicity of petroleum ether and methanol extract of *L. camara* against *C. chinensis* at 1, 2.5 and 5 % concentrations, resulting 10–43 % mortality [25, 26]. This is in accordance with the present results. Hence, this plant may be exploited for development of bio-rational pest control of stored product insect pests.

Conclusion

The leaf extract of *L. camara* is toxic. This plant is proved to be useful for development of biorational insecticide. Further analysis is required to isolate the active principles and optimum dosages, responsible for ovicidal and larvicidal activity in *C. cautella*. The products of these plants can be well utilized for preparing biocides or phytochemicals. These plants would be eco-friendly and may serve suitable alternative to synthetic insecticides as they are relatively safe, inexpensive and are readily available in many areas of the world.

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