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Fumigant Toxicity of Essential Oil from *Lantana camara* Against Almond Moth, *Cadra cautella* (Walker)

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Abstract: *Lantana camara* is a common weed species in tropics and sub tropics and coastal locations of Asia. The fumigant toxicity of essential oil extracted from leaves of plant was tested against almond moth, *Cadra cautella* (Walker) (Lepidoptera, Phycitidae). Concentrations of essential oil relative to the wheat grains were 5, 10, 25, 50, 100 and 200 µl/l used against early eggs (0-24 hrs.), late eggs (2-3 days), early larvae (5-7 days), mid larvae (10-12days), late larvae (18-20 days) and early adult (0-24 hrs.) and late adults (2-3 days) stage of *C. cautella.* The different concentrations of essential oil were applied to Whatman No. 1 with 2.5 cm in diameter filter paper and then pasted on the inner surface of the lid with adhesive tape in the bottom of fumigation flask ten eggs, larvae and adults of different ages with 20 wheat seeds were kept before keeping the treated filter paper, the lid was closed and sealed to create airtight condition in the chamber. The concentrations of essential oil were tested against different stages of test insect and one control with insect and wheat grain but without essential oil was maintained. The experiment was repeated thrice and observation was made at 6, 12, 24 and 48 hrs. of exposure period. Early eggs showed lower LC_{50} value of 48.56 µl/L compared to 77.75 µl/L with late eggs. The larval LC_{50} values ranged between 29-39 µl/L. Susceptibility of adult stage was also increased and the LC_{50} values being 15.23 and 11.21 µl/L for early and late adult stages, respectively.

Key words: *Cadra cautella*, *Lantana camara*, Essential oil, Bioassay, Exposure period, Fumigant toxicity.

Introduction

The almond moth, *Cadra cautella* (Walker) (Lepidoptera: Phycitidae) is one of the most economically important stored product insect pest. If not managed properly, this insect cause's serious damage directly from larval feeding on a variety of dried fruits and stored vegetable materials, additionally frass and silk filled larval galleries cause significant contamination of the commodity [1].

Essential oils of plant origin are among the best known substances to have attracted attention in recent years as potential pest control agents due to their insecticidal, repellent and antifeedant properties [8]. These oils are usually safe to humans and the environment [20]. Insecticides of plant origin are expected to be target selective and biodegradable leading to fewer harmful effects on human and other animals and are environmentally safe as compared to synthetic compounds [9,14].

Lantana camara (Verbenaceae) is an evergreen hairy shrub, height ranges from 1.22 to 3.00 m planted as an ornamental hedge, widely found in tropics and subtropics and coastal locations of

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Asia. Hydro-distilled and steam-distilled oils from the leaves and flowers of *L. camara* were analysed by combination of GC and GC-MS. Forty four components in the hydrodistilled oil and 25 components in the steam-distilled oil were identified. The main constituents characterized were sabinene (16.5 % and 7.3 %), β-caryophyllene (24.0 % and 22.5 %), 1,8-cineole (10 % and 6 %), bicyclogermacrene (8.1 % and 8.4 %) and α-humulene (6 % and 0.8 %) in the hydro and steam distilled oils respectively [16]. More than twenty components were identified in volatile essential oil from the aerial parts of *L. camara* and active constituents of this plant are lanthanine (or lantadene A), lantadene B, icterogenin and essential oil. Chemically these are triterpenes α-amyrine, β-sitosterol and rehmannic acid. Other constituents are citral and other sesquiterpenes, caryophyllene, α-phelandrene, dipentene, terpeneol, geraniol, linalool, eugenol, methyl-3-oxo-ursulal lantanol acid and lantanic acid [5].

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 [19]. Out of these two fumigants former was withdrawn in 2005 according to the Montreal Protocol on substances that deplete the ozone layer and later has developed resistance against most of the stored grain insects. 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 [7]. One of the most promising approaches to control storage insects is the use of natural insecticides produced by plants. Botanical insecticides have a broad spectrum of pest control, are biodegradable with low mammalian toxicity and pose low danger to the environment [2].

The aim of this study is to evaluate the fumigant activity of essential oil extracted from the wild sage (*Lantana camara*) against different stages of Almond moth, *C. cautella*. The reason of the selection of *C. cautella* for the present study was based on its economic importance. Control of *C. cautella* population worldwide is primarily dependent on repeated application of contact insecticide 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 insecticide have caused multiple and over dosed treatments, fostering safety issues for human health by residue in stored products and serious environmental concerns. Thus there is a need to search ecofriendly protectants as well as fumigants to control storage insect pests.

Materials and methods

Biological material

A colony culture of Almond moth, *C. cautella* was developed in controlled environment, *i.e.* 26-28°C temperature and 65-75 % relative humidity on susceptible HS-420 wheat flour in storage entomology laboratory of Indian agricultural Research Institute (IARI), New Delhi. Eggs were initially kept in petri plate with the wheat flour and after 3-4 days larvae were shifted to glass jars containing wheat flour. Freshly emerged adults of both males and females were released in oviposition jar and it is 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.

Extraction of essential oil

Green fresh leaves of *L. camara* were collected from Forest Research Institute (FRI) campus, Dehradun of Uttaranchal State, India. Five hundred grams of fresh green leaves were washed thoroughly, chopped into small pieces and placed in a conical flask and soaked overnight in distilled water. Next day it was agitated with a mechanical stirrer for 30 min. and placed in a Clevenger's apparatus with distilled water and kept over an electric oven at 45°C. The essential oil fraction was extracted from fresh leaves by hydro-distillation method [12]. The essential oil was accumulated at the neck region of the apparatus and collected after 6 h. The collected oil was dehydrated with anhydrous sodium sulphate and then stored in a refrigerator at 4°C before use.

Bioassay for fumigant activity

The fumigation chamber of flat bottom flasks of 250 ml capacity provided with screw lid was used. The desired concentrations of 5, 10, 25, 50,100, and 200 µl/L of essential oil were applied to Whatman No. 1 with 2.5 cm in diameter filter paper and then pasted on the inner surface of the lid with adhesive tape and the lid was closed and sealed to create airtight condition in the chamber. In the bottom of flask ten eggs and larvae of different ages with 20 wheat seeds were kept. Similarly ten adults were released in the flask before keeping the treated filter paper, for experiments the 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) were used. The concentrations of essential oil were tested against different stages of test insect and one fumigation chamber without oil treatment was considered as control to decide their level of susceptibility. All treatments were replicated thrice. The observation was made at 6, 12, 24 and 48 hrs of exposure period. Larvae were pressed softly with brush to know their movement, for eggs mortality and hatching was observed. The LC_{50} values were calculated by probit analysis.

Results

Efficacy of essential oil against different stages of *C. cautella* was assessed based on its LC_{50} values. The concentrations of 5 to 50 µl/L, mortality could not be observed against early and late adult stage. Mortality was observed against egg and larval stage with six hours exposure period. Adults' mortality appeared only at highest dose of 100 and 200 µl/L. Larval stage was more susceptible as compared to eggs and adult stage. Early eggs showed lower LC_{50} value of 48.56 µl/L compared to late eggs 77.75 µl/L. As the larvae hatched from eggs the susceptibility of neonates increased, being 54.10 µl/L. LC_{50} values against early larval stage, as the larvae grew the LC_{50} values further decreased to 34.06 µl/L at late larval stage. Among the various stages, matured larvae were most susceptible (Table 1). As the exposure period was increased from 6 to 12 h, adult stage was found to be most tolerant followed by eggs and larval stage based on mortality and their LC_{50} values. It was observed that early egg stage were again more susceptible with 30.40 µl/L. The LC_{50} values as compared to late eggs 33.97 µl/L. Larval LC_{50} values ranged between 29-39 µl/L whereas it was 56.60 µl/L with early adult stage. The lethal concentration of essential oil was decreased as exposure period increased from 6 to 12 h of exposure period. The complete mortality could be observed at highest dose of 200 µl/L. at 6 h exposure period against early adult stage.

The lethal concentrations of essential oil required to kill 50 percent population of *C. cautella* was further reduced as the exposure period was increased from 12 to 24 h. Similar to previous results the adult stage was most resistant. Early and mid-larval stages showed higher LC_{50} values compared to egg stages. The maximum dose of 200 µl/L at adult stages showed complete mortality whereas the egg stage could not show more than 90 percent mortality. The mortality was very much dependent on dose and exposure period apart from stage specificity. Complete mortality of adult and larval stages of *C. cautella* was observed at maximum concentration dose of 200 µl/L when exposure period was increased to 48 h. The calculated LC_{50} values showed that as the exposure period was increased, susceptibility of adult stage also increased and the LC_{50} values being 15.23 and 11.21 for early and late adult stages, respectively. Larval stages showed LC_{50} values ranging between 18.25 to 26.50 µl/L. lethal concentrations for early and late egg stages were 19.87 and 23.56 µl/L, respectively.

Discussion

The essential oils are the most economical and convenient tool for managing stored grain insect pests. Furthermore, studies have shown that they are readily biodegradable [3] and less detrimental to non-target organisms than pesticides [21]. Plant essential oils and their constituents in relation to contact [4,6] and fumigant actions have been well demonstrated against stored product pests. Compared with investigation the present study reveals that early eggs showed lower LC_{50} value as compared to late eggs [10]. As the exposure period was increased from 6 h to 12 h it was observed that

Table 1. Toxicity of *Lantana camara* essential oil against Almond moth, *Cadra cautella* at different exposure Period

Stage	Heterogeneity *df*	X^2	SE±b	Regression equation	LC_{50} (µl/L)	Fiducial limits (95 %) Min.	M
Six hours exposure							
Early eggs	7.132	4	0.103	3.226+1.052x	48.563	38.170	61.785
Late eggs	6.316	4	0.100	3.482+0.807x	77.755	53.542	107.18
Early larva	6.651	4	0.107	2.975+1.167x	54.103	43.274	67.642
Mid larva	0.602	4	0.106	3.031+1.152x	51.145	40.897	63.961
Late larva	5.134	4	0.101	3.520+0.965x	34.061	26.474	43.821
Early adults	Complete mortality was observed up to 200 µl/L of concentration						
Late adults	Complete mortality was observed up to 200 µl/L of concentration						
Twelve hours exposure							
Early eggs	4.470	4	1.102	3.508+1.005x	30.404	23.842	38.775
Late eggs	3.423	4	0.104	3.296+1.112x	33.971	27.213	42.402
Early larva	9.394	4	0.103	3.336+1.042x	39.412	31.093	45.957
Mid larva	1.867	4	0.103	3.415+1.077x	29.559	23.511	37.164
Late larva	8.927	4	0.102	3.461+1.014x	32.836	25.790	41.807
Early adults	3.850	4	0.135	3.072+1.104x	56.603	41.149	75.133
Late adults	1.888	4	0.137	2.931+1.214x	50.541	38.817	65.806
Twenty four hours exposure							
Early eggs	2.837	4	0.099	3.760+0.889x	24.714	18.740	35.591
Late eggs	6.051	4	0.100	3.654+0.930x	27.949	21.502	36.329
Early larva	0.990	4	0.127	3.642+0.863x	37.270	26.864	51.706
Mid larva	5.289	4	0.102	3.453+1.031x	31.591	24.915	40.056
Late larva	3.250	4	0.135	3.107+1.328x	26.584	21.650	32.643
Early adults	3.544	4	0.129	3.413+0.935x	49.648	35.438	69.557
Late adults	3.727	4	0.129	3.451+0.908x	50.624	35.680	71.826
Forty eight hours exposure							
Early eggs	3.381	4	0.098	3.996+0.773x	19.871	14.296	27.618
Late eggs	7.582	4	0.100	3.791+0.880x	23.568	17.798	31.257
Early larva	4.538	4	0.127	3.793+0.956x	18.258	13.806	24.146
Mid larva	1.849	4	0.127	3.662+0.956x	25.032	19.014	32.954
Late larva	0.896	4	10.130	3.415+1.150x	23.829	18.905	30.035
Early adults	1.235	4	0.129	3.742+1.062x	15.235	11.709	19.822
Late adults	1.581	4	0.132	3.829+1.114x	11.210	8.495	14.810

early eggs stage were again more susceptible.

Comparison of results presented here with earlier investigations demonstrates that mortality of *C. cautella* eggs, larvae and adults stages due to fumigant activity of essential oil was dose dependent [17]. Larval stage was more susceptible as compared to eggs and adults stage. As the larvae hatched from eggs, susceptibility of neonates increased. The LC_{50} values further decreased at late larval stage. Among the various stages matured larvae were most susceptible. The stage specificity of stored product insects to essential oils were also reported earlier [18]. Complete mortality of adult and larval stage of *C. cautella* was observed at maximum concentration dose of 200 µl/L. when exposure period was increased to 48 h. When fumigant activity of dichlorovos was studied at similar dose and similar exposure period against

various doses. The LC_{50} of all the stages at 48h exposure period could not be calculated as complete mortality occurred at lowest dose of 25 µl/L dose. The most susceptible stage was adults. This showed complete mortality at 5 µl/L dose when a least of 6h exposure period was given. The essential oil *Mentha microphylla* showed the strongest toxicity among the test oils against *Sitophilus oryzae* with LC_{50} value of 0.21 µl/L. The toxicity of *M. microphylla* was 150 fold higher than the two next most active oils of *L. camara* (LC_{50}= 29.47 µl/L) and *Eucalyptus camaldulensis* (LC_{50}=30.81 µl/L). The oils of *Schinus terebenthifolius* and *Citrus reticulata* exhibited moderate fumigant toxicity against *S. oryzae*, while the oils of *Artemisia judaica* and *Majorana hortensis* were not toxic to the insect at the highest concentration tested of 100 µl/L. In the case of *Tribolium castaneum*, the oil of *M. microphylla* (LC_{50}=4.51 µl/L) was the most effective, followed by *Citrus reticulata* (LC_{50}=19.47 µl/L) and *Schinus terebenthifolius* (LC_{50}=20.50 µl/L) [13]. The calculated LC_{50} values showed that as the exposure period was increased, susceptibility of adult stage was also increased. The odors from *L. camara* was capable of suffocation of storage insects in bags, mortality of adults might be due to either inhalation of toxic compounds through spiracles or direct absorption through cuticle [15]. The essential oils of *Zataria multiflora* and *Satueja hortnsis* showed dose dependent mortality against *C. maculatus*. Increase in concentrations of these essential oil, the egg and adults mortality increased while its LC_{50} values decreased [22].

Although essential oil of *L. camara* was less effective than synthetic pyrethroids and chemical insecticides but still it can be used due to their greatest impact in future IPM because of their safety to non-target organism and environment. The essential oils are mainly nerve poisons as these interfere with octopaminergic nervous system in insects. Most of the essential oils are safe to mammals and fish as they are not shared with insect nervous system, hence they can be used as ecofriendly pesticides [11]. Recent investigations indicate that some chemical constituents of these oils interfere with the octopaminergic nervous system in insects. As this target site is not shared with mammals, most essential oil chemicals are relatively non-toxic to mammals and fish in toxicological tests, and meet the criteria for reduced risk pesticides [11].

Conclusion

The present investigation revealed that use of *L. camara* essential oil could be utilized for sustainable management of *C. cautella*. The essential oil of *L. camara* look promising and effective alternative to chemical insecticides against this pest and at the same time it is ecofriendly. Though many of the plant natural products including *L. camara* utilized against insect pests under storage conditions as fumigants and grain protectants. However the complete studies on essential oil and their volatile, chemical constituents, formulations are need to be further studied to exploit the plant products in commercial scale.

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