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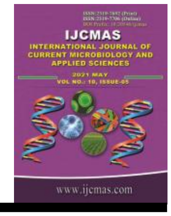

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Chemical Composition and Efficacy of *Lantana camara* L. Essential Oil against Post harvest Invasion of Chickpea by Pulse Beetle (*Callosobruchus chinensis* L.)

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A B S T R A C T

Pulse beetle (*Callosobruchus chinensis* L.) invasion results qualitative and quantitative losses of chickpea seeds during storage. Most of the synthetic chemicals used as preservative are having adverse effects. Therefore, insecticidal potential of *Lantana camara* L. essential oil (LcEO) was evaluated to find out an eco-friendly substitute of synthetic pesticides. The chemical profile of LcEO exhibited 31 known components. Germacrene-D (37.12%) was found as major component followed by β-Caryophyllene (25.18%) and Germacrene-B (16.35%). LcEO oil showed potent insecticidal activity against *C. chinensis* at different concentrations and exposure time. The oviposition by *C. chinensis* was completely checked at 10 µl L^{-1} while F_1 emergence was completely inhibited at 200 µl L^{-1}. During *in situ* experiments, 94.05% protection of the chickpea from *C. chinensis* by LcEO showed superiority over organophosphate insecticide malathion where 90.75% protection was recorded. The LcEO showing potent insecticidal efficacy and may be recommended as plant based preservative in the management of insect infestation of chickpea and other pulses during storage.

Keywords

Lantana camara; Essential oil; *Callosobruchus chinensis*; Insecticidal; GC-MS

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Introduction

Postharvest losses of food commodities in Indian subcontinent is a burning issue because 20-60% of stored grains are deteriorated by various pests viz. insects, fungi, bacteria, rodents etc. (Shaaya *et al.,* 1997). Hot and humid climatic conditions of tropical region are most conducive for infestation of stored pulse commodities by insects especially bruchidaes (Shimizu and Hori, 2009). The pulse beetle, *Callosobruchus* has numerous species causes enormous damage to economically important legumes during storage in tropics (Shukla *et al.*, 2007). To overcome such storage problems, an effective control measure have to develop which minimize the quantitative losses due to infestation by different insect pests.

The application of various prevalent synthetic pesticides since last few decades has made a significant contribution in management of such storage losses but has also raised adverse effects on environmental and human health (Rajkumar *et al.*, 2019). Therefore, some safer alternatives of synthetic chemicals should be explored. Now days, some botanical products are explored as safer and eco-friendly alternative of various synthetic pesticides to protect stored crops and their products (Isman, 2006). Recently, essential oils (EOs) of various plant species are gaining attention because of their bioactivity as insecticidal (Upadhyay *et al.*, 2019), antibacterial (Pandey *et al.*, 2017), antifungal, antioxidant and bioregulatory properties (Holley and Patel, 2005). Therefore, in the present study, efficacy of *Lantana camara* L. (Family-Verbenaceae) essential oil (LcEO) against pulse beetle *Callosobruchus chinensis* L. causing significant deterioration of chickpea (*Cicer arietinum* L.) has been explored to minimize postharvest invasion of pulses as well as enhancement of their shelf life.

Materials and Methods

Sampling

Chickpea seeds (variety- Radha) of about 4-6 months of storage were procured and collected in sterilized polythene bags to avoid further invasion.

Isolation of *L. camara* leaf essential oil

Leaves (500 g) of *L. camara* was thoroughly washed with water and subjected to Clevenger's hydro-distillation apparatus for extraction of EO. The extracted LcEO was dehydrated with anhydrous sodium sulphate and stored in dark clean glass vial at 4±2°C (Kumar *et al.*, 2013).

GC-MS analysis of LcEO

Chemical profile of LcEO was performed at Central Institute of Medicinal and Aromatic Plants, Lucknow, India. To determine chemical profile, LcEO was subjected to GC-MS (Perkin Elmer Turbomass Gold MA, USA) using 60mm × 0.32mm × 0.25mm capillary column.

The GC was performed with injection temperature 250ºC; detector temperature 270ºC; column temperature program isotherm at 70ºC for 2 min, 3ºC/min gradient to 250ºC, isotherm duration was 10 min and flow rate of carrier gas (He) was 1 ml/min. The components of LcEO were identified by comparing their retention times and mass spectra with authentic reference compounds in the literature available in mass spectral libraries of Wiley, NIST and NBS (Adams, 2007).

Insect repellent activity of LcEO

The insect repellency of LcEO was tested against *C. chinensis* following Upadhyay *et al.*, (2019) using 'Y' shaped Olfactometer. The repellency experiment was carried out at room temperature (27±2ºC). A cotton swab soaked separately with requisite amount of LcEO to find out the desired concentration viz. 10, 50, 100, 200 and 500 µl/L with respect to the aerial volume of Olfactometer was plugged in treatment arm whereas in control arm cotton swab without LcEO was plugged.

Thirty adult insects were inserted separately from the base of the Olfactometer. After 30 min the number of insects in the treatment and control arm was recorded.

Insecticidal activity of LcEO

The insecticidal activity of LcEO against *C. chinensis* was assessed by direct contact method. The requisite amount of LcEO was soaked on two layered Whatman no. 1 filter paper placed in a Petri dish (90 mm) to find out the final concentrations i.e. 0.1, 0.5, 1, 5, 10, 50, 100 and 200 µl/L as per aerial volume of Petri plate. Thirty insects of equal age (5-7 days) were inserted separately in Petri dishes fumigated with LcEO. Control sets were kept parallel to treatment sets without LcEO. Percent mortality of insects in each set was recorded on 2, 4, 6, 8 and 10 hour exposure (Kumar *et al.*, 2009).

Oviposition deterrent activity of LcEO

The LcEO was tested for its effect on oviposition of *C. chinensis*. Healthy seeds (50 g) of chickpea were taken in plastic containers (250 ml) and fumigated separately with requisite concentrations viz. 0.1, 0.5, 1.0, 2.0, 5.0 and 10.0 µl/L of LcEO.

After an hour, 30 bruchids of almost same age were introduced in each container separately for egg laying. The control sets run along with treatment set without LcEO. Insects were removed after 24 hours and laid eggs were counted after 3-4 day of release. The effect of LcEO in reduction of egg laying was calculated following Sabbour (2019).

Ovicidal activity of LcEO

The LcEO was tested for its inhibitory effect on F_1 adult emergence of *C. chinensis*. Chickpea seeds containing eggs were taken in plastic containers (250 ml) and fumigated separately with requisite concentrations viz. 5, 10, 20, 50, 100, 200, 500 and 1000 µl/L of LcEO. The fumigated sets along with control were incubated at 27±2°C and RH 80±5%. The number of pulse beetles of F_1 generation emerging from chickpea seeds from fumigated and control sets was recorded up to 30 days. The effect of LcEO on adult development (F_1 generation) was determined following Kumar *et al.*, (2009).

Determination of feeding deterrence index

To determine its practical applicability, 5.0 kg of chickpea seeds were fumigated with 1.0 ml LcEO in plastic containers (10 L) for six months. Along with treatment sets, one positive control fumigated with malathion, an organophosphate insecticide at 1.0 g/10 L and one negative control without fumigation was also kept. All the containers were infested with 25.0 g of previously infested chickpea seeds. The level of insect infestation and grain damage was monitored after six months by quantifying the feeding deterrence index (Brari and Kumar, 2019).

Seed germination test

The percent germination of chickpea seeds, fumigated with LcEO, was tested after six month of storage. Hundred uninfested seeds were taken from each fumigated set, soaked in distilled water for 3 h.

Thereafter, seeds were aseptically transferred to Petri dishes (15 cm diameter) containing moist filter paper and incubated at 25±2°C. Hundred healthy and uninfested seeds were procured from the market as control for comparison. The number of seeds germinated within a week was recorded as viable (Kumar *et al.*, 2009).

Data Analysis

All the treatments were in triplicate and the data presented are mean ± Standard Error (SE). The data for bioactivity of LcEO against *C. chinensis* were analysed using software SPSS version 16.0.

Results and Discussion

The LcEO was extracted through hydrodistillation and characterized with its pungent smell, yellow green colour and 0.96 % yield (w/w). The GC/GC-MS analysis of LcEO showed 31 considerable peaks. The GC-MS analysis exhibited Germacrene-D (37.12%) as major component followed by β-Caryophyllene (25.18%) and Germacrene-B (16.35%) were recorded as major components. β-Pinene (1.40%), β-Caryophyllene (1.32%), Caryophyllene oxide (1.22%), β-Elemene (1.21%), α-Terpineol (1.19%), Terpinene-4-ol (1.10%), Viridiflorol (1.06%), δ-Cadinol (1.03%) and Valencene (1.10%) were recorded in small quantity while rest other identified components were found in trace amount (Table 1; Fig. 1).

The percent insect repellency of LcEO against *C. chinensis* increased with increasing concentration. The percent repellency increased upto 100 µl/L and beyond this became almost constant (Fig. 2).

Similarly, LcEO exhibited potent insecticidal activity which was found directly proportional to concentration and exposure period. During the study 100% mortality of the insect was recorded at 200 µl/L on two hour exposure to oil while complete mortality was noted at 1 µl/L when exposure period was increased to 10 hour (Fig. 3).

In addition, oviposition deterrency and ovicidal activity of LcEO was also evaluated. LcEO altered the egg laying behavior of *C. chinensis*. The oviposition deterrency increased with increasing LcEO concentration and egg laying was completely checked at 10 µl/L (Fig. 4). The LcEO potentially inhibited the adult emergence from the eggs. The F_1 emergence decreased with increasing EO concentration and completely checked at 200 µl/L (Fig. 5).

During *in situ* experiments conducted to observe efficacy of LcEO as feeding deterrent, 89.46% protection of the chickpea from *C. chinensis* infestation was recorded showing slight superiority of LcEO over the prevalent organophosphate insecticide malathion where 88.63% protection was recorded (Table 2). The treated chickpea seeds having no significant losses in their viability even after six months of fumigation. LcEO treated chickpea seeds showed 73.52% germination while malathion exhibited 72.81% (Table 3) also showing minor superiority over malathion.

L. camara L. plants are luxuriously growing as weed is an invasive species found in most part of Uttar Pradesh (Kumar *et al.,* 2010). The plant contains significant amount of EO, highly demanded in various industries. The chemical composition of EOs varies with age of the plant, season of collection, geographical area and soil characteristics (Rawat *et al.,* 2020). Hence, the extracted LcEO was standardized to determine its chemical profile through GC–MS analysis. Plant EOs are complex mixtures of terpenic (especially mono- and sesquiterpenes and mono- and sesquiterpenoids), aromatic, and aliphatic components (Ebadollahi *et al.,* 2020).

Plant EOs or their volatile components cause critical defense strategies against herbivorous pests. They also have a vigorous role in plant–plant interactions and attraction of pollinators (Theis and Lerdau, 2003; Tholl, 2006). EOs exhibit a wide spectrum of pesticidal activities from lethal to sublethal effects against a wide range of insects and mites (Campos *et al.,* 2019). Pesticidal effects of essential oils extracted from different plant families such as Apiaceae, Asteraceae, Chenopodiaceae, Cupressaceae, Lamiaceae, Lauraceae, Myrtaceae, Zingiberaceae, Umbelliferae, and Geraniaceae have been documented (Ebadollahi *et al.,* 2020)

Table.1 Chemical composition of LcEO

RT	Compounds	Percentage
7.201	3-Methyl-2-heptanone	**0.18**
8.401	2-Methylcyclopentanol acetate	**Tr***
10.476	2,6-Dimethyl-2,7-octadiene-1,6-diol	**Tr***
11.177	6-Methyl-5-hepten-2-one	**0.32**
11.301	α-Pinene	**0.73**
11.951	Sabinene	**Tr***
12.286	Caryophyllene diepoxide	**0.57**
12.501	Linalol oxide	**0.27**
12.901	β-Pinene	**1.40**
13.226	Terpinolene	**0.41**
13.401	2-Nonyne	**0.36**
14.351	Terpinene-4-ol	**1.10**
14.751	α-Terpineol	**1.19**
14.926	δ-Elemene	**0.68**
15.922	**β-Caryophyllene**	**25.18**
16.551	α-Copaene	**0.87**
17.126	Linalyl acetate	**Tr***
18.301	β-Elemene	**1.21**
18.951	β-Caryophyllene	**1.32**
19.451	γ-Elemene	**0.77**
20.134	**Germacrene-D**	**37.12**
26.526	Valencene	**0.68**
24.676	Trans carvone oxide	**1.01**
28.776	**Germacrene-B**	**16.35**
31.401	3-Heptadecen-5-yne	**Tr***
38.276	Caryophyllene oxide	**1.22**
44.224	2,3,4,5-Tetramethyl cyclopent-2-en-1-ol	**Tr***
48.217	β-Costal	**0.69**
51.202	Viridiflorol	**0.36**
54.652	α-Farnesene	**0.38**
55.727	δ-Cadinol	**1.03**
	Total	**95.%**

RT= Retention time; Tr*- Trace amount (<0.10%)

Table.2 Comparative efficacy of LcEO and malathion on feeding behaviour of *C. chinensis*

Fumigants	Dose	FDI (%)
LcEO	100 µl L^{-1}	89.46 ± 4.18
Malathion	100 mg L^{-1}	88.63 ± 3.28

Values are mean (n = 3) ± standard error

Table.3 Comparative efficacy of LcEO and malathion on germination of chickpea seeds

Treatment	Germination (%)
Control	84.72 ± 4.14
LcEO	73.52 ± 5.26
Malathion	72.81 ± 3.42

Values are mean (n = 3) ± standard error

Fig.1 GC-MS chromatogram of LcEO

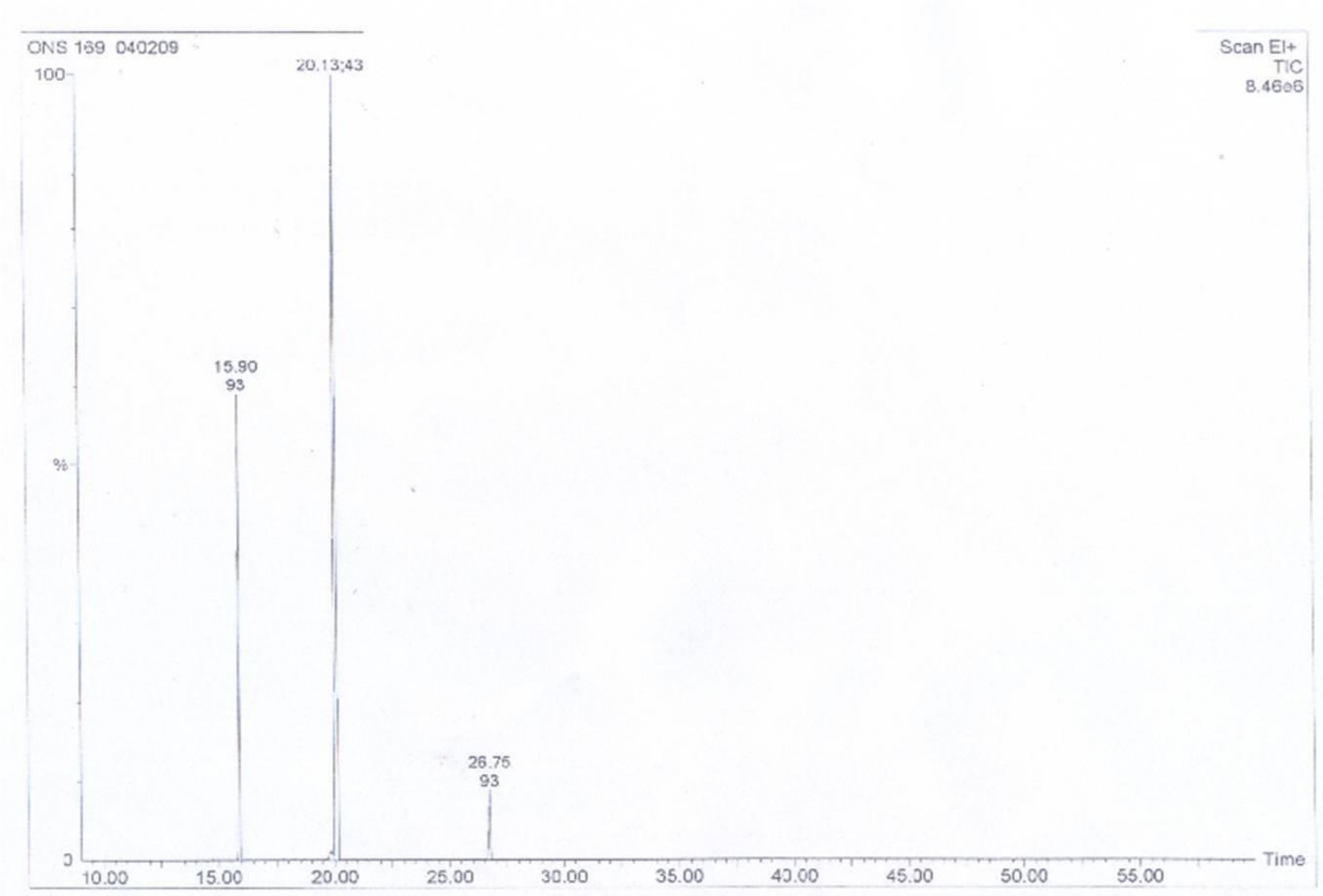


Fig.2 Insect repellent activity of LcEO against *C. chinensis*

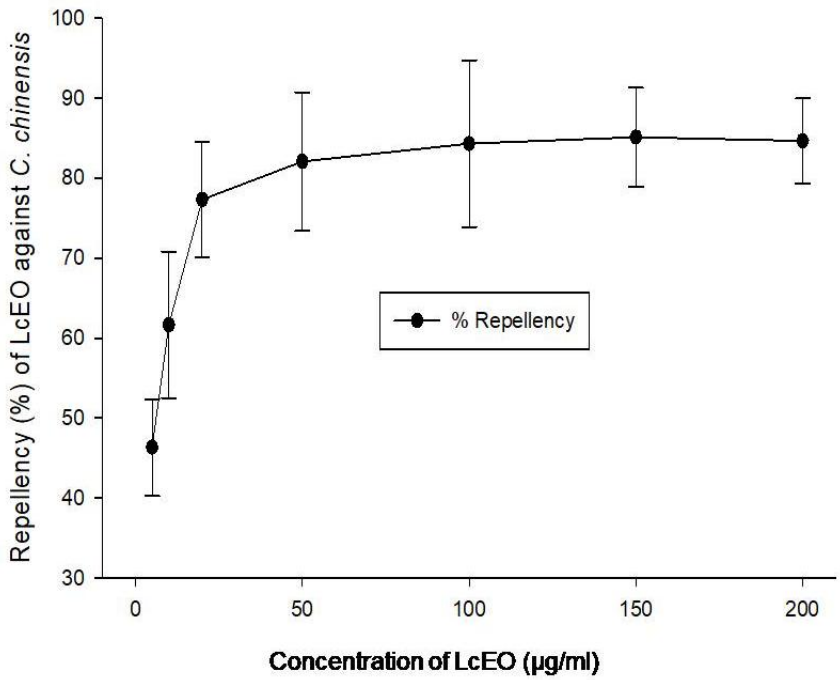


Fig.3 Oviposition deterrent activity of LcEO against egg laying of *C. chinensis*

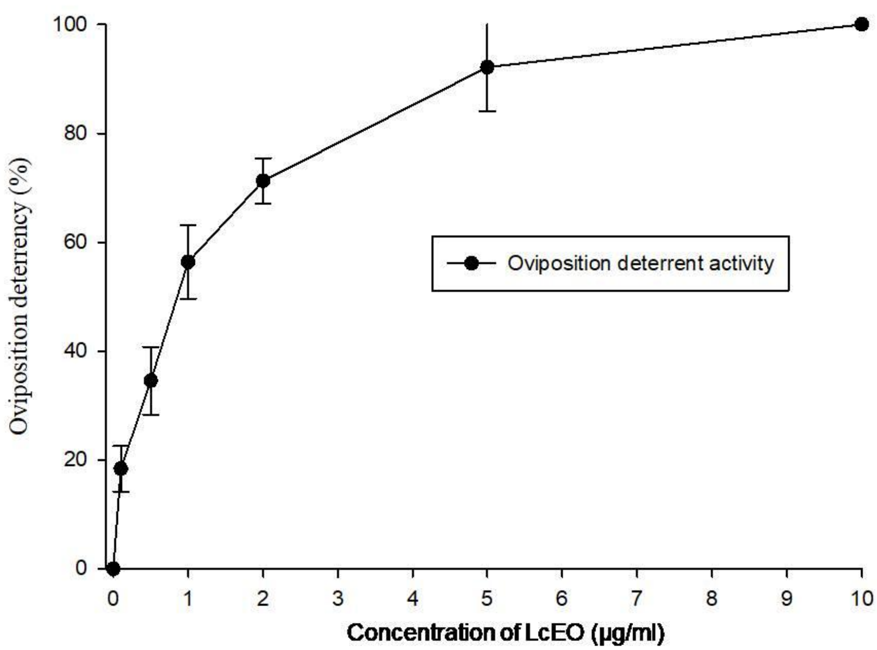


Fig.4 Ovicidal activity of LcEO against *C. chinensis*

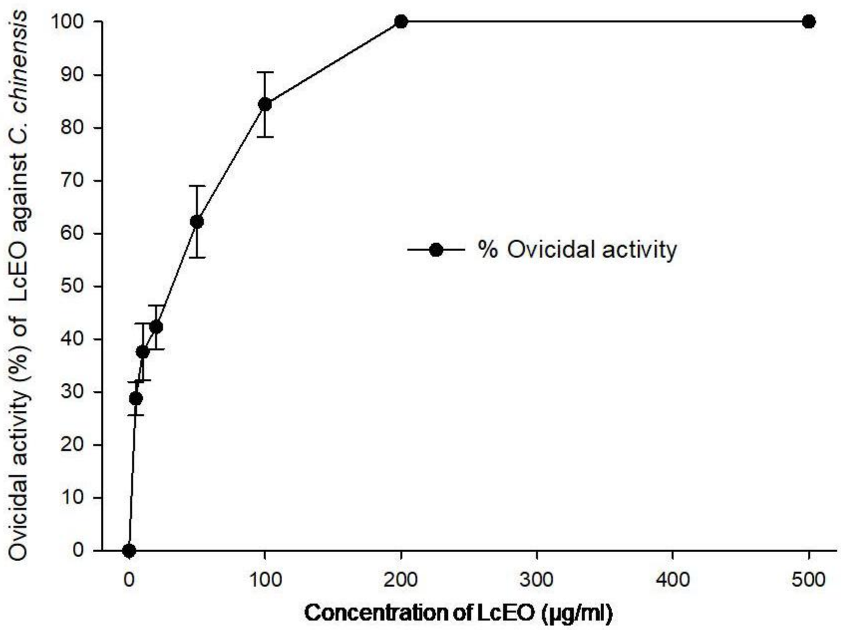
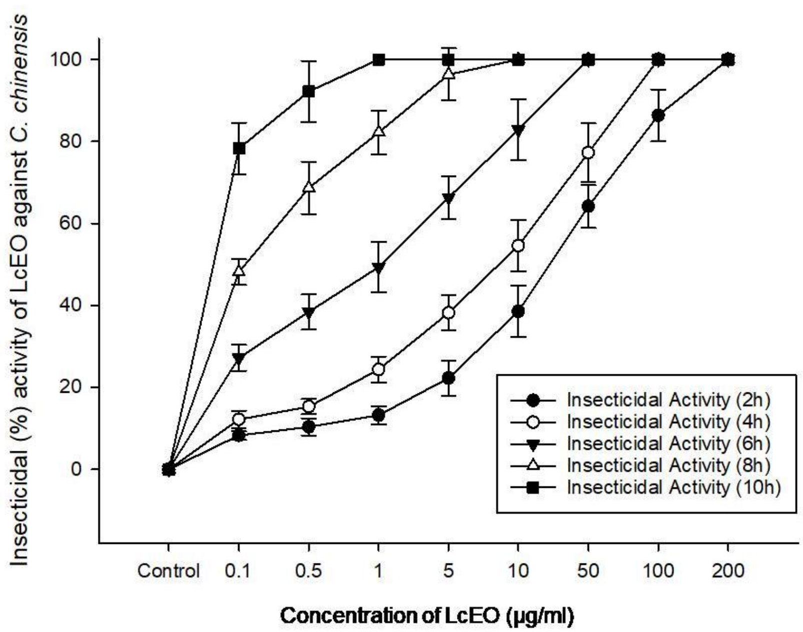


Fig.5 Insecticidal activity (%) of LcEO against *C. chinensis*



In the present investigation, an effort has been made to evaluate the efficacy of LcEO against pulse beetle *C. chinensis* severely infested stored chickpea seeds. Although, some plant products like azadirachtin (Chaudhary *et al.*, 2017), pyrethrum (Sun *et al.*, 2020), rotenone (Huang *et al.*, 2018), sabadilla (Isman, 2006), ryania (Jefferies *et al.*, 1992), termeron (Leyva *et al.*, 2020) etc. are in large scale use in control of different insect pests. The LcEO in the present investigation showed potent insecticidal efficacy. Such a virtue of LcEO would increase its market value if formulated as botanical pesticide in protection of stored food commodities.

The degree of susceptibility of *C. chinensis* adults to LcEO varies with concentration and exposure period. By increasing the exposure duration (10 hr), the oil showed complete mortality of the insects even at 1 µl/L.

Thus, even lower doses of the LcEO may be sufficient to protect the commodities from insect infestation in case of their storage for longer duration. Similarly, oviposition deterrency is also directly related with LcEO concentration.

The volatiles present in the LcEO may be altering the egg laying behaviour of the insects as reported by Autran *et al.,* (2009). Such products in pest management are termed as 'behavior altering chemicals or "semiochemicals" and are recommended in integrated pest management. The use of such 'behavior altering chemicals' would solve the problem of resistance development in target pests which is frequently reported by use of different prevalent synthetic chemicals which act through cidal (lethal) mode of action (Isman, 2006). Likewise, the reduction in F_1 emergence from the treated eggs may be due to mortality of developing embryos in the eggs exposed to the LcEO.

The findings of the present investigation may draw the attention regarding large scale exploitation of LcEO as plant based insecticide for stored food commodities. The attraction of modern society towards 'green consumerism' (Smid and Gorris, 1999) desiring fewer synthetic chemicals in protection of food commodities and recommendation of herbal products as 'generally recognized as safe' (GRAS) in the developed countries (Burt, 2004). The significant bioactivity of LcEO against pulse beetle may lead scientific interest for its exploitation in postharvest storage technology of food commodities in complete protection from insect pests. The LcEO may be a safer alternative to prevalent insecticides in control of postharvest insect invasion of food commodities and pulses. Because of luxuriant growth of *L. camara* in central India, sufficient amount of raw materials would be available for the extraction of EO. Further investigations are needed to determine its safety profile and the exact mode of action of the active ingredients.

The findings of present investigation reveal that, the LcEO exhibited strong insect repellent, insecticidal, oviposition deterrent and ovicidal activity against pulse beetle *C. chinensis* strengthen its possible exploitation as an indigenous, safe plant-based green pesticide for postharvest storage of food commodities to enhance their shelf life against insect pests.

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