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Bioactivity of Trans-cinnamaldehyde against Cigarette Beetle, *Lasioderma serricorne* (Fabricius) (Coleoptera: Anobiidae)

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Abstract: Insecticidal activity of the trans-cinnamaldehyde was studied against the cigarette beetle, *Lasioderma serricorne* (F.) under laboratory conditions. The egg stage was the most tolerant stage for both contact and fumigant toxicity. The mortality of *L. serricorne* grubs was highest (96.67%) at 2000 ppm of trans-cinnamaldehyde when applied topically. In case of adults, highest mortality of 98.33 per cent was observed at 4000 and 5000 ppm of trans-cinnamaldehyde after 48 hours after treatment. Trans-cinnamaldehyde caused 96.67 and 98.33 per cent mortality of grubs and adults of *L. serricorne,* respectively at 50000 ppm after 48 hours of exposure over treated surface. In case of surface treatment, the LC_{50} values of trans-cinnamaldehyde for grubs and adults of *L. serricorne* were 18303.78 and 10747.42 ppm, respectively after 48 h of exposure. In fumigant toxicity study, 60.00 and 98.33 per cent mortality were observed against grubs and adults of *L. serricorne,* respectively at 50000 ppm after 48 h of exposure. The LC_{50} values of trans-cinnamaldehyde for grubs and adults of *L. serricorne* when tested for fumigant toxicity were 9406.68 and 16550.70 ppm, respectively after 48 h of exposure.

Keywords: *Lasioderma serricorne,* Trans-cinnamaldehyde, Bioactivity, fumigant toxicity and LC_{50} value

INTRODUCTION

During recent years, some plant essential oils and their components have received increased attention as natural pesticides, and are considered among the most promising alternatives to residual insecticides. This is due in part to the fact that most of the phytochemicals act as general toxicants against various life stages of an insect. They may also hinder the insect olfactory receptors, triggering attraction or repulsion, or interfere with their growth and reproduction (Gonzalez-Coloma *et al.,* 2011). Essential oil components have even been evaluated against different insect pest from more than 100 years; their practical use is rather limited. Among essential oil components, trans-cinnamaldehyde is a phenyl proponiod which has an increasing interest in the storage pest control. It is pale yellow, viscous liquid occurs naturally in the bark of cinnamon trees and other species of the genus *Cinnamomum* like camphor and cassia (Pubchem, 2019). It is the main constituent of cinnamon which gives its flavor and odor.

Trans-cinnamaldehyde is also used in agriculture because of its low toxicity (Olsen, 2014) and has been tested as a safe and effective insecticide against mosquito larvae. A concentration of 29 ppm of trans-cinnamaldehyde kills half of *Aedes aegypti* mosquito larvae in 24 hours (Cheng, 2004). Trans-cinnamaldehyde works as a potent fumigant and practical repellant for adult mosquitos (Ma, 2014). It also has antibacterial (Vasconcelos, 2018) and antifungal properties (Shreaz, 2016). Trans-cinnamaldehyde have a lot of advantages such as low mammalian toxicity, rapid degradation, local availability, and they have been used traditionally in medicine, herbal beverages, pharmacy, and as natural flavorings (Li *et al.,* 2010). As the majority of the essential oils and their constituents are classified as Generally Recognized as Safe (GRAS), their use in stored grains for controlling stored grain pests and in foods as preservatives is often now-a-days. Hence, it became necessary to evaluate the insecticidal properties of trans-cinnamaldehyde against storage pests where chemical pesticides cannot be used. The objective of this study was to test both the contact and fumigant toxicity of trans-cinnamaldehyde at different concentrations against the life stages of cigarette beetle, *Lasioderma serricorne* (Fabricius) (Coleoptera: Anobiidae).

MATERIALS AND METHODS

Trans-cinnamaldehyde (trans-3-Phenyl-2-propenal) with 99 % purity and Mol. Wt: 132.16) obtained from Sigma-Aldrich Chemicals Ltd, India was used to study the bioactivity activity against different life stages of *L. serricorne*.

Collection of eggs: Fifty freshly emerged 3-5 day old healthy and unsexed adults of cigarette beetle were randomly chosen and released into a 250 ml empty plastic jar for egg laying. After two days, the released adults were removed and eggs were separated under microscope for further utilization in the experiments.

Collection of grubs: The collected eggs were released into coriander powder and left undisturbed for 20 days. The eggs were hatched in 6-8 days and 12- 14 days grubs were collected and used for the experiments.

Collection of Adults: Cigarette beetles were cultured on coriander seeds in 1 lit plastic bottles under laboratory conditions. Freshly emerged adults from the rearing medium were collected in separate jars and used for further experiments.

Topical Application: The method from Liu and Ho (1999) was employed for the evaluation of contact toxicity against *L. serricorne*. Different concentrations (1000, 2000, 3000, 4000 and 5000 ppm) of trans-cinnamaldehyde were prepared in acetone. Egg, grub and adult stages were tested at different concentrations with four replications. Petriplates of diameter 9 cm were used for the experiment with 20 numbers (eggs/ grubs/ adults of *L. serricorne*) in each replication. Before experimentation, the test insects were refrigerated for 10 minutes to inactivate them for the topical application. An aliquot of 0.5 µL of different concentrations of trans-cinnamaldehyde was applied topically on the dorsal side of thorax of grubs and adults as direct application with the help of micropipette. In case of eggs, 0.5 µL aliquots of different concentrations of trans-cinnamaldehyde were applied topically on the surface of the egg under the microscope. Both treated and control insects were then transferred to plastic jars with rearing media (20 insects per jar). Observations on unhatched eggs after 10 days of treatment and mortality of grub and adult stages after 24, 48 h of treatment were recorded.

Contact toxicity: The contact toxicity of trans-cinnamaldehyde, was studied against *L. serricorne*, as described by Zhou *et al.* (2012). Petri plates of diameter 9 cm were used for surface treatment of trans-cinnamaldehyde to study the contact toxicity against egg, grub and adult stages of cigarette beetle. An aliquot of 1000 µL of different concentrations (10000, 20000, 30000, 40000 and 50000 ppm) of trans-cinnamaldehyde were applied evenly on inner surfaces of petriplate and allowed to shade dry. 20 numbers (eggs/ grubs/ adults of *L. serricorne*) were released into each pertiplate and each concentration was replicated four times. After 24 and 48 hours of exposure, observed mortality was documented.

Fumigation toxicity

The fumigant toxicity against *L. serricorne* was tested as described by Liu and Ho (1999). Different concentrations (10000, 20000, 30000, 40000 and 50000 ppm) of trans-cinnamaldehyde were prepared in acetone for fumigation studies. Fumigation was done in small plastic jars (height 12 cm and diameter of 7 cm) with twenty numbers (eggs/ grubs/ adults of *L. serricorne*) in each. A filter paper disc (6 cm diameter) treated with 100 µL of sample solution was placed at the bottom of the cap and sealed tightly to form a closed chamber. Mortality was recorded at 24 and 48 h period of exposure.

Mortality values were determined and their absolute values were transformed to arcsine values before subjecting to Analysis of variance (ANOVA). The mean percentage mortality values were compared at P = 0.05 level of significance. LC_{50} values for topical application, contact toxicity and fumigant toxicity of trans-cinnamaldehyde were calculated by using Probit analysis (Finney, 1971) using MS Excel. Samples for which the 95% fudicial limits did not overlap were considered to be significantly different.

RESULTS

Topical Application

The mortality data of *L. serricorne* at different concentrations of trans-cinnamaldehyde were furnished in Table 1. Eggs were not hatched after 10 days at all concentrations of trans-cinnamaldehyde and even in control (acetone) when applied topically. An increasing trend was observed on per cent mortality of grubs and adults of *L. serricorne* with increase in concentrations of trans-cinnamaldehyde. At 24 h of exposure, the mortality of *L. serricorne* grubs was 63.33 per cent at 1000 ppm of trans-cinnamaldehyde and increased to 96.67 per cent with increase in concentration of trans-cinnamaldehyde to 2000 ppm. Further increase in concentration of trans-cinnamaldehyde to 3000, 4000 and 5000 ppm exhibited 96.67 per cent mortality of *L. serricorne* grubs with no mortality observed in control. Similar trend was observed on per cent mortality of *L. serricorne* grubs with increase in concentrations of trans-cinnamaldehyde at 48 h of exposure. The highest mortality of *L. serricorne* grubs was 96.67 per cent at 2000 ppm of trans-cinnamaldehyde after exposing for 48 h which was on par with the mortality rates at 3000, 4000 and 5000 ppm of trans-cinnamaldehyde (Table 1).

The highest mortality at 5000 ppm of trans-cinnamaldehyde was 93.33 per cent and lowest was 13.33 per cent at 1000 ppm when tested against adults of *L. serricorne* after exposing for a period of 24 h. The highest mortality at 5000 ppm of trans-cinnamaldehyde was 98.33 per cent which was found to be on par with the mortality at 4000 ppm followed by 78.33 per cent

Table 1. Insecticidal action of trans-cinnamaldehyde on cigarette beetle (topical application)

Treatment	Mortality (%)			
	Grubs		Adults	
	24 HAT	48 HAT	24 HAT	48 HAT
1000 ppm	63.33 (53.07)[b]	70.00 (57.00)[b]	13.33 (21.34)[c]	31.67 (34.23)[c]
2000 ppm	96.67 (81.94)[a]	96.67 (81.94)[a]	28.33 (32.14)[b]	36.67 (37.26)[c]
3000 ppm	96.67 (81.94)[a]	96.67 (81.94)[a]	35.00 (36.24)[b]	78.33 (62.91)[a]
4000 ppm	96.67 (81.94)[a]	96.67 (81.94)[a]	86.67 (69.55)[a]	98.33 (83.78)[a]
5000 ppm	96.67 (81.94)[a]	96.67 (81.94)[a]	93.33 (77.14)[a]	98.33 (83.78)[a]
control	0.00 (2.87)[c]	0.00 (2.87)[c]	0.00 (2.87)[d]	1.67 (6.22)[d]
SED	7.02	6.59	4.82	4.73
CD (0.05)	14.75	13.85	10.12	9.93

Figures in the parantheses are arcsine transformed values.
Values in the same column followed by the same letter(s) do not differ significantly (p=0.05) in ANOVA test.
HAT – Hours after treatment

Table 2. Insecticidal action of trans-cinnamaldehyde on cigarette beetle (surface treated)

Treatment	Mortality (%)			
	Grubs		Adults	
	24 HAT	48 HAT	24 HAT	48 HAT
10000 ppm	3.33 (8.06) [de]	26.67 (31.00)[d]	13.33 (21.15)[d]	50.00 (45.00)[c]
20000 ppm	10.00 (18.44) [cd]	53.33 (46.92)[c]	20.00 (26.45)[cd]	76.67 (61.22)[b]
30000 ppm	16.67 (23.86)[c]	73.33 (59.00)[b]	31.67 (34.15)[bc]	98.33 (83.78)[a]
40000 ppm	36.67 (37.14)[b]	80.00 (63.44)[b]	38.33 (38.19)[b]	98.33 (83.78)[a]
50000 ppm	60.00 (51.15)[a]	96.67 (81.94)[a]	55.00 (47.88)[a]	98.33 (83.78)[a]
Control	0.00 (2.87)[e]	3.33 (8.06)[e]	1.67 (6.22)[e]	1.67 (6.22)[d]
SED	5.60	4.92	3.78	4.23
CD (0.05)	11.77	10.33	7.95	8.88

Figures in the parantheses are arcsine transformed values.
Values in the same column followed by the same letter(s) do not differ significantly (p=0.05) in ANOVA test.
HAT – Hours after treatment

Table 3. Fumigant action of trans-cinnamaldehyde on grubs and adults of cigarette beetle

Treatment	Mortality (%)			
	Grubs		Adults	
	24 HAT	48 HAT	24 HAT	48 HAT
10000 ppm	21.67 (27.22)[b]	63.33 (52.78)[c]	5.00 (11.41)[cd]	35.00 (36.24)[d]
20000 ppm	15.00 (22.29)[b]	60.00 (50.77)[c]	11.67 (19.89)[bc]	55.00 (47.88)[c]
30000 ppm	18.33 (25.00)[b]	76.67 (61.22)[b]	15.00 (22.02)[b]	76.67 (61.15)[b]
40000 ppm	55.00 (47.88)[a]	98.33 (83.78)[a]	20.00 (26.45)[ab]	75.00 (60.08)[b]
50000 ppm	60.00 (50.85)[a]	98.33 (83.78)[a]	28.33 (32.14)[a]	91.67 (73.40)[a]
Control	0.00 (2.87)[c]	0.00 (2.87)[d]	0.00 (2.87)[d]	1.67 (6.22)[e]
SED	4.68	3.36	4.20	3.02
CD (0.05)	9.83	7.05	8.81	6.34

Figures in the parantheses are arcsine transformed values.

Values in the same column followed by the same letter(s) do not differ significantly (p=0.05) in ANOVA test.

HAT – Hours after treatment

at 3000 ppm when tested against adults of *L. serricorne* after exposing for a period of 48 h. The lowest mortality was 31.67 per cent at 1000 ppm followed by 36.67 per cent mortality at 2000 ppm when tested against adults of *L. serricorne* after exposing for a period of 48 h.

When the concentrations of trans-cinnamaldehyde and mortality rates of grubs and adults of *L. serricorne* were subjected to probit analysis, the LC_{50} values were exhibited as 653.01 and 2597.14 ppm, respectively after 24 h of exposure when applied topically (Fig 1(a)). The LC_{50} values of trans-cinnamaldehyde for grubs and adults of *L. serricorne* were 532.24 and 1742.06 ppm, respectively after 48 h of exposure when applied topically (Fig 1(b)).

Contact toxicity

The contact toxicity of the trans-cinnamaldehyde on the survival of grubs and adults of *L. serricorne* was concentration dependent but all the eggs were hatched normally after 6-10 days. Trans-cinnamaldehyde caused 60 per cent and 96.67 per cent mortality to grubs of *L. serricorne* at concentration of 50000 ppm after 24 and 48 hours of treatment, respectively. The lowest mortalities of grubs of *L. serricorne* (3.33% and 26.67%) were recorded at 10000 ppm of trans-cinnamaldehyde after 24 and 48 h of exposure. The mortality increased with increased period of exposure for both grubs and adults of *L. serricorne* (Table 2). The LC_{50} values of trans-cinnamaldehyde for grubs and adults of *L. serricorne* were 48458.65 and 53283.46 ppm, respectively after 24 h of exposure (Fig 1(c)).

An increasing trend was observed on per cent mortality (13.33, 20.00, 31.67, 38.33 and 55.00%) of adults of *L. serricorne* with increase in concentrations of trans-cinnamaldehyde from 10000 to 50000 ppm after exposing for 24 h. Increase in the exposure period to 48 h, increased the mortality of *L. serricorne* adults to 50.00 per cent at 10000 ppm of trans-cinnamaldehyde and 76.67 per cent mortality was observed with increase in concentration to 20000 ppm. Further increase in concentration of trans-cinnamaldehyde to 30000, 40000 and 50000 ppm exhibited maximum mortality of 98.33 % at 48 h of exposure. *L. serricorne* adults showed 1.67 per cent mortality in control for both 24 h and 48 h exposure period (Table 2). The LC_{50} values of trans-cinnamaldehyde for grubs and adults of *L. serricorne* were 18303.78 and 10747.42 ppm, respectively after 48 h of exposure (Fig 1d)).

Fumigation toxicity

Trans-cinnamaldehyde caused highest mortalities of 60 per cent and 98.33 per cent against grubs of *L.*

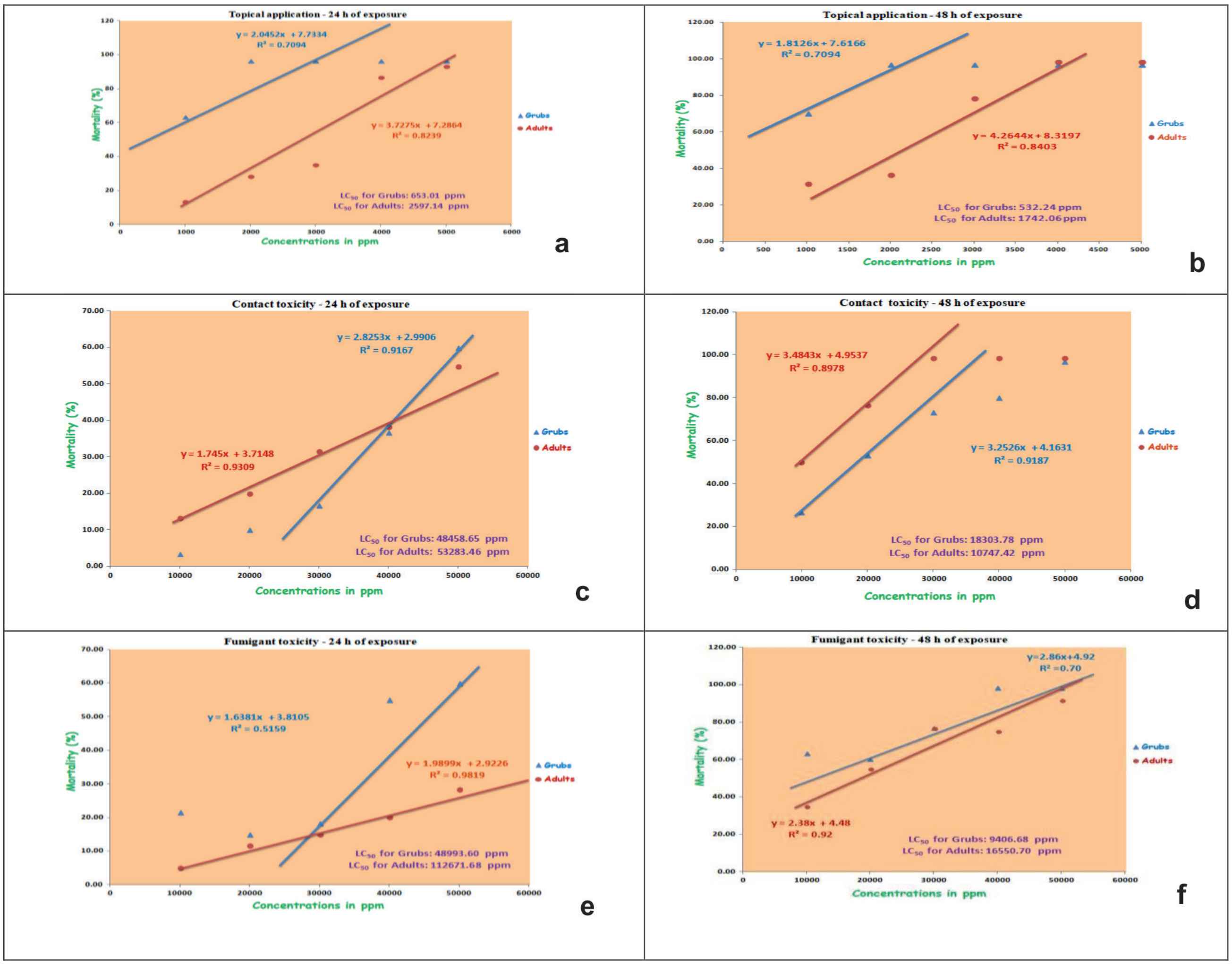

Fig 1. Dose Response Curves of Trans-cinnamaldehyde against *Lasioderma serricorne*

serricorne at 50000 ppm after 24 and 48 h of exposure, respectively. The lowest mortalities of grubs of *L. serricorne* (15% and 60%) were observed at 20000 ppm of trans-cinnamaldehyde after 24 and 48 h of exposure (Table 3). Trans-cinnamaldehyde showed fumigant toxicity against *L. serricorne* grubs and adults with LC_{50} values of 48993.60 and 112671.68 ppm, respectively after 24 h of exposure (Fig 1(e)). But no effect of trans-cinnamaldehyde was observed against the eggs of *L. serricorne* and all the eggs hatched normally.

The effect of trans-cinnamaldehyde exhibited minimum and maximum mortalities of 5 and 28.33% at 10000 and 50000 ppm respectively against adults with no mortality observed in control at 24 h exposure (Table 3). Trans-cinnamaldehyde caused 35% and 91.67% mortality against adults of *L. serricorne* at 10000 and 50000 ppm after 48 hours of exposure, respectively with control mortality of 1.67 per cent. The LC_{50} values of trans-cinnamaldehyde for grubs and adults of *L. serricorne* when tested for fumigant toxicity were 9406.68 and 16550.70 ppm, respectively after 48 h of exposure (Fig 1f)).

DISCUSSION

There is a wide variation among the insects in their ability to survive against different essential oils and their components. It is often difficult to compare the insecticidal action and LC_{50} values of different phytocompounds against store grain pests. Comparing between the studies can be difficult, because insects are not tested at the same doses, or presented at different probit estimations of mortality. However broad trends can be seen. Wang *et al.* (2018|) studied contact toxicity and repellency revealed that the LD_{50} values of trans-cinnamaldehyde against *T. castaneum* and *L. serricorne* were 5.78 and 3.24 µg/adult, respectively while in the present study showed the LC_{50} value of 2597.14 ppm against adults of *L. serricorne*. The active components of *Cinnamomum cassia* extract produced by steam distillation were tested in spray and leaf-dipping bioassays against *Ricania* sp. nymphs and adults. At 48 and 72 hours after exposure, trans-cinnamaldehyde was the most lethal to both nymphs and adults, followed by *C. cassia* oil (Jeon *et al.,* 2017). In various aspects, such as fumigation bioassays, repellency potential, and egg hatch, *Cinnamomum aromaticum* oil comprising of 53.90 per cent trans-cinnamaldehyde proved to be remarkably effective against *Callosobruchus maculatus.* (Islam *et al.,* 2009).

The variety, chemical composition and relative amount of plant extracts are closely related to their insecticidal properties. Trans-cinnamaldehyde as the principal component of these oils could be the leading factor for the potent insecticidal activity. It was also reported that this compound was potentially responsible for the effective control of the carmine spider mite (Chen and Dai, 2015). Rajendran and Sriranjini (2008) stated that the toxicity of bio-fumigants against insect pests will be less in with-food conditions due to low vapour pressure (≤1 mm Hg) and high sorption by the grains. Present findings, considered together with previous research, suggest that the trans-cinnamaldehyde is a potent compound presenting both contact and fumigant toxicity for grubs and adults of *L. serricorne.* The egg stage was the most tolerant stage and no effect of trans-cinnamaldehyde was seen on the eggs except when topically applied.

CONCLUSION

Many of the research findings revealed that trans-cinnamaldehyde tested in the present study have contact, fumigant and repellent activities not only against *L. serricorne* but also against many of the stored grain pests. In the current study, trans-cinnamaldehyde exhibited both contact and fumigant activity and the results also revealed that bioactivity is directly proportional to concentration of the compound. Thus, the compound tested has the potential for development as biopesticide for managing the cigarette beetle if proper formulations with low cost and reasonable application strategies are developed. Nevertheless, their high production costs, low vapour pressure, high volatility, low persistence, strong odour, low efficacy, especially against large chewing insects, and phytotoxicity limit their use in pest control (Isman *et al.,* 2010), but they may be the best alternative for storage pests.

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