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Repellency of different phytochemicals against cigarette beetle, *Lasioderma serricorne* (Fabricius)

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Abstract
Experiments were conducted to study the repellent activity of the phytochemicals *viz.*, citral, β - Citronellol, trans - cinnamaldehyde, eucalyptol, geraniol, β - myrecene and thymol against the cigarette beetle, *Lasioderma serricorne* (F.) under laboratory conditions. Trans - cinnamaldehyde and eucalyptol showed cent per cent repellent effect whereas β – myrecene, thymol and geraniol exhibited 97.5, 70.0 and 57.5 per cent repellent effect against adults of cigarette beetle at 31.0 nL/cm^2 after exposing for 2 h, respectively. In contrary to the behavioural response of *L. serricorne* adults to trans - cinnamaldehyde, eucalyptol, geraniol, β - myrecene and thymol, citral (-22.5%) and β - citronellol (-30%) attracted the adults of cigarette beetle at very low concentrations *viz.*, 1.5 nL/cm^2 and 7.8 nL/cm^2 even after 2 h exposure but repelled insects at 31.0 nL/cm^2 after exposing for 2 h. But the repellent effect of all phytochemicals at different concentrations decreased after exposing for a period of 24 h.

Keywords: Repellent activity, behavioural response, phytochemicals

Introduction
The cigarette beetle, *Lasioderma serricorne* (Fabricius) (Coleoptera: Anobiidae) ranks as one of the most serious pests of stored products in the world (Kim *et al.* 2003) [6]. Beside its main host tobacco and cigarettes, it has also been recorded on turmeric, ginger, castor beans, wheat, coconut meal, pepper, cardamom, mustard, chilli, fennel, cumin and opium leaves (Dimetry *et al*. 2004; Sharma, 2007; Mahroof and Phillips, 2008) [2, 12, 9]. *L. serricorne* larvae commonly cause damage to tobacco by eating it and penetrating deep into the bulk, leaving little round holes in the tobacco and its products. Control of *L. serricorne* populations primarily dependent upon continued applications of phosphine. However, its repeated and intensive use for decades has disrupted biological control by natural enemies and led to insecticide residue, outbreaks of other insect species, development of insecticide resistance, undesirable effects on non-target organisms and environmental and human health concerns (White and Leesch, 1995) [18]. The development and implementation of environmentally friendly control tactics and integrated pest management (IPM) systems has recently been viewed as the only long-term approach to combat pesticide resistance. (Kim *et al*. 2003) [6].
Behavioral manipulation is an important insect control method and insect behavioral responses were influenced by several factors *viz.*, insect species, developmental stages, strains, rates, compound components, application methods, and particular environmental conditions (Kanzaki, 1996; Watson and Barson, 1996; Fields *et al.* 2001) [5, 17, 3]. In particular, repellents and attractants are frequently used to alter insect behaviour, which can effectively reduce insect infestation of crops and stored products (Burkholder and Ma, 1985; Trematerra, 2012) [1, 15] or used as attractants (Nansen and Phillips, 2004) [10]. Insect repellants, have been developed from compounds that may significantly repel insects are used mainly in insect-resistant packaging. Some substances have been proven to be effective attractants or repellents in practical applications. Many plant essential oils and their components are pleasantly aromatic with antimicrobial and anti-inflammatory properties. Many of the research findings revealed that they also have tendency to repel insect pests and also have toxic effect on insects. Therefore, the goal of this study was to assess the behavioural reaction of *L. serricorne* adults to different phytochemicals.

Materials and Methods
Seven different phytochemicals (citral, β - citronellol, trans - cinnamaldehyde, eucalyptol,

geraniol, β - myrecene and thymol) obtained from Sigma-Aldrich were used to study the repellent activity against *L. serricorne*. Cigarette beetles were cultured on turmeric and coriander under laboratory conditions. Freshly emerged 3-5 day old healthy and unsexed adults of cigarette beetle were randomly chosen for the repellent bioassays.

Repellency test

The behavioral response of *L. serricorne* adults to citral, β - citronellol, trans - cinnamaldehyde, eucalyptol, geraniol, β - myrecene and thymol was evaluated by using the area preference method given by Lu and Liu (2016) [8]. All the compounds were dissolved in acetone (1.5, 7.8, 15.0, 23.0 and 31.0 nL/cm^2). Petri dishes were used to study the repellency effect of different phtochemicalson insects. Whatman No.1 Filter paper with a diameter of petridish (9 cm) was cut and this circular disc was cut into two halves. One half was applied with 500µl of each concentration and other half of the filter paper was applied with 500µl of acetone as uniformly as possible by using micro pipette. 20 insects were released at the centre of the petridish and the experiment was replicated four times. The insect count on each half was taken at 2, 4, 8 and 24 h after exposure and the percent repellency (PR) of the essential oil was calculated using the formula:

$$PR\ (\%) = [(N_c - N_t) / (N_c + N_t)] \times 100$$

Where
Nc = insects present in the control half
Nt = insects present in the treated half.

The negative values of percentage repellency represent the attractiveness. The same method was repeated for all the compounds at all prepared concentrations to find out Per cent Repellency (PR) for different phtochemicals (citral, β - citronellol, trans - cinnamaldehyde, eucalyptol, geraniol, β - myrecene, thymol). Repellency values were determined and their absolute values were transformed to arcsine values before subjecting to Analysis of variance (ANOVA). The mean percentage repellency values were compared at P = 0.05 level of significance.

Results and Discussion

Percentage Repellency of *L. serricorne* adults to different phtochemicals significantly varied depending on tested concentrations at 2 h of exposure period. Repellency increased with increase in concentration. But the repellent effect of trans - cinnamaldehyde, eucalyptol and β - myrecene decreased after exposing for 24 h. Among all the treatments tested, trans - cinnamaldehyde, eucalyptol and β - myrecene showed strong repellent effect towards adults of cigarette beetle at all test concentrations for 2 h, 4 h and 8 h exposure periods. Trans - cinnamaldehyde and eucalyptol showed similar trends of repellency *viz.*, minimum at 1.5 nL/cm^2 (65%) and maximum at 31.0 nL/cm^2 test concentration (100%) after 2 h exposure period (Table 1). The Percentage Repellency of *L. serricorne* adults to trans - cinnamaldehyde (72.5% at 1.5 nL/cm^2 and 100% at 31.0 nL/cm^2) and eucalyptol (60% at 1.5 nL/cm^2 and 97.5% at 31.0 nL/cm^2) increased upto 4 hrs exposure period at all test concentrations and then the repellent effect was decreased after 24 h of exposure. The repellency percentage of cigarette beetle adults to trans - cinnamaldehyde decreased from 72.5% to 27.5% at 1.5 nL/cm^2 and from 100% to 77.5% at 31.0 nL/cm^2 after exposing to 24 h. The repellency percentage of cigarette beetle adults to eucalyptol decreased from 60% to 25% at 1.5 nL/cm^2 and from 97.5% to 65% at 31.0 nL/cm^2 after exposing to 24 h.

β - myrecene exhibited lowest percent repellency (62.5%) at 1.5 nL/cm^2 and highest per cent repellency (97.5%) at 31.0 nL/cm^2 concentration after 2 h exposure period (Table 1). The behavioural response of cigarette beetle adults were decreased after 4h, 8h and 24 h of exposure to β - myrecene. The repellency percentage of cigarette beetle adults to β - myrecene decreased from 62.5% to 32.5% at 1.5 nL/cm^2 and from 97.5% to 62.5% at 31.0 nL/cm^2 after exposing to 24 h. Whereas geraniol and thymol exhibited medium repellent effect towards adults of cigarette beetle at all test concentrations. The per cent repellency values of geraniol were 25, 7.5, 22.5, 47.5 and 57.5% at 1.5, 7.8, 15.0, 23.0 and 31.0 nL/cm^2 test concentrations, respectively after 2 h of exposure (Table 5). Similarly, the percent repellency values of thymol were 27.5, 17.5, 50, 52.5 and 70% at 1.5, 7.8, 15.0, 23.0 and 31.0 nL/cm^2 test concentrations, respectively after 2 h of exposure (Table 7). The repellent effect of both geraniol and thymol persisted even after 24 h period of exposure at all different concentrations (Table 1).

Trans – cinnamaldehyde and eucalyptol were found to be the best repelling agents against adults of cigarette beetle while Wang *et al.* (2018) [16] also observed the supercritical carbon dioxide (SFE) extraction of *Cinnamomum cassia* bark and trans - cinnamaldehyde repelled over 90% at 78.63 and 15.73 nL/cm^2 for *L. serricorne* adults, at 2 h post-exposure. Trans - cinnamaldehyde also showed repellency against *Tribolium castaneum* (Wang *et al.* 2018) [16], *Psoroptes cuniculi* (Shen *et al.* 2012) [13] and *Liposcelis bostrychophila* (Liu *et al.* 2014) [7]. *Artemisia mongolica* essential oil and its isolated constituents (eucalyptol, verbenol, camphor, 4-terpineol and *α*-terpineol) exhibited comparable repellent activity against *L. serricorne* adults. Among which *α*-Terpineol, 4-terpineol, verbenol and eucalyptol showed the strongest repellency at different test concentrations. Eucalyptol showed 46% repellency at 0.06 nL/cm^2 and 82% of repellency at 39.32 nL/cm^2 at 2 h and 4 h of exposure, respectively (You *et al.* 2015) [19, 20]. Sun *et al* (2020) [14] showed that β-myrcene in *Peucedanum terebinthaceum* essential oil exhibited only 14% repellency even at higher concentrations (78.63 nL/cm^2) whereas in the current study, β-myrcene (90% pure) exhibited 97.5% repellency at 31.0 nL/cm^2 concentration after 2 h exposure. Hori (2003) [4] revealed that perillaldehyde, carvacrol, trans - cinnamaldehyde and thymol repelled the cigarette beetle adults at a dose of 0.1 µl while citral attracted the beetles at same dose. Geraniol showed 50% repellency at 3.125 µl/ cm^2 at 3 h of exposure to cigarette beetle adults (Ramadan *et al.* 2020) [11] while in present study exhibited 57.5% repellency at 31 nL/cm^2 at 2 h exposure.

Behavioural response of *L. serricorne* adults to citral, β - citronellol significantly varied depending on test concentrations at 2 h of exposure period. Both citral and β - citronellol showed attractiveness towards the adults of cigarette beetle at very low concentrations and with the increase in concentration attractiveness decreased and repellency increased. The citral exhibited slight attractant activity of -22.5% at the low concentration of 1.5 nL/cm^2 followed by -12.5% attractance at 7.8 nL/cm^2 concentration. However, it showed repellent activity of 7.5, 32.5 and 50% at high concentrations of 15.0, 23.0 and 31.0 nL/cm^2 at 2 hours after exposure period (Table 1). Cigarette beetle adults had

similar behavioural response to β - citronellol at different test concentrations (Table 1). The percent attractant or repellant activity of β - citronellol at 1.5, 7.8, 15.0, 23.0 and 31.0 nL/cm^2 concentrations were -30, -27.5, 0, 57.5 and 67.5%, respectively. Similar trends of responses were observed when adults of cigarette beetle exposed to citral and β - citronellol at different test concentrations for 4 hours exposure period. Later on further exposure to 8 h and 24 h, both citral and β - citronellol effect decreased and the repellency percentages not differed significantly (Table 1). Previous investigations also revealed that, *L. serricorne* adults when exposed to citronellal, citral (1:10, 1:50, 1:100 and 1:1000 (citronellal: ethanol, v/v)) and rutin (10, 30 and 90 g/m2) for 1, 2, 12 and 24 h, respectively showed repellent potential at the higher rates and both citral and β - citronellol showed attractivity at very low concentrations (Lu *et al.* 2016) [8].

However, more research on the toxicity to stored product insects, the safety of essential oils/compounds to people, and formulation development are needed to make use of the phytochemicals in the management of stored product insects.

Table 1: Repellent action of *L. serricorne* adults to different phtochemicalsat different concentrations after 2, 4, 8 and 24 h of exposure

Citral (nL/cm^2)	Per cent Repellency			
	2 hrs after exposure	4 hrs after exposure	8 hrs after exposure	24 hrs after exposure
1.5	-22.50 ± 6.26^{ab}	-20.00 ± 9.33^{a}	-17.50 ± 8.22^{a}	-27.50 ± 5.11^{a}
7.8	-12.50 ± 9.34^{a}	0.00 ± 7.53^{a}	-5.00 ± 10.04^{a}	-17.50 ± 8.22^{a}
15.0	7.50 ± 10.08^{a}	2.50 ± 8.83^{a}	-5.00 ± 10.04^{a}	2.50 ± 18.79^{a}
23.0	32.50 ± 5.71^{ab}	15.00 ± 13.70^{a}	17.50 ± 17.52^{a}	-7.50 ± 15.13^{a}
31.0	50.00 ± 6.53^{c}	32.50 ± 3.84^{a}	22.50 ± 16.88^{a}	10.00 ± 15.09^{a}
β - citronellol (nL/cm^2)				
1.5	-30.00 ± 15.68^{bc}	-32.50 ± 5.72^{b}	-52.50± 5.02^{a}	-62.50 ± 2.83^{b}
7.8	-27.50 ± 10.99^{ab}	-12.50 ± 21.39^{b}	-15.00 ± 14.87^{a}	-5.00 ± 13.55^{a}
15.0	0.00 ± 7.53^{a}	-2.50 ± 4.61^{a}	-12.50 ± 13.54^{a}	-12.50 ± 9.34^{a}
23.0	57.50 ± 6.66^{c}	52.50 ± 12.51^{b}	5.00 ± 15.27^{a}	-20.00 ± 14.34^{a}
31.0	67.50 ± 4.64^{c}	60.00 ± 4.35^{b}	15.00 ± 13.03^{a}	-2.50 ± 14.32^{a}
Trans - cinnamaldehyde (nL/cm^2)				
1.5	65.00 ± 11.87^{a}	72.50 ± 10.00^{a}	60.00 ± 4.35^{a}	27.50 ± 10.00^{a}
7.8	70.00 ± 10.64^{a}	77.50 ± 8.65^{a}	67.50 ± 3.84^{ab}	45.00 ± 5.64^{a}
15.0	82.50 ± 10.53^{a}	87.50 ± 8.73^{a}	65.00 ± 5.19^{a}	55.00 ± 5.09^{a}
23.0	90.00 ± 8.04^{a}	90.00 ± 8.04^{a}	85.00 ± 7.18^{bc}	60.00 ± 12.75^{a}
31.0	100.00 ± 0.00^{a}	100.00 ± 0.00^{a}	87.50 ± 6.26^{c}	77.50 ± 9.26^{a}
Eucalyptol (nL/cm^2)				
1.5	65.00 ± 1.74^{a}	60.00 ± 3.89^{a}	55.00 ± 1.67^{a}	25.00 ± 5.62^{a}
7.8	62.50 ± 4.35^{a}	75.00 ± 3.17^{ab}	62.00 ± 4.57^{ab}	40.00 ± 3.40^{ab}
15.0	85.00 ± 9.59^{b}	85.00 ± 7.18^{bc}	60.00 ± 4.14^{ab}	50.00 ± 4.14^{bc}
23.0	87.50 ± 6.80^{b}	92.50 ± 6.70^{c}	80.00 ± 3.02^{bc}	52.50 ± 6.06^{bc}
31.0	100.00 ± 0.00^{b}	97.50 ± 4.61^{c}	82.50 ± 7.36^{c}	65.00 ± 5.32^{c}
Geraniol (nL/cm^2)				
1.5	25.00 ± 16.92^{a}	15.00 ± 10.64^{a}	20.00 ± 11.54^{a}	27.50 ± 9.69^{a}
7.8	7.50 ± 6.70^{a}	27.50 ± 13.32^{a}	20.00 ± 8.26^{a}	12.50 ± 8.73^{a}
15.0	22.50 ± 10.94^{a}	40.00 ± 9.26^{a}	37.50 ± 5.28^{a}	35.00 ± 7.94^{a}
23.0	47.50 ± 8.02^{a}	32.50 ± 3.84^{a}	22.50 ± 5.19^{a}	37.50 ± 3.84^{a}
31.0	57.50 ± 4.61^{a}	32.50 ± 5.32^{a}	35.00 ± 5.61^{a}	32.50 ± 4.57^{a}
β - myrecene (nL/cm^2)				
1.5	62.50 ± 6.27^{ab}	52.50 ± 6.24^{a}	55.00 ± 1.67^{a}	32.50 ± 5.62^{a}
7.8	60.00 ± 4.35^{a}	62.50 ± 5.14^{ab}	62.50 ± 4.57^{a}	37.50 ± 4.57^{a}
15.0	82.50 ± 7.88^{bc}	82.50 ± 3.57^{bc}	55.00 ± 3.77^{a}	45.00 ± 3.77^{a}
23.0	90.00 ± 5.62^{c}	90.00 ± 5.62^{c}	72.50 ± 5.61^{a}	52.50 ± 6.06^{a}
31.0	97.50 ± 4.61^{c}	95.00 ± 5.32^{c}	72.50 ± 5.61^{a}	62.50 ± 4.57^{a}
Thymol (nL/cm^2)				
1.5	27.50 ± 13.32^{a}	20.00 ± 6.64^{a}	5.00 ± 5.32^{a}	25.00 ± 13.50^{a}
7.8	17.50 ± 10.99^{a}	25.00 ± 13.50^{a}	30.00 ± 6.02^{a}	25.00 ± 5.62^{a}
15.0	50.00 ± 7.53^{a}	25.00 ± 12.99^{a}	40.00 ± 15.09^{a}	22.50 ± 9.26^{a}
23.0	52.50 ± 8.45^{a}	47.50 ± 10.94^{a}	20.00 ± 8.74^{a}	27.50 ± 13.32^{a}
31.0	70.00 ± 8.45^{a}	60.00 ± 7.05^{a}	37.50 ± 2.83^{a}	47.50 ± 5.02^{a}

Means (PR ± SE) in the same column followed by the same letters do not differ significantly (p=0.05) in ANOVA test.
Percent Repellency was subjected to an arcsine square-root transformation before ANOVA.

Conclusions

Many of the research findings revealed that all the compounds 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, all the compounds exhibited repellent activity but among them trans-cinnnamaldehyde and eucalyptol acted as quite promising compounds as they exhibited highest repellent effect against adults of cigarette beetle. Whereas citral and β - citronellol exhibited some attractance towards the adults of *L. serricorne* at very low concentrations and can be developed as attractants. Thus, the compounds tested have the potential for development as attractants and repellents for managing the stored product insects if proper formulation with low cost and reasonable application strategies are developed.

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