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Contact and ovicidal toxicity of plant essential oils against pulse beetle, *Callosobrucus chinensis* Linn. (Coleoptera: Bruchidae)

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Abstract
Contact and ovicidal toxicity of the nine plant essential oils, *viz*., *Cinnamonum camphora*, *Cymbopogon citrates*, *Cymbopogon flexuosus*, *Mentha longifolia*, *Lavender agustifolia*, *Ocimum basilicum*, *Polargonium graveolens*, *Elethia cardmomum* and *Foeniculum vulgare* on *C. chinensis* adults. Residual film bioassay was employed in Petri dish (5 cm dia.) for contact and ovicidal studies and inner surface of petri dish (9 cm diameter, surface 63.6 cm2) were used for testing contact and ovicidal activities. Maximum contact toxicity was recorded in lavender oil at 24, 48, 72 and 96 h after exposure different concentrations, the values being (10.00, 10.00, 12.23 and 17.75%), (13.75, 13.75, 28.61 and 42.87%) and (13.75, 17.26, 17.57 and 48.11%), respectively. The highest percent hatching inhibition rate was recorded in lavender oil at all the three dose of 0.25, 0.5 and 1% after 35 days, the values being 37.17, 58.37 and 89.80%, respectively.

Keywords: pulse beetle, ovicidal activity, contact activity and essential oils

1. Introduction

Pulse beetle, *Callosobruchus chinensis* L. (Coleoptera: Bruchidae) is one of the three species that causes significant damage to the stored legumes causing up to 55.7% of damage in severe infestations (Chaubey, 2008) [3]. In recent years, control of *Callosobruchus chinensis* L. (Coleoptera: Bruchidae) has gained great attention because it is widely distributed throughout the tropical and sub-tropical regions and it is an important pest of several plant products including chickpea (*Cicer arietinum* L.), cowpea [*Vigna unguiculata* (L.) Walp.], lentil (*Lens culinaris* Medik.), soybean (*Glycine max* Mer.) and haricot beans (*Phaseolus vulgaris* L.). Control of these pests is primarily dependent upon repeated application of synthetic insecticides (Hasan and Reichmuth, 2004) [5]. Pulse beetle control is currently based on heavy use of many insecticides, which damage the environment and pose a threat to public health via food residues. The problems caused by pesticides and their residues have amplified the need for effective and biodegradable pesticides with great selectivity (Hazaa & Alam EL-Din, 2011) [6]. Among various classes of natural substances that introduced as natural biopesticides are essential oils from aromatic plants (Prakash *et al.*, 2014) [11]. The advantage of using plant essential oils is that they are easily available and they have been used extensively for medicinal purposes, implying that they have low or no toxicity to humans (Upadhyay, 2013) [13]. The deleterious effects of plant products on insects can be manifested in several manners including toxicity, mortality, antifeedant growth inhibitor, suppression of reproductive behavior and reduction of fecundity and fertility, growth inhibition, perturbation of reproductive behavior. The present work was carried out to evaluate the toxic activities of some natural plant essential oils on pulse beetle, *Callosobruchus chinensis* (L.) under controlled laboratory conditions for possible use as a safe biological method and alternative to chemical pesticides within the means of integrated pest control program.

2. Material and methods

A Laboratory experiment was conducted on contact and ovicidal activity caused by different plant essential oils on *Callosobruchus chinensis* (L.) in the Department of Entomology, G.B. Pant University of Agriculture and Technology, Pantnagar during year 2015.

Rearing and maintenance of culture

The Pulse beetle, *Callosobruchus chinensis* L. was used as test insect in the present study. The nucleus culture of the test insect was obtained from the infested samples of chickpea in NEB Crop Research Centre farm store at G.B. Pant University of Agriculture and Technology, Pantnagar. The culture of pulse beetle was maintained on chickpea at room temperature in the laboratory, Department of Entomology.

Evaluation of the bioactivity of plant essential oils against pulse beetle, *C. chinensis*

The experiment was included nine plant essential oils**.** These were subjected to evaluation of the bioactivity against *Callosobruchus chinensis* L in the laboratory. They can be successfully exploited as contact activity and ovicidal activity against *C. chinensis.* Admixture of plant essential oils (camphor, citronella, mentha and lemongrass oils) were obtained from the AICRP medicinal and aromatics plant, department plant physiology, RAU, Pusa, Samastipur (Bihar) and lavender, geranium, cardamom, basil and funnel oils from local market, Haldwani, Nainital (Uttarakhand).

Contact activity

A series of dilutions of each essential oil (0.25, 0.5 and 1% each) was prepared using ethanol as solvent as described by Tapondjou *et al.* (2005) [12]. Aliquot of each dilution was separately 0.5mL applied on inner surface of Petri dish (9 cm diameter, surface 63.6 cm2). Each dish was left without direct sunlight for 2 min, after which 10 newly unsexed adults *C. chinensis* with 20 chickpea seeds were placed in each one were introduced into the each Petri dish separately. After incubation at 28 ± 2ºC temperature and 24, 48, 72 and 96 h exposure, mortality was observed after starting the test. The insects were considered to be dead as no leg or antennal movements were observed. A control experiment was maintained in which treatment was made with ethanol. Three replicates of each control and treatment set were made. Data obtained were corrected using Abbott's formula (1925).

$$CM = [(TM – CM) / (100 – CMt)] \times 100$$

Where,

TM = treated mortality, CM = control mortality

Ovicidal activity

The nine plant essential oils were tested for their *in vivo* effects on ovicidal activity following Kumar *et al*. (2007) [10] with some modification**.** A series of dilutions of each essential oil (0.25, 0.50 and 1% each) was prepared using ethanol as solvent. 0.5 ml of each concentration of the essential oils was applied. One hundred 0-24 hrs old eggs of *C. chinensis* with average of three to four eggs per seed were put in Petri dishes (9 cm diameter) and replicated three times.

The seeds were gently mixed for five minutes for proper mixing of the oils on the seeds. For control sets the seeds were dressed in requisite amount of acetone in place of the oil. The treated samples were kept in B.O.D. to control temperature and humidity (27±2 °C and RH 70 ± 5%). The number of adult insects emerged in chickpea samples in each treated and control set was counted after 35 days. Percent Hatching Inhibition Rate (% HIR) was calculated as:

$$\text{Per cent Hatching Inhibition Rate} = \frac{Cn - Tn}{Cn} \times 100$$

Where,

Cn – number of adults in control, and Tn – number of adults in test.

3. Results and discussion

The contact activity of plant essential oils was evaluated against *C. chinensis* under laboratory conditions presented in (Table 1-3). Percentage of accumulative mortality of *C. chinensis* increased gradually with the increase of the exposure intervals. The data indicated that contact activity treatment with all plant essential oils caused significant mortality as compared to control. The highest percentage of accumulative mortality was recorded in lavender oil at the three doses (0.25, 0.5 and 1%) after 24 h, the value being 0.2 per cent. The lowest percentage of accumulative mortality was recorded in funnel oil all the three doses (0.25, 0.5 and 1%) after 24 h, the value being 24.36, 34.59 and 47.2 per cent, respectively. All the remaining essential oils mortality ranged between 28.03 to 37.69, 34.59 to 69.30 and 57.25 to 71.52 per cent respectively, which was significantly different as compared to control where there was no mortality.

The ovicidal activity of plant essential oils was evaluated against *C. chinensis* under laboratory conditions presented in (Table 4). The data indicated that ovicidal treatment with all plant essential oils caused significant percent hatching inhibition rate as compared to control. The highest percent hatching inhibition rate was recorded in lavender oil at all the three dose of 0.25, 0.5 and 1% after 35 days, the values being 37.17, 58.37 and 89.80 per cent, respectively. The lowest percent hatching inhibition was recorded in funnel oil at all the three dose of 0.25, 0.5 and 1% after 35days, the value being 10.99, 28.66 and 27.77 per cent, respectively. All the remaining essential oils reflected percent hatching inhibition ranged between 13.08 to 25.29, 19.14 to 39.59 and 27.77 to 68.50 per cent, respectively which was significantly different as compared to control where there is no inhibition on hatching.

Thus, the results of the present contact and ovicidal studies revealed that different plant essential oils showed different potencies against *C. chinensis*. All the essential oils with 1 per cent concentration contact activity to the adult *C. chinensis* except the funnel oil. The percentage of mortality after 96 h was higher when filter paper treated with essential oil of lavender 1 per cent compared with other essential oils and concentrations. Among the treatments, lavender oil caused the highest mortality followed by citronella, camphor, mentha, lemongrass, geranium, cardamom and basil oils have potential for use in the integrated management of adult *C. chinensis*. Further, it was observed that mortality of adult beetles due to treatment of plant essential oils were directly related to application dosages and the time of exposure. It indicated that higher dosage and longer exposure periods are required to achieve appreciable management of *C. chinensis*. Likewise, different workers reported (Ketoh *et al.*, 2006; Jayasingha *et al.*, 1999 and Kumar *et al.*, 2009) [9, 7] essential oils of *Cymbopogon citratus* and *Cymbopogon nardus* caused more than 90 % mortality of bruchids within 24 h of exposure at a concentration higher than 33.3µl/l. A similar result was also reported Aboua *et al.* (2010) [2] who reported the contact activity of *Agastache foeniculum*, *Anethum graveolens*, *Cuminum cyminum*, *Foeniculum vulgare* and *Satureja hortensis* had strong effects on adults 67 % and 100 % mortality of *C. maculates and C. chinensis*. Haidri *et al.* (2014) [4] also checked for the effectiveness of *Azadirachta indica* and *Murraya koenigii* towards pulse beetle and found the definite potential of the plants towards causing mortality of test insect.

Table 1: Mortality of *C. chinensis* adults caused by contact toxicity of plant essential oils at 0.25% concentration

Treatment	No. of released insects	Mortality ± SE (%) hours after insect release				
		24 h	48 h	72 h	96 h	Total mortality
Camphor oil	30	8.90	10.00	9.94	8.85	37.69
Citronella oil	30	10.00	12.23	12.14	12.85	44.33
Mentha oil	30	8.90	10.00	9.94	6.65	36.59
Lemongrass oil	30	7.77	10.00	8.81	9.98	34.33
Lavender oil	30	10.00	17.77	12.14	7.75	47.66
Geranium oil	30	7.77	8.90	9.94	5.52	34.03
Cardamom oil	30	7.77	8.90	7.71	7.77	29.89
Basil oil	30	7.77	8.90	6.61	3.32	28.03
Funnel oil	30	6.67	6.67	6.61	4.42	24.36
Control	30	0.00	0.00	0.00	0.00	0.00
	SEm±	0.761	0.053	0.058	0.587	0.911
	CD at 5%	2.273	0.159	0.174	1.761	2.733

Table 2: Mortality of *C. chinensis* adults caused by contact toxicity of plant essential oils at different time and 0.5% concentrations

Treatment	No. of released insects	Mortality ± SE (%) hours after insect release				
		24 h	48 h	72 h	96 h	Total mortality
Camphor oil	30	3.41	10.34	16.6	35.57	65.92
Citronella oil	30	6.92	16.6	6.6	42.87	72.99
Mentha oil	30	13.75	10.34	16.6	28.61	69.3
Lemongrass oil	30	10.34	13.75	10.0	32.15	66.24
Lavender oil	30	13.75	13.30	10.0	39.33	76.38
Geranium oil	30	6.92	10.34	10.0	28.61	55.87
Cardamom oil	30	6.92	8.90	16.6	28.61	52.13
Basil oil	30	3.41	6.67	16.6	14.25	34.26
Funnel oil	30	0.0	10.34	10.0	14.25	34.59
Control	30	0.0	0.0	0.0	0.0	0.0
	SEm±	0.131	0.145	0.143	0.494	0.801
	CD at 5%	0.388	0.428	0.423	1.458	1.458

Table 3: Mortality of *C. chinensis* adults caused by contact toxicity of plant essential oils at different time and 1% concentrations

Treatment	No. of released insects	Mortality ± SE (%) hours after insect release				
		0.25%	0.5%	1%	96 h	Total mortality
Camphor oil	30	16.23	37.64	63.88	29.55	68.09
Citronella oil	30	25.29	19.14	68.50	44.44	75.76
Mentha oil	30	22.32	22.88	59.24	22.22	68.02
Lemongrass oil	30	20.57	25.60	33.33	33.33	71.52
Lavender oil	30	37.17	58.37	89.80	48.11	82.84
Geranium oil	30	14.13	39.59	45.35	29.55	61.24
Cardamom oil	30	17.95	38.85	47.22	33.33	64.72
Basil oil	30	13.08	36.05	44.44	22.22	57.25
Funnel oil	30	10.99	28.66	27.77	25.88	47.23
Control	30	6.66	6.66	6.66	0.0	0.0
	SEm±	0.083	0.146	0.222	0.453	0.889
	CD at 5%	0.246	0.431	0.657	1.336	2.624

Table 4: Per cent hatching inhibition rate of *C. chinensis* adults due to plant essential oils at different concentrations

Treatments	No. of eggs	Hatching Inhibition Rate (% HIR)		
		0.25%	0.5%	1%
Camphor oil	100	16.23 ± 0.33^{e}	37.64 ± 0.29^{g}	63.88 ± 0.42^{f}
Citronella oil	100	25.29 ± 0.03^{i}	19.14 ± 0.27^{b}	68.50 ± 1.50^{g}
Mentha oil	100	22.32 ± 0.51^{h}	22.88 ± 0.42^{c}	59.24 ± 1.29^{e}
Lemongrass oil	100	20.57 ± 0.21^{g}	25.60 ± 0.07^{d}	33.33 ± 0.21^{c}
Lavender oil	100	37.17 ± 0.66^{j}	58.37 ± 0.30^{j}	89.80 ± 2.29^{h}
Geranium oil	100	14.13 ± 0.13^{d}	39.59 ± 0.64^{i}	45.35 ± 0.97^{d}
Cardamom oil	100	17.95 ± 0.45^{f}	38.85± 0.22^{h}	47.22 ± 1.01^{d}
Basil oil	100	13.08 ± 0.18^{c}	36.05± 0.30^{f}	44.44 ± 0.56^{d}
Funnel oil	100	10.99 ± 0.26^{b}	28.66± 0.51^{e}	27.77 ± 0.32^{b}
Control	100	6.66 ± 0.00^{a}	6.66 ± 0.00^{a}	6.66 ± 0.00^{a}
	SEm±	0.34	0.36	1.10
	CD at 5%	1.01	1.09	3.28

*Means in a column followed by the same letter(s) do not differ significantly at the 5% level by DMRT

4. Conclusion

It can be concluded from the experiment that use of essential oils as an alternative in insect pest management programmes is a sustainable alternative as they can be obtained from nature. Essential oils cause contact and oviposition inhibitory activities and act on various levels in the insects, so that possibility of generating resistance is low. Thus, plant essential oils can be considered as a natural alternative in the control of stored grains insects.

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