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Insecticidal property of methanolic leaf extract of Sweet Basil, *Ocimum basilicum* (L.) against cigarette beetle, *Lasioderma serricorne* (Fab.) (Coleoptera: Anobiidae)

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Abstract
The present study was conducted to evaluate the insecticidal property of *Ocimum basilicum* (L.) against cigarette beetle, *Lasioderma serricorne* (Fab.). The bioactive compounds from the leaves were extracted using Soxhlet apparatus with methanol. Five different concentrations *viz.*, 1%, 2%, 3%, 4%, 5% were used against adult beetles of *L. serricorne*. In contact toxicity, highest mean mortality was effected by 5% methanol extract (66.67%), followed by 4% methanol extract (58.33%), 3% methanol extract (52.22%), 2% methanol extract (47.22%) and 1% methanol extract (43.33%). In repellency test, 5% methanol extract produced highest mean repellency (86.88%) (Class V) followed by 4% methanol extract (80.00%) (Class V), 3% methanol extract (73.75%) (Class IV), 2% methanol extract (68.13%) (Class IV) and 1% methanol extract (64.38%) (Class IV).

Keywords: *Ocimum basilicum*, *Lasioderma serricorne*, methanol extract, insecticidal activity, contact toxicity, repellency

Introduction
Cigarette beetle, *Lasioderma serricorne* Fab. (Coleoptera: Anobiidae) occurs all over the world from tropics to sub-tropics [7]. They are also called as tobacco beetle, since their main host is tobacco. They also infest a wide range of products like turmeric, seed spices, paprika seeds, onion seeds, sesame seeds, wheat flour, corn flour, dried yeast, biscuits, milk powder, dried flowers, processed cereal products and even materials like books, museum specimens, dead insects, fish meal, animal meal *etc.*, The annual loss caused by *L. serricorne* on stored spices at normal unprotected condition is 58.02 per cent on fennel, 49.58 per cent on coriander, 47.75 per cent on ajowan, 51.02 per cent on cumin and 39.0 per cent on dill [8]. Synthetic pesticides and fumigation are commonly used to control this notorious pest in godowns and ware houses. Excessive use of fumigants like methyl bromide, carbonyl sulphide and aluminium phosphide has lead to development of insecticide resistance, disruption of natural enemies, high mammalian toxicity, *etc.*, Hence, it is necessary to discover an ecofriendly management strategies to reduce the usage of chemical pesticides.
Sweet basil, *O. basilicum* (L.) is an herbaceous perennial plant which belongs to the family Lamiaceae. They are distributed generally in tropical areas of Asia, Africa, Central and South America and cultivated in India over an area of 25,000 ha. The *O. basilicum* plant has been reported to show insecticidal properties against *Sitophilus oryzae*, *Lasioderma serricorne*, *Acanthoscelides obtectus*, *Tribolium castaneum*, *etc.*, [12, 9, 16]. Compounds like linalool, estragole, 1,8-cineloe, α-pinene, β-pinene, farnesene, germacrene, hexadecenoic acid, menthol pulegone, ocimene, D-camphor *etc.*, were identified in *O. basilicum* which may be the inherent character for its toxic nature to insects [2, 11]. Moreover, these plant derived compounds are biodegradable, have low mammalian toxicity, are selective in action and also delay the development of pesticide resistance in insects. The present study reveals the insecticidal activity of *O. basilicum* against *L. serricorne*.

Materials and Methods

The laboratory experiments were conducted at Natural Pesticide Laboratory, Department of Agricultural Entomology, Agricultural College and Research Institute, Tamil Nadu Agricultural University, Madurai during August 2019 to March 2020.

Mass culturing of the cigarette beetle *L. serricorne*

Adults of cigarette beetle were collected from Department of Agricultural Entomology, Tamil Nadu Agricultural University, Coimbatore and from various infested spice products at Madurai local markets. They were mass multiplied on suitable rearing media like sterilized wheat flour + 5% dried yeast and coriander seeds contained in plastic containers of 1.5 l capacity. The top of the plastic containers was covered with muslin cloth and secured with rubber band. The culture was maintained in the laboratory at room temperature of 30 ± 2º C and 70 ± 5 per cent relative humidity till the emergence of adults [17]. After a lapse of about 45 to 50 days, the adults emerged from stock culture were collected and used for carrying out various laboratory studies.

Extraction of bioactive compounds from *O. basilicum* leaves:

The leaves of *O. basilicum* were collected and washed thoroughly with water. They were air dried at room temperature (30±2º C) till the moisture level reduced completely. Air dried leaves were ground into fine powder and sieved in 60 mesh sieve to get fine powder. The ground powder was stored in air tight glass containers till extraction. The bioactive compounds were extracted by Soxhlet method of extraction using methanol [10]. About 20 g of sample was taken in Soxhlet thimble and extracted with 200 ml of solvent at 60º C for four hours. After extraction, the extracts were concentrated under vacuum using rotary vacuum evaporator at 45º C to obtain the crude extract and then stored in refrigerated condition (4º C) until further use.

Contact toxicity test

Contact toxicity bioassay was conducted by dry film method (Busvine, 1971) [3]. The Petri dishes (9 cm diameter) were applied with 1ml of different concentrations *viz.*, 1%, 2%, 3%, 4% and 5% of the methanol extract. The Petri dishes were gently swirled to get a uniform thin film over the surface and then air dried to evaporate the solvent. The *L. serricorne* adults (10 pairs) were released into the Petri dish and covered with lid. The treatments were replicated thrice. The untreated check was maintained with 1ml of methanol alone and malathion 50 EC 1% was used as the standard check. The observations on mortality were recorded at 24, 48 and 72 hrs after treatment (HAT). The insects were considered as dead, if appendages did not move or respond when disturbed with a brush. Mortality percentage was calculated by using the formula followed by Abbott (1925) [1].

$$\text{Percent Mortality} = \frac{X-Y}{X} \times 100$$

Where,
X = Per cent living in the check
Y = Per cent living in the treatment
X-Y = Per cent killed by the treatment

Repellency test

Repellency bioassay was carried out using area preference method described by Tapondjou *et al.* (2005) [20]. Whatman filter papers (9cm diameter) were cut into two halves fitting into the Petri plates. One half of the filter paper was applied with 1ml of different concentrations *viz.*, 1%, 2%, 3%, 4% and 5% of the methanol extract as uniformly as possible using a micro-pipette, while the other half was applied with 1ml of methanol alone and it was maintained as untreated check, while neem oil 1% was used as standard check. The filter paper strips were air dried to evaporate the solvent and then attached lengthwise to form a full disc and fitted on the bottom of the Petri plates. The treatments were replicated thrice. The number of beetles on the treated and untreated halves of the filter paper were counted after 1, 2, 3 and 6 hrs. Percent repellency was calculated using the formula as followed by Soujanaya *et al*. (2016) [18].

$$\text{Percent repellency} = \frac{Nc-Nt}{Nc+Nt} \times 100$$

Where,
Nc = Number of insects in the untreated half
Nt = Number of insects in the treated half

Based on the average percent repellency, the concentrations were grouped into different class using the scale which was followed by Talukdar and House (1993) [19].

Categorization of scale based on percent repellency against *L. serricorne*

Percent repellency	Class
>0.01 - < 0.1	0
0.1 - 20	I
20.1 - 40	II
40.1 - 60	III
60.1 - 80	IV
80.1 - 100	V

Statistical analysis

The data on mortality and repellency were subjected to arc sine transformation [5]. The analysis of variance (ANOVA) was carried out by using AGRES Statistical package (version 3.01) to differentiate the transformed mean values by using Fisher's Least Significant Difference (LSD) at 5% probability level.

Results and Discussion

Contact toxicity of methanolic leaf extract of *O. basilicum* against *L. serricorne*

The insecticidal activity of methanolic leaf extract of *O. basilicum* against *L. serricorne* is given in Table 1. The results clearly indicate that *O. basilicum* has significant toxic activity against the cigarette beetle, *L. serricorne*. Among the different concentrations of the *O. basilicum* extract tested, highest mean mortality was effected by 5% methanol extract (66.67%), followed by 4% methanol extract (58.33%), 3% methanol extract (52.22%), 2% methanol extract (47.22%) and 1% methanol extract (43.33%). Also, mortality increased with increase in concentration. No mortality was observed in untreated check while the standard check Malathion 1% recorded mean mortality of 98.33 per cent.

Several similar studies have been done for the insecticidal effects of *O. basilicum* against storage insects like

Callosobruchus maculatus, *Sitophilus oryzae*, *Acanthoscelides obtectus etc*., [12, 9, 16]. Similarly, methanol extract produced significantly high mortality when compared to other solvent extracts [4]. Essential oil obtained from *O. kilimandscharicum* caused 100 per cent morality in *Sitophilus zeamais* and *Rhyzopertha dominica* [6]. Studies have indicated that camphor, linalool and eugenol present in *O. basilicum* plants were toxic to storage insects [21, 14, 13].

Repellency effect of methanolic leaf extract of *O. basilicum* against *L. serricorne*

The repellency activity of methanolic leaf extract of *O. basilicum* against *L. serricorne* is given in Table 2. Among the different concentrations of *O. basilicum* extract, 5% methanol extract produced highest mean repellency (86.88%) (Class V) followed by 4% methanol extract (80.00%) (Class V), 3% methanol extract (73.75%) (Class IV), 2% methanol extract (68.13%) (Class IV) and 1% methanol extract (64.38%) (Class IV) which showed the least repellency, while the standard check neem oil 1% produced 89.38 per cent repellency. Similar to contact toxicity, increasing the concentration of the extract, increased the repellency effect significantly.

Ouko *et al*. (2017) [15] reported that methanol and hexane blend extract of *O. basilicum* produced 86.50 per cent repellency against *S. oryzae*, followed by methanol extract with 84 per cent repellency which is similar to the present results. Similarly, Mishra *et al*. (2012) [12] reported that repellent effect of *O. basilicum* against *Tribolium castaneum* and *S. oryzae* were 85 per cent and 81.6 per cent respectively. Methanol is more polar than other solvents used for extraction and this may yield high number of phytochemicals in the extract which causes them to be more insecticidal in nature.

The results revealed the promising insecticidal activity of methanol extract of *O. basilicum* leaves against cigarette beetle, *L. serricorne*. Thus, it can be utilized for the eco-friendly management of *L. serricorne* and thereby reduces the losses caused by them in stored products.

Table 1: Contact toxicity of methanolic leaf extract of *O. basilicum* against *L. serricorne*

Treatments	Mortality (%)			Mean mortality (%)
	24 HAT	48 HAT	72 HAT	
T_1 - 1% leaf extract of Ocimum basilicum	20.00 (26.55)^{e}	41.67 (40.19)^{f}	68.33 (55.76)^{e}	43.33 (41.15)^{f}
T_2 - 2% leaf extract of Ocimum basilicum	23.33 (28.87)^{e}	46.67 (43.07)^{e}	71.67 (57.82)^{e}	47.22 (43.39)^{e}
T_3 - 3% leaf extract of Ocimum basilicum	28.33 (32.15)^{d}	51.67 (45.94)^{d}	76.67 (61.09)^{d}	52.22 (46.26)^{d}
T_4 - 4% leaf extract of Ocimum basilicum	33.33 (35.25)^{c}	58.33 (49.78)^{c}	83.33 (65.88)^{c}	58.33 (49.78)^{c}
T_5 - 5% leaf extract of Ocimum basilicum	41.67 (40.19)^{b}	68.33 (55.76)^{b}	90.00 (71.54)^{b}	66.67 (54.71)^{b}
T_6 - Standard check (Malathion 1%)	95.00 (77.05)^{a}	100.00 (89.96)^{a}	100.00 (89.96)^{a}	98.33 (82.55)^{a}
T_7 – Untreated check	0.00 (0.33)^{f}	0.00 (0.33)^{g}	0.00 (0.33)^{f}	0.00 (0.33)^{g}
SEd	1.13	1.16	1.21	0.71
CD (0.05)	2.41	2.49	2.58	1.52

Numeric data represents the mean value of three replications.
HAT-Hours after treatment.
Figures in the parentheses are arc sine transformed values.
Mean values followed by the same alphabet in a column are not significantly different (P < 0.05) by LSD.

Table 2: Repellency effect of methanolic leaf extract of *O. basilicum* against *L. serricorne*

Treatments	Repellency (%)				Mean repellency (%)	Repellency class
	1 HAT	2 HAT	3 HAT	6 HAT		
T_1 - 1% leaf extract of *Ocimum basilicum*	55.00 (47.85)^{c}	60.00 (50.75)^{d}	67.50 (55.22)^{d}	75.00 (59.98)^{b}	64.38 (53.33)^{d}	IV
T_2 - 2% leaf extract of *Ocimum basilicum*	57.50 (49.29)^{c}	65.00 (53.71)^{cd}	72.50 (58.35)^{d}	77.50 (61.66)^{b}	68.13 (55.60)^{d}	IV
T_3 - 3% leaf extract of *Ocimum basilicum*	62.50 (52.22)^{bc}	70.00 (56.77)^{c}	77.50 (61.66)^{cd}	82.50 (65.24)^{b}	73.13 (58.75)^{c}	IV
T_4 - 4% leaf extract of *Ocimum basilicum*	70.00 (56.77)^{b}	77.50 (61.66)^{b}	82.50 (65.24)^{bc}	87.50 (69.27)^{b}	79.38 (62.96)^{b}	V
T_5 - 5% leaf extract of *Ocimum basilicum*	77.50 (61.66)^{a}	85.00 (67.19)^{a}	90.00 (71.54)^{ab}	95.00 (77.05)^{a}	86.88 (68.73)^{a}	V
T_6 – Standard check (Neem oil 1%)	82.50 (65.24)^{a}	87.50 (69.27)^{a}	92.50 (74.08)^{a}	95.00 (77.05)^{a}	89.38 (70.95)^{a}	V
SEd	2.16	2.27	3.26	4.75	1.12	
CD (0.05)	4.54	4.76	6.84	9.98	2.35	

Numeric data represents the mean value of three replications.
HAT-Hours after treatment.
Figures in the parentheses are arc sine transformed values.
Mean values followed by the same alphabet in a column are not significantly different (P < 0.05) by LSD.

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