SUPPLEMENTARY MATERIAL

Insecticidal potential of *Ocimum canum* plant extracts against *Anopheles stephensi*, *Aedes aegypti* and *Culex quinquefasciatus* larval and adult mosquitoes (Diptera: Culicidae)

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Abstract

Mosquitoes have developed resistance to various synthetic insecticides, making their control increasingly difficult. Insecticides of botanical origin may serve as suitable natural control. The present study, evaluates the toxic potential of *Ocimum canum* (Sims) leaf extract and powder against *Anopheles stephensi* (Liston), *Aedes aegypti* (Lin) and *Culex quinquefasciatus* (Say) larval and adult mosquitoes. Larval mortality was observed after 24h recovery period and adult smoke toxicity observed for 40 mins duration at 10 mins interval. Methanol extract of *O. canum* showed highest larval mortality against the larvae of *Cx. quinquefasciatus* $LC_{50}=28.3225$; $LC_{90}=44.1150$, *Ae. aegypti* $LC_{50}=43.327$; $LC_{90}=61.249$ and *An. stephensi* $LC_{50}=30.2001$; $LC_{90}=48.2866$ ppm. The smoke toxicity were 93% mortality in *Cx. quinquefasciatus*, 74%, *Ae. aegypti* 79% and *An. stephensi* adults respectively, whereas 100% mortality was recorded in the commercial mosquito control. Our results suggest that *O. canum* leaf extract and powder are natural insecticide, ideal eco friendly approach for mosquito control

Keywords: Plant derived extracts; Bioassay; Larvicidal activity; Smoke toxicity; *Ocimum canum*

Experimental

Plant materials

The leaves of *Ocimum canum* (Fig.S1) were collected from yarcurd Hills of the Eastern Ghats of Tamil Nadu. It is located in the Salem district of the Southeast Tamil Nadu and lies between (10°12' - 11°7'N, 76° - 77°56'E and Altitude 1300 meters).

Fig.S1. *Ocimum Canum*

Preparation of plant extracts

The leaves were shade dried for 7–10 days. The dried leaves were powdered mechanically using commercial electrical stainless steel blender and the powdered leaves (300g) were extracted with chloroform (400ml), ethyl acetate (400ml), acetone (400ml) petroleum benzene and methanol (300ml) in a Soxhlet apparatus (boiling point range 50–80°C) for 8h. The extract was concentrated under reduced pressure 22–26mm Hg at 45°C and the residue obtained was stored at room temperature

Maintenance mosquito larvae

Anopheles stephensi, *Aedes aegypti* and *Culex quinquefasciatus* mosquito larvae were procured from Institute of Vector Control (IVC), Banahalli, Tamil Nadu, India. The larvae were kept in plastic trays containing tap water and were maintained in the laboratory, and all the experiments were carried out at 27±2°C and 75–85% relative humidity under 14:10 light and dark photoperiod cycles. Larvae were fed with dog biscuit and yeast powder in the ratio of 3:1. They were maintained and reared in the laboratory.

Mosquito larvicidal bioassay

The larvicidal activity of the plant crude extracts was evaluated as per the method recommended by World Health Organization (2005). 25 fourth instar larvae were transferred to a small disposable paper cups, in 249 ml of water and 1.0 ml of the desired plant extract at different concentration (10, 50, 150, 200 and 250ppm), an equal number of controls were set up simultaneously using tap water. The numbers of dead larvae were counted after 24 h of exposure, and the percentage mortality was reported from the average of three replicates. The LC₅₀ (lethal concentration that kills 50 % of the exposed larvae) and LC₉₀ (lethal concentration that kills 90 % of the exposed larvae) values were calculated after 24 h by probit analysis (Finney, 1971).

Mosquito coil preparation

Mosquito coils were prepared by following methods of (Ramkumar et al., 2015; Singha et al., 2011). The composition of mosquito coils were 4 grams of shade dried plant powder containing active ingredients, 2 grams of sawdust and 2 grams of charcoal powder. All the materials were thoroughly mixed with distilled water to form a semi-solid paste and the paste was used for the preparation of 0.5 cm thickness mosquito coils. The mosquito coils were dried in shade and used for further repellency experiment.

Smoke toxicity test

Smoke toxicity experiment was conducted in a glass chamber measuring 60x40x35cm with mid bottom of the chamber. Fifty blood fed adult mosquitoes were released to the chamber and the mosquitoes were exposed to the smoke of burning coils for 40 mins and the mortality data were recorded after every 10mins. The smoke toxicity was compared with the commercially available mosquito coil as tested above (Ramkumar et al., 2015). The commercial mosquito coil active ingredients contain extract allethrin as active ingredient.

Statistical analysis

The average larval mortality data were subjected to probit analysis for calculating LC₅₀, LC₉₀ and other statistics at 95 % confidence limits of upper confidence limit (UCL) and lower confidence limit (LCL) values and chi square test were calculated using the SPSS13.0 (Statistical Package of Social Sciences) software version 13.0.

Reference

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Table S1; Larvicidal activity of *Ocimum canum* leaf extracts against fourth instar larvae of *Cx. quinquefasciatus*

Species	Sample	n ^a	LC ₅₀ (LCL-UCL) (95% confidence limit) ppm	LC ₉₀ (LCL-UCL) (95% confidence limit) ppm	df
<i>Culex quinquefasciatus</i>	Acetone	375	32.5810 (30.212-34.823)	55.2801 (50.211-64.888)	3
	Ethyl Acetate	375	58.533 (56.1011-62.413)	72.524 (68.365-77.512)	3
	Methanol	375	28.3225 (28.127-30.1864)	44.1150 (42.2326-47.1661)	3
	Chloroform	375	34.1286 (33.822-39.177)	60.2532 (58.421-62.511)	3

n^a -Number of larvae, LC₅₀ - Lethal concentration 50% mortality, LC₉₀- Lethal concentration 90% mortality, LCL- lower confidence limits, UCL- upper confidence limits, χ^2 -chi square, df - degrees of freedom

Table S2; Larvicidal activity of *Ocimum canum* leaf extracts against fourth instar larvae of *An stephensi*

Species	Sample	n ^a	LC ₅₀ (LCL-UCL) (95% confidence limit) ppm	LC ₉₀ (LCL-UCL) (95% confidence limit) ppm	df
<i>Anopheles stephensi</i>	Acetone	375	33.292 (31.222-35.434)	48.5821 (46.255-50.721)	3
	Ethyl Acetate	375	44.584 (43.531-46.577)	52.312 (50.298-55.2123)	3
	Methanol	375	30.2001 (28.433-33.185)	48.2866 (46.311-51.216)	3
	Chloroform	375	33.521 (32.150-38.4145)	49.3201 (47.2391-58.3221)	3

n^a - Number of larvae, LC₅₀ - Lethal concentration 50% mortality, LC₉₀- Lethal concentration 90% mortality, LCL- lower confidence limits, UCL- upper confidence limits, df - degrees of freedom

Table S3; Larvicidal activity of *Ocimum canum* leaf extracts against fourth instar larvae of *Ae. aegypti*

Species	Sample	n ^a	LC ₅₀ (LCL-UCL) (95% confidence limit) ppm	LC ₉₀ (LCL-UCL) (95% confidence limit) ppm	Df
<i>Aedes aegypti</i>	Acetone	375	66.324 (61.231-68.441)	81.228 (8.771-85.348)	3
	Ethyl Acetate	375	52.1854 (49.77-54.321)	66.321 (61.357-69.001)	3
	Methanol	375	43.327 (41.119-45.551)	61.249 (59.82-64.321)	3
	Chloroform	375	49.118 (47.321-50.734)	63.766 (61.382-65.743)	3

n^a - Number of larvae, LC₅₀ - Lethal concentration 50% mortality, LC₉₀- Lethal concentration 90% mortality, LCL- lower confidence limits, UCL- upper confidence limits, df - degrees of freedom

Table.S4. Smoke toxicity of *Ocimum canum* plant powder mosquito coil compared with commercial mosquito coils and mosquito coil without any plant powder on major mosquito vectors

Mosquito species	Observation (In mins)	% Mortality from plant powder mosquito coil	% Mortality from Commercial mosquito coil (allethrin)	% Mortality in control
<i>Cx. quinquefasciatus</i>	10	48	54	1
	20	58	78	2
	30	76	100	3
	40	93	100	3
<i>Ae. aegypti</i>	10	43	43	1
	20	67	54	1
	30	70	100	1
	40	74	100	1
<i>An. stephensi</i>	10	65	32	0
	20	68	77	0
	30	77	98	0
	40	79	98	0