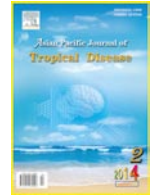




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Extraction of mosquitocidals from *Ocimum canum* leaves for the control of dengue and malarial vectors

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ABSTRACT

Objective: To assess the potentiality of *Ocimum canum* (*O. canum*) (Lamiaceae) in larvicidal, pupicidal, adulticidal, and repellent activities against the malarial vector, *Anopheles stephensi* (*An. stephensi*) and the dengue vector, *Aedes aegypti* (*Ae. aegypti*).

Methods: The mosquitocidal activity of methanol extracts from *O. canum* against immature and adult *An. stephensi* and *Ae. aegypti* (L.) were studied. Standard WHO bioassays were used to evaluate the effectiveness of the plant extract against mosquitoes.

Results: The methanol extract of *O. canum* was very effective against the immature stages of *An. stephensi* (LC₅₀=193.280, 240.551, 303.409, 374.936 and pupa 469.547 mg/L) and *Ae. aegypti* (LC₅₀=242.071, 287.277, 332.668, 394.061 and pupa 457.879 mg/L). Smoke toxicity assay showed significant mortality rate against adult *An. stephensi* (86.6%) and *Ae. aegypti* (84.78%). The number of eggs laid by the females was strictly reduced after exposure to smoke.

Conclusions: From the observed results we conclude that *O. canum* can be used as an effective larvicidal and repellent agent against the malarial and dengue vectors.

1. Introduction

Mosquitoes are insect vectors responsible for the transmission of parasitic and viral infections to millions of people worldwide with substantial morbidity and mortality. Infections transmitted by mosquitoes include malaria, yellow fever, chikungunya, filariasis and other arboviruses[1]. *Aedes aegypti* (*Ae. aegypti*) is known to transmit dengue and yellow fever while malaria is transmitted by *Anopheles* species[2].

Anopheles stephensi (*An. stephensi*) is a primary mosquito vector of malaria in urban India. *An. stephensi* mosquitoes transmit malaria parasites among humans[3]. In 2010, over 1.2 million global malaria deaths were reported in both children and adults[4].

Dengue is considered to be one of the deadly mosquito

borne human viral infections. *Ae. aegypti* is the vector that spread dengue fever. In Asian region, most of the deaths of children are due to the dengue haemorrhagic fever[5]. Dengue affects 50 million people annually with approximately 20 000 deaths[6]. Dengue fever is the most important re-emerging arboviral disease, causing an estimated 390 million infections every year worldwide, of which nearly 100 million require medical attention, and more than 500 000 require hospitalization[7].

Repellents have an important place in protecting man from the bites of insect pests. An effective repellent will be useful in reducing human vector contact and interrupting disease transmission. A repellent compound should be toxic, non-irritating and long lasting. Amides, imides, esters and other poly functional compounds are known to be good repellents[8]. Plants could be an alternative source for mosquito repellents because they are constituted by a potential source of bioactive chemicals and typically are free from harmful effects[9].

Plant products have been used traditionally by human communities in many parts of the world against the

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pests and vectors. The phytochemicals derived from plant sources can act as larvicides, insect growth regulators, and repellents^[10–12]. Plants are considered as a rich source of bioactive chemicals and they may be an alternative source of mosquito control agents^[13]. The phytochemicals derived from plant sources possess a complex of chemicals with unique biological activity.

Ocimum canum (*O. canum*) Sims. (Lamiaceae), commonly known as wild basil, is called “koti” in Cameroon and its leaves are used locally as spice in an ethno dietary soup known as “Mbongo tchobi”. Traditional practitioners in Cameroon apply this plant for abdominal pain, malaria, diarrhea, and stomach-related disorders. It has been demonstrated that its leaf essential oil shows antimicrobial properties^[14,15]. *O. canum* was studied for volatile oil composition, and the compounds identified were α -thujene, myrcene, α -pinene, sabinene, α -phellandrene, α -terpinene, limonene, γ -terpinene, terpinolene, β -caryophyllene, trans- α -bergamotene, α -caryophyllene, germacrene D, β -seliene, biocyclogermacrene, estragole, thymol and carvacrol^[16]. This study was undertaken to assess the potentiality of *O. canum* (Lamiaceae) in larvicidal, pupicidal, adulticidal, and repellent activities against the malarial vector, *An. stephensi* and the dengue vector, *Ae. aegypti*.

2. Materials and methods

2.1. Collection of plant materials and preparation of extracts

Leaves of *O. canum* (Lamiaceae) were collected from in and around Bharathiar University, Coimbatore, Tamilnadu, India. The plants were authenticated at Botanical Survey of India and the voucher specimens were deposited at Zoology Department, Bharathiar University, Coimbatore, India. Leaves of *O. canum* were washed with tap water, shade dried at room temperature and powdered by an electrical blender. The active compounds were extracted with 300 mL of methanol for 8 h in a Soxhlet apparatus^[17]. The crude plant extracts were evaporated to dryness in rotary vacuum evaporator and diluted to different concentrations for bioassays.

2.2. Mosquito rearing

The eggs of *An. stephensi* and *Ae. aegypti* were collected from drinking water bodies and water stored containers in and around Coimbatore District, Tamil Nadu, India. These eggs were returned to the laboratory and transferred (in approximately the same aliquot numbers of eggs) to 18 cm L \times 13 cm W \times 4 cm D enamel trays containing 500 mL of water where they were allowed to hatch.

Mosquito larvae were reared at (27 \pm 2) °C and 75%–85% relative humidity in a 14:10 (L:D) photoperiod. Larvae were

fed 5 g ground dog biscuit and brewer’s yeast daily in 3:1 ratio. Pupae were collected and transferred to plastic containers with 500 mL of water. The container was placed inside a screened cage (90 cm L \times 90 cm H \times 90 cm W) to retain emerging adults, for which 10% sucrose in water solution (v/v) was available *ad libitum*. On Day 5 post emergence, the mosquitoes were provided access to a rabbit host for blood feeding. The shaved dorsal side of the rabbit was positioned on the top of the mosquito cage in contact with the cage screen (using a cloth sling to hold the rabbit) and held in this position overnight. Glass Petri dishes lined with filter paper and contained 50 mL of water were subsequently placed inside the cage for oviposition by female mosquitoes.

2.3. Larvicidal and pupicidal bioassay

A laboratory colony of *An. stephensi* and *Ae. aegypti* larvae and pupae were used for the larvicidal and pupicidal activity. Hundred numbers of I, II, III and IV instar larvae and pupae were kept in 500 mL glass beaker containing 250 mL of dechlorinated water with desired concentrations of *O. canum* methanolic leaf extract. For each tested concentration, 3 trials were made and each trial consisted of three replicates. The control was set up by mixing acetone with dechlorinated water. Mortality was corrected by using Abbott’s formula^[18].

$$\text{Corrected mortality} = \frac{\text{Observed mortality in treatment} - \text{Observed mortality in control}}{100 - \text{Control mortality}} \times 100$$

$$\text{Percentage mortality} = \frac{\text{Number of dead larvae/pupae}}{\text{Number of larvae/pupae treated}} \times 100$$

The values of LC₅₀, LC₉₀ and their 95% confidence limit (CL) of upper confidence limit and lower confidence limit and *Chi*-square values were calculated by using probit analysis^[19].

2.4. Repellent activity of *O. canum*

Repellent activity of *O. canum* leaf extract was tested with human volunteers using percentage protection in relation to dose dependent method^[7]. Three to four days old blood starved female of adult mosquitoes (100) were kept in a net cage. The arms of the test person cleaned with isopropanol. After air-drying the 25 cm² of the dorsal side of the skin on each arm exposed, the remaining area were covered by rubber gloves.

O. canum was dissolved in isopropanol and this alcohol served as control. *O. canum* at 0.49, 0.99 and 1.99 mg/L concentrations were applied. The control and treated arms were introduced simultaneously into the cage. The number of bites counted for 60 min every 5 min at 18:00 to 02:00 for *An. stephensi* and 09:00 to 18:00 for *Ae. aegypti*. The

experiment was replicated for five times. The percentage protection was calculated by using the following formula.

$$\% \text{Protection} = \frac{\text{Number of bites received by control arm} - \text{Number of bites received by treated arm}}{\text{Number of bites received by control arm}} \times 100$$

2.5. Ovicidal assay

Freshly laid eggs were collected by providing ovitraps in mosquito cages. Ovitrap were kept in the cages for 2 d after the female mosquitoes were given a blood meal. The eggs were laid on filter paper lining provided in the ovitrap. After scoring, 100 gravid female mosquitoes were placed in a screen cage where ten oviposition cups were introduced for oviposition 30 min before the start of the dusk period. Of these ten cups, eight were filled with test solution of 0.49, 0.99, 1.49, 1.99 and 2.49 mg/L respectively and one was filled with 100 mL of respective solvent containing water and polysorbate 80 that served as a control. A minimum of 100 eggs was used for each treatment, and the experiment was replicated five times. After treatment, the eggs were sieved through muslin cloth, thoroughly rinsed with tap water, and left in plastic cups filled with dechlorinated water for hatching assessment after counting the eggs under microscope^[20]. The percent egg mortality was calculated on the basis of non hatchability of eggs with unopened opercula^[21]. The hatching rate of eggs was assessed after 98 h post-treatment^[22].

2.6. Oviposition deterrence assay

To study the ovipositional deterrence effect and the number of eggs deposited in the presence of different solvent extracts of experimental plants, a multiple concentration test was carried out. For bioassay test, 20 males and 20 females were separated in the pupal stage (by size of the pupae) and were introduced into screen cages (45 cm×45 cm×40 cm) in a room at (27±2) °C and 75%–85% relative humidity with a photoperiod of light:dark cycles of 14:10 h. The pupae were allowed to emerge into adults in the test cages. Adults were provided continuously with 10% sucrose solution in a plastic cup with a cotton wick. They were blood fed (from rabbit) on Day 5 after emergence. In the multiple concentration test, five cups, each containing 100 mL distilled water with a 9-cm piece of white filter paper for oviposition as well as solvent extracts at a concentration of 0.49, 0.99, 1.49, 1.99 and 2.49 mg/L were placed in each cage. A sixth cup without extract was served as a control. The positions of the plastic cups were alternated between the different replicates so as to nullify any effect of position on oviposition. Five replicates for each concentration were run with cages placed side by side for each bioassay. After 24 h, the number of eggs laid in treated and control cups were counted under a stereomicroscope. The percent effective repellency for each concentration was calculated using the following

formula.

$$\text{ER}(\%) = \frac{\text{NC} - \text{NT}}{\text{NC}} \times 100$$

Where, ER means effective repellency, NC refers to number of eggs in control, and NT means number of eggs in treatment^[22].

The oviposition experiments were expressed as mean number of eggs and oviposition activity index (OAI), which was calculated using the following formula.

$$\text{OAI} = \frac{\text{NT} - \text{NS}}{\text{NT} + \text{NS}} \times 100$$

Where, NT refers to total number of eggs in the test solution and NS means total number of eggs in the control solution. Oviposition active index of +0.3 and above are considered as attractants while those with –0.3 and below are considered as repellents^[23]. Positive values indicate that more eggs were deposited in the test cups rather than in the control cups and that the test solutions were attractive. Conversely, negative values indicate that more eggs were deposited in the control cups rather than in the test cups and that the test solutions were a deterrent.

2.7. Statistical analysis

The data from bioassay were subjected to statistical analysis. The SPSS software package (Version 14) was computing all the data including confidential limits, *Chi*-square values and mean of the sample.

3. Results

Table 1 illustrates the larval (I to IV) and pupal mortality of *An. stephensi* after the treatment of *O. canum* leaf extract at different concentrations (99.88, 199.77, 299.65, 399.54 and 499.42 mg/L). The mortality of 36% was observed against the I instar larva at 99.88 mg/L concentration, whereas it has been increased to 92% at 499.42 mg/L. Similar trend has been noticed for all larval instars of malarial vector, *An. stephensi*. The LC₅₀ values of I, II, III and IV instars larvae are 193.280, 240.551, 303.409, 374.936 mg/L, and LC₉₀ values of I, II, III and IV instar larvae are 503.274, 585.828, 721.643, 827.598 mg/L, respectively. Similarly LC₅₀ and LC₉₀ values for the pupae are 469.547 and 977.814 mg/L, respectively.

The effect of *O. canum* leaf extract at different concentrations on I to IV instars and pupa of *Ae. aegypti* showed significant mortality rates (Table 2). Higher mortality (86%) was observed at the 499.42 mg/L concentration. The LC₅₀ and LC₉₀ values of *O. canum* are represented as follows: LC₅₀ of I instar was 242.071 mg/L, II instar was 287.277 mg/L, III instar was 332.668 mg/L, IV instar was 394.061 mg/L and pupa was 457.879 mg/L, respectively. The LC₉₀ value of I instar was 567.413 mg/L, II instar was 647.979 mg/L, III instar was 729.020 mg/L,

Table 1Larvicidal activity of *O. canum* methanolic leaf extract against malarial vector, *An. stephensi*.

Larval instars and pupae	Larval mortality (%)±SD					LC ₅₀ (LC ₉₀)	Regression equation	95% CL		Chi-square value (χ ²)
	99.88 (mg/L)	199.77 (mg/L)	299.65 (mg/L)	399.54 (mg/L)	499.42 (mg/L)			LC ₅₀	LC ₉₀	
I Instar	36.00±0.42	53.00±0.82	63.00±1.04	79.00±0.26	92.00±0.32	193.280 (503.274)	0.004 13	154.139–224.298	454.383–575.118	1.592
II Instar	32.00±1.21	46.00±0.63	52.00±0.34	72.00±0.42	86.00±0.11	240.551 (585.828)	0.003 71	203.748–272.060	523.072–682.188	2.759
III Instar	27.00±0.32	39.00±0.74	47.00±0.45	61.00±0.35	74.00±0.50	303.409 (721.643)	0.003 06	265.161–342.172	626.402–882.574	0.469
IV Instar	23.00±0.21	31.00±0.53	39.00±0.86	53.00±0.41	65.00±0.23	374.936 (827.598)	0.002 83	334.004– 428.196	705.351–1 045.674	0.420
Pupae	16.00±0.90	28.00±0.44	31.00±0.82	45.00±0.20	52.00±0.61	469.547 (977.814)	0.002 52	413.268–564.064	807.863–1 312.250	1.178

Table 2Larvicidal activity of *O. canum* methanolic leaf extract against dengue vector, *Ae. aegypti*.

Larval instars and pupae	Larval mortality (%)±SD					LC ₅₀ (LC ₉₀)	Regression equation	95% CL		Chi-square value (χ ²)
	99.88 (mg/L)	199.77 (mg/L)	299.65 (mg/L)	399.54 (mg/L)	499.42 (mg/L)			LC ₅₀	LC ₉₀	
I Instar	28.00±0.54	46.00±0.44	58.00±0.24	71.00±1.22	86.00±0.62	242.071 (567.413)	0.003 94	207.567–271.956	510.140–653.152	0.784
II Instar	24.00±0.41	41.00±1.03	50.00±0.43	65.00±0.42	78.00±0.11	287.277 (647.979)	0.003 55	253.252–320.052	574.824–762.759	0.675
III Instar	21.00±0.32	37.00±0.74	44.00±0.45	58.00±0.84	71.00±0.28	332.668 (729.020)	0.003 23	297.285–371.994	636.637–881.536	0.883
IV Instar	17.00±0.82	30.00±1.83	37.00±0.46	52.00±0.52	62.00±0.64	394.061 (810.830)	0.003 07	354.880–446.296	699.065–1 002.542	0.617
Pupae	13.00±0.40	26.00±0.24	35.00±0.65	42.00±0.82	54.00±1.60	457.879 (911.197)	0.002 83	408.445–535.166	769.219–1 171.552	1.255

L, IV instar was 810.830 mg/L and pupa was 911.197 mg/L, respectively.

The repellent activity of *O. canum* methanolic leaf extract on malarial vector, *An. stephensi* is shown in Table 3. The repellent activity was carried out in the evening from 18:00 to 02:00. The repellency effect was very low (63.33% protection) at 0.49 mg/L. The percentage protection was 77.77% and 86.66% at 0.99 mg/L and 1.99 mg/L, respectively. Similarly, Table 4 shows the repellent activity of *O. canum* methanolic leaf extract on dengue vector, *Ae. aegypti*. The repellent activity was carried out in the morning from 09:00–18:00. The repellency effect was very low (50.00% protection) at 0.49 mg/L, whereas it has been increased to 71.73% and 84.78% at 0.99 and 1.99 mg/L, respectively.

Table 3Repellent activity of *O. canum* methanolic leaf extract against malarial vector, *An. stephensi*.

Repellent activity observation time	No. of mosquitoes fed			
	0.49 mg/L	0.99 mg/L	1.99 mg/L	Control
18:00–19:00	0	0	0	0
19:00–20:00	5	3	3	14
20:00–21:00	4	2	2	15
21:00–22:00	2	4	1	16
22:00–23:00	5	3	2	10
23:00–24:00	4	2	1	9
24:00–01:00	7	4	1	14
01:00–02:00	6	2	2	12
Fed mosquito	33	20	12	90
Unfed mosquito	67	80	88	10
Percentage of mosquito repellency	63.33%	77.77%	86.66%	–

Table 4Repellent activity of *O. canum* methanolic leaf extract against dengue vector, *Ae. aegypti*.

Repellent activity observation time	No. of mosquitoes fed			
	0.49 mg/L	0.99 mg/L	1.99 mg/L	Control
09:00–10:00	0	0	0	0
10:00–11:00	7	4	2	15
11:00–12:00	5	2	3	12
12:00–13:00	4	3	2	10
13:00–14:00	7	5	2	15
14:00–15:00	6	4	1	16
16:00–17:00	8	5	2	11
17:00–18:00	9	3	2	13
Fed mosquito	46	26	14	92
Unfed mosquito	54	74	86	8
Percentage of mosquito repellency	50.00%	71.73%	84.78%	–

Table 5Ovicidal activity of *O. canum* methanolic leaf extract against eggs of *An. stephensi* and *Ae. aegypti*.

Concentration (mg/L)	Percentage of egg hatchability±SD	
	<i>An. stephensi</i>	<i>Ae. aegypti</i>
99.88	81.00±0.34	76.00±0.42
199.77	53.00±0.82	39.00±0.64
299.65	26.00±0.65	15.00±0.24
399.54	12.00±0.42	NH
499.42	NH	NH
Control	100.00±0.00	100.00±0.00

NH: No hatchability (100% mortality).

Table 5 shows the percentage of egg hatchability after the treatment of *O. canum* methanolic leaf extract at different concentrations. *O. canum* methanolic leaf extract

exerted zero hatchability (100% mortality) at 399.54 and 499.42 mg/L by *Ae. aegypti* and *An. stephensi*, respectively. The percentage egg hatchability after treatment with 99.88, 199.77, 299.65, 399.54 and 499.42 mg/L were 81%, 53%, 26%, 12% and no hatchability, respectively. Likewise in *Ae. aegypti* 99.88 mg/L concentration exerted 76% of egg hatchability, 199.77 mg/L exerted 39%, 299.65 mg/L exerted 15%, 399.54 and 499.42 mg/L exerted no hatchability.

Oviposition deterrent activity of *O. canum* methanolic leaf extract at different concentration against gravid females of *Ae. aegypti* and *An. stephensi* is shown in Table 6. Among the two species, the highest oviposition deterrent activity (99.13%) was recorded in *An. stephensi* at 499.42 mg/L concentration and lowest oviposition deterrent was observed in *Ae. aegypti*. The percentage of effective repellency at different concentrations of *O. canum* methanolic leaf extract against *An. stephensi* were 51.19%, 68.20%, 79.19%, 92.80% and 99.13%, respectively. *Ae. aegypti* showed 44.98%, 62.48%, 74.74%, 91.05% and 98.45% repellency against the different concentrations of leaf extract.

Table 6

Oviposition deterrent activity of *O. canum* methanolic leaf extract against *An. stephensi* and *Ae. aegypti*.

Concentration (mg/L)	No. of <i>An. stephensi</i> eggs laid			ER (%)	OAI	No. of <i>Ae. aegypti</i> eggs laid			ER (%)	OAI
	Treated	Control				Treated	Control			
99.88	483.6	990.8	51.19	-0.34	547.6	995.4	44.98	-0.29		
199.77	310.8	975.1	68.20	-0.51	363.4	968.6	62.48	-0.45		
299.65	185.4	889.2	79.19	-0.65	221.0	875.2	74.74	-0.59		
399.54	63.2	878.6	92.80	-0.86	76.3	852.6	91.05	-0.83		
499.42	6.8	790.4	99.13	-0.98	12.4	804.2	98.45	-0.97		

ER: Effective repellency; OAI: Oviposition active index.

4. Discussion

Mosquito control lies in personal protection by using repellents and community education as the most economical method, and application of eco-friendly larvicides. Synthetic insecticides are no doubt having quick actions, but it received wide public concern for their adverse effects to the environment, like insecticide resistance, environmental pollution, toxic hazards to human and other non-target organisms[24].

Human beings have used plant parts, products and secondary metabolites of plant origin in pest control since early historical times. Vector control has been practiced since the early 20th century. During the pre-DDT era, reduction of vector mosquitoes mainly depended on environmental management of breeding habitats, i.e., source reduction. During that period, some botanical insecticides used in different countries were chrysanthemum, pyrethrum, derris, quassia, nicotine, hellebore, anabasine, azadirachtin, d-limonene camphor, turpentine, etc[25].

Botanicals are basically secondary metabolites that serve as a means of defence mechanism of the plants to

withstand the continuous selection pressure from herbivore predators and other environmental factors. Several groups of phytochemicals such as alkaloids, steroids, terpenoids, essential oils and phenolics from different plants have been reported previously for their insecticidal activities[25]. These compounds can be divided into different chemical groups like alkaloids, phenolic, terpenoids, rare amino acids, plant amines and glycosides. These compounds also play an important role as anti-nutritional components of food and animal feed with a number of phenolic compounds. Plant terpenoids have been studied for their activities against a number of insects[26].

In this study higher mortality (92%) was observed at the 499.42 mg/L concentration. LC₅₀ values is 303.409 mg/L III instar larvae of *An. stephensi*. Earlier report on larval mortality after 24 h exposure to ethyl acetate extract of *Leucas aspera* was similar to the present study with LC₅₀ value of 352.84 mg/L.

In this present study the observed LC₅₀ (193.280, 240.551, 303.409, 374.936 and 469.547 mg/L, respectively) and LC₉₀ (503.274, 585.828, 721.643, 827.598 and 977.814 mg/L) values of *O. canum* against *An. stephensi*, were also in concordance with the results of Arivoli *et al*[27]. Various plant species have been screened for their larvicidal activity against different species of mosquitoes[25,28–30].

Larvicidal activities of crude hexane, ethyl acetate, petroleum ether, acetone, and methanol extracts of *Abutilon indicum*, *Aegle marmelos*, *Euphorbia thymifolia*, *Jatropha gossypifolia*, and *Solanum torvum* were assayed[31], as well as the extracts of peel and leaf extracts of *Camellia sinensis*, *O. canum*, *Ocimum sanctum* and *Rhinacanthus nasutus* were assayed against mosquitoes[32].

Our results clearly indicate that extracts from *O. canum* are more toxic for larval population of *Ae. aegypti*, endorsed by larval mortality in lesser concentration with minimum time interval. The plant derivatives are rich source of some primary as well as secondary metabolites that adds impact to the potential of the extract[31]. Bagavan *et al.* 2009 reported that the presence of chemicals in the leaves of *O. canum* justifies the local use of this plant for the treatment of various ailments[32]. The leaves are rich in flavonoids, saponins and tannins with considerable amount of phenolics and alkaloids. Flavonoids are polyphenolic compounds that are biologically active against liver toxins, microorganisms, inflammation, tumor and free radicals. The earlier literature survey on the plant revealed that the major compounds isolated from *O. canum* were volatile components like terpinol-4 (21%–30%), linalool (17%–19%) and γ -terpinene (7%–11%). Mathur, 2003 reported that the essential oil of *O. canum* was characterized by its high content of trans-methyl cinnamate[33].

A large number of synthetic chemicals have been tested for their repellent activity against mosquitoes. However, the prohibitive retailers' cost of proprietary formulations

of chemicals like DEET (N,N-diethyl-m-toluamide) restricts their usage by the poor in countries such as India. Hence, the search for a safer, better, and cheaper repellent is an ongoing effort. Since cost is an important factor, investigation on the use of local plants as repellents is strongly recommended^[34]. Repellents of plant origin should be nontoxic, nonirritating and long lasting. Plants of terrestrial origin have been reported to be a source of mosquito repellents^[35].

Plant derived smoke contains an array of chemicals with different modes of action, which kill mosquitoes. Curtis (1990) reported that smoke from burning various dry materials has been used since early times to deter insects especially mosquitoes^[36].

Majority of the interviewed households (66.7%) showed preference to *Ocimum* species rather than other anti-mosquitoes plants. *Ocimum* plants are common as the post-harvest weed in the area around Coimbatore District, Tamil Nadu, India. Most common method of application is burning of plant leaves for protection before going to bed and hanging the repellent plants inside the house^[37,38]. Application of repellents was mostly done from 7 pm to 10 pm, such timing corresponds with the mosquito active biting cycle in the evening^[39].

Smoke of *O. canum* contains an array of chemicals which have been used to deter mosquitoes and it is cheap target specific and highly toxic to adult mosquitoes at low dose. It also affects the egg production and egg hatchability. Smoke toxicity from *Moringa oleifera*^[12], *Mesua ferra*^[40], and *Artemisia parviflora* affects the neuroendocrine system to inhibit the hatchability of eggs and reduces the egg laying capacity as well as the egg hatchability of the mosquitoes^[41].

In our study *O. canum* showed 86.66% and 84.78% protection for more than 8 h against *An. stephensi* and *Ae. aegypti* respectively. Someshwar *et al.* reported 78% and 92% repellency by *Mesua ferra* against the adult females of *An. stephensi* and *Ae. aegypti*^[42]. Some botanicals are comparable to, or even better than synthetic repellents; nonetheless, repellents based on essential oils tend to be short-lived in their effectiveness, due to their volatility. Consequently, there is a need for effective ecofriendly repellents^[43].

Hence, *O. canum* leaf extract can be used as biopesticides for the control of mosquito vectors. The result of this study indicates that *O. canum* leaf extracts enhances the larvicidal, pupicidal and smoke repellency activity and hence it may act as an effective alternative to conventional synthetic insecticides for the control of *An. stephensi* and *Ae. aegypti*.

Conflict of interest statement

We declare that we have no conflict of interest.

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