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Larvicidal and repellent properties of some essential oils against *Culex tritaeniorhynchus* Giles and *Anopheles subpictus* Grassi (Diptera: Culicidae)

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ABSTRACT

Objective: To investigate the larvicidal and repellent properties of essential oils from various parts of four plant species *Cymbopogan citrates, Cinnamomum zeylanicum, Rosmarinus officinalis* and *Zingiber officinale* against *Culex tritaeniorhynchus (Cx. tritaeniorhynchus)* and *Anopheles subpictus (An. subpictus).* **Methods:** Essential oils were obtained by hydro-distillation method. The mosquitoes were reared in the vector control laboratory and twenty five late third instar larvae of *Cx. tritaeniorhynchus* and *An. subpictus* were exposed to based on the wide range and narrow range test, essential oil tested at various concentrations ranging from 25 to 250 ppm. The larval mortality was observed after 24 h under the laboratory conditions. The repellent efficacy was determined against two mosquito species at three concentrations viz., 1.0, 2.5 and 5.0 mg/cm^2 under laboratory conditions. **Results:** Results showed all the four plant essential oil produced significant larval mortality against two mosquito species. However, the highest larvicidal activity was observed in the essential oil from *Zingiber officinale* against *Cx. tritaeniorhynchus* and *An. subpictus* with the LC_{50} and LC_{90} values as 98.83, 57.98 ppm and 186.55, 104.23 ppm, respectively. All the four essential oil shows significant repellency against *Cx. tritaeniorhynchus* than *An. subpictus*. Among four essential oil tested the highest repellency was observed in *Zingiber officinale*, a higher concentration of 5.0 mg/cm^2 provided 100% protection up to 150 and180 min against *Cx. tritaeniorhynchus* and *An. subpictus*, respectively. **Conclusions:** In this work, it can be concluded that four essential oils which were distilled from *Cymbopogan citrates, Cinnamomum zeylanicum, Rosmarinus officinalis* and *Zingiber officinale* showed promising larvicidal and repellent agent against *Cx. tritaeniorhynchus* and *An. subpictus.*

1. Introduction

Blood-feeding female mosquitoes are responsible for the intolerable biting nuisance and the transmission of a large number of diseases, such as malaria, yellow fever, dengue, filariasis, chikengunya and encephalitis, causing serious health problems to humans and obstacles to socioeconomic development of developing countries, particularly in the tropical region[1]. *Anopheles subpictus (An. subpictus)* is known to transmit malaria and filariasis, in an isolated study of multiple host-feeding in field populations, and its specific role in transmitting malaria in Sri Lanka revealed that multiple blood feeding within the same gonotrophic cycle was attributed to a local "frequent feeding strategy" in this primarily zoophagic and endophilic malaria vector. On the contrary, in Indonesia, *An. subpictus* is a potential vector of bancroftian filariasis and fed on microfilaraemia carriers that harbored *Wuchereria bancrofti* larvae[2]. In India, malaria is one of the most important causes of direct or indirect infant, child and adult mortality. About two million confirmed malaria cases and 1 000 deaths are reported annually, although 15 million cases and 20 000 deaths are estimated by the World Health Organisation (WHO) Southeast Asia Regional Office. India contributes 77% of the total malaria in Southeast Asia[3]. *Culex tritaeniorhynchus (Cx. tritaeniorhynchuss)* Giles is an important vector of JE in India and South East Asian countries[4] . JE is endemic in few states of India and highly endemic in few districts of Tamil Nadu, Southern India[5, 6]. Keiser *et al*[7] have reported that approximately 1.9 billion people currently live in rural JE-prone areas of the world, the majority of them in China (766 million) and India (646 million). In the last few years, there was an increase of

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public concern on the safety of many chemical products that instigated a renewed interest on the use of natural products from plant origin for vector management. New botanical natural products are effective, environment-friendly, easily biodegradable, inexpensive, and readily available in many areas of the world, no ill effect on non-target organisms and have novel modes of action[8]. In addition to application as general toxicants against immature mosquitoes, phytochemicals may also have potential uses as larvicidal, repellents, ovicidal and oviposition deterrents, and growth and reproduction inhibitors[9-16].

Plant essential oils in general have been recognized as an important natural resource of insecticides[17]. Their lipophilic nature facilitates them to interfere with basic metabolic, biochemical, physiological and behavioural functions of insects[18]. Jantan and Zaki[19] evaluated four essential oils of *Litsea elliptica*, *Cinnamomum mollissimum*, *Cymbopogon nardus*, and *Pogostemom cablin*, respectively, for their repellency effect against *Aedes aegypti (Ae. aegypti)*. Barnard[20] tested the repellency of five essential oils (*Bourbon geranium*, cedarwood, clove, peppermint, and thyme) singly applied at different concentrations (5%, 10%, 25%, 50%, 75%, and 100%) or in combinations against two mosquito species *Ae. aegypti* and *Anopheles albimanus*. Thyme and clove oils were the most effective mosquito repellents. Palsson and Jaenson[21] conducted research in several villages in Guinea Bissau (West Africa) on eight plant species being used traditionally as mosquitoes repellents by native people [*Hyptis suaveolens* (Lamiaceae), *Daniellia oliveri* (Caesalpiniaceae), *Elaeis guineensis* (Arecaceae), *Parkia biglobosa* (Mimosaceae), *A. indica* (Meliaceae), *Eucalyptus* sp. (Myrtaceae), *Ocimum canum* (Lamiaceae), and *Senna occidentalis* (Caesalpiniaceae)]. Essential oils, secondary metabolites of the plants, are odor components that can be extracted from plant tissue by water steam distillation or supercritical fluid extraction. Most of them are complex mixtures of mono- and sesquiterpenes and biologically related phenolic compounds. Essential oils and their volatile constituents are widely used in the prevention and treatment of human illnesses. Various essential oils have also been documented to exhibit acute toxic effects against insects, including mosquitoes[22]. The present study was an attempt to assess the larvicidal and repellent properties of four essential oils against *Cx. tritaeniorhynchus* Giles and *An. subpictus Grassi*.

2. Materials and methods

2.1. Plant material & essential oil distillation

Various parts of four medicinal plants: *Cymbopogan citrates (C. citrates)* (leaves), *Cinnamomum zeylanicum (C. zeylanicum)* (bark), *Rosmarinus officinalis (R. officinalis)* (shoot), and *Zingiber officinale (Z. officinale)* (rhizome) were collected from Erode, Tamil Nadu. India. The various plant parts were subjected to steam distillation. The essential oils were collected and stored at 4 ℃ for further experimentation.

2.2. Mosquitoes

The mosquitoes, *Cx. tritaeniorhynchus* and *An. subpictus* were reared in the vector control laboratory, Department of Zoology, Annamalai University. The larvae were fed on dog biscuits and yeast powder in the 3:1 ratio. Adults were provided with 10% sucrose solution and one week old chick for blood meal. Mosquitoes were held at (28±2) ℃, 70%-85% relative humidity (RH), with a photo period of 14 h light, 10 h dark.

2.3. Larvicidal activity

Larvicidal activity of four essential oils against *Cx. tritaeniorhynchus* and *An. subpictus* was assessed by using the WHO standard method[23]. For experimental treatment, 1 mL of essential oil was dissolved in 100 mL distilled water using acetone. Various concentrations of dissolved oils were prepared in distilled water. After, 25 larvae of late third instar taken on a strainer with fine mesh were transferred gently to the test medium by topping. The control experiments were also run parallel with each replicate. The larval mortality was calculated after 24 h of the exposure period. The corrected percent of mortality was calculated by applying Abbott's formula[24]. The data were subjected to probit analysis in order to estimate the LC_{50}, and LC_{90} values[25].

2.4. Repellent activity

The repellency of four essential oils was evaluated using the human-bait technique to stimulate the condition of human skin to which repellents will be eventually applied[26]. Evaluation was carried out in a net cage (45 cm × 30 cm ×25 cm) containing 100 blood starved female *Cx. tritaeniorhynchus* and *An. subpictus* of 3-4 days old at (28±2) ℃ and relative humidity of 65% - 80%. The volunteer had no contact with lotions, perfumes, oils or perfumed soaps on the day of the assay. The arms of the volunteer, only 25 cm^2 dorsal side of the skin on each arms was exposed and the remaining area covered by rubber gloves. The essential oils were applied at 1.0, 2.5 and 5.0 mg/cm^2 separately in the exposed area of the fore arm. Only ethanol served as control. Each bioassay was conducted between 19:00 and 05:00 h. The control and treated arm were introduced simultaneously into the mosquito cage, and gently tapping the sides on the experimental cages, the mosquitoes were activated. Each test concentration was repeated six times. The volunteer conducted their test of each concentration by inserting the treated and control arm into the same cage for one full minute for every 5 min[27]. The mosquitoes that landed on the hand were recorded and then shaken off before imbibing any blood; making out a 5-min protection. The percentage of repellency was calculated by the following formula

$$\% \text{ Repellency} = [(Ta-Tb)/Ta] \times 100$$

Where Ta is the number of mosquitoes in the control group and Tb is the number of mosquitoes in the treated group.

2.5. Statistical analysis

The average larval mortality data were subjected to probit analysis for calculating LC_{50}, LC_{90} and other statistics at 95% confidence limits of upper confidence limit and lower confidence limit, and chi-square values were calculated using the SPSS12.0 (Statistical Package of Social Sciences) software. Results with $P<0.05$ were considered to be statistically significant.

3. Results

The results indicated that the yields of essential oils ranged from 1.9 to 4.5 mL/kg. The yields of *C. citrates*, *C. zeylanicum*, *R. officinalis*, and *Z. officinale* were 4.5, 3.1, 2.4 and 1.9 mL/kg, respectively. The toxicities of four plant essential oils to late third instar *Cx. tritaeniorhynchus* and *An. subpictus* larvae were noted, and the LC_{50}, LC_{90}, 95% confidence limits of LCL and UCL and chi-square were also calculated (Table 1, 2). Essential oils of four plants screened for larvicidal activity, revealed highest activity in the essential oil of *Z. officinale* against the *Cx. tritaeniorhynchus* and *An. subpictus* mosquito species with the LC_{50} and LC_{90} values as 98.83, 57.98 ppm and 186.55, 104.23 ppm, respectively (Figure 1&2). All the four essential oils were found to be more effective against *An. subpictus* than *Cx. tritaeniorhynchus*. Thus larvicidal assay indicated the order of larvicidal potential in the essential oils as *Z. officinale* > *R. officinalis* > *C. zeylanicum*> *C. citrates*. The repellency of four essential oils against *Cx. tritaeniorhynchus* and *An. subpictus* under laboratory condition is given in Table 3, 4. All the four essential oil shows significant repellency against *Cx. tritaeniorhynchus* and *An. subpictus*. Among four essential oil tested the highest repellency was observed in *Z.officinale*, a higher concentration of 5.0 mg/cm^2 provided 100% protection up to 150 and180 min against *Cx. tritaeniorhynchus* and *An. subpictus*, respectively. In the repellency results, increases in the concentration of the essential oils from 1 to 5 mg/cm^2 were found to increase the repellency time. The essential oils of four plants used in this study did not cause skin irritation, hot sensations or rashes on the arms of the test volunteers during the study period.

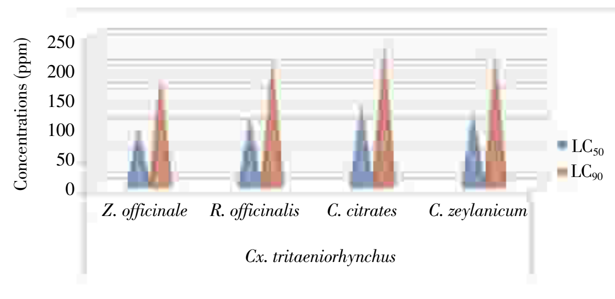

Figure 1. LC_{50} and LC_{90} values of *Cx. tritaeniorhynchus*.

Table 1

Larvicidal activity of four essential oils against *Cx. tritaeniorhynchus*.

Name of the plant	Concentration(ppm)	Mortality (%)	LC_{50} (ppm) (LCL-UCL)	LC_{90} (ppm) (LCL-UCL)	χ^2
Z. officinale Rosc.	Control	0.0±0.0	98.83 (65.81-128.20)	186.55 (152.42-257.18)	20.238*
	50.0	34.5±0.8			
	100.0	56.3±1.1			
	150.0	72.3±1.5			
	200.0	89.6±1.7			
	250.0	100.0±0.0			
R. officinalis L.	Control	0.0±0.0	115.38 (85.27-144.10)	211.53 (176.37-280.69)	17.083*
	50.0	28.7±1.9			
	100.0	46.9±1.6			
	150.0	61.6±1.2			
	200.0	83.9±0.8			
	250.0	98.2±1.4			
C. citrates Stapf.	Control	0.0±0.0	136.58 (111.62-162.57)	243.18 (208.29-306.00)	11.678*
	50.0	21.6±2.1			
	100.0	38.2±1.6			
	150.0	51.7±2.3			
	200.0	74.8±1.7			
	250.0	92.7±0.8			
C.zeylanicum L.	Control	0.0±0.0	124.70 (95.65-153.54)	225.36 (189.15-295.85)	15.816*
	50.0	25.8±0.8			
	100.0	42.4±1.9			
	150.0	56.9±2.3			
	200.0	79.2±1.8			
	250.0	96.8±1.2			

Each value ($\overline{\chi}$ ±SD) represents mean of four values; *Significant at $P<0.05$ level.

Table 2
Larvicidal activity of four essential oils against *An. subpictus*.

Name of the plant	Concentration(ppm)	Mortality (%)	LC_{50} (ppm) (LCL–UCL)	LC_{90} (ppm) (LCL–UCL)	χ^2
Z. officinale Rosc.	Control	0.0±0.0	57.98 (42.07–73.25)	104.23 (86.17–141.63)	19.712*
	25.0	26.5±1.2			
	50.0	47.2±1.9			
	75.0	61.7±2.3			
	100.0	82.6±1.6			
	125.0	100.0±0.0			
R. officinalis L.	Control	0.0±0.0	64.50 (49.96–79.16)	113.74 (95.58–149.57)	16.487*
	25.0	19.9±1.5			
	50.0	43.6±1.9			
	75.0	57.8±1.3			
	100.0	74.2±1.4			
	125.0	97.6±0.8			
C. citrates Stapf.	Control	0.0±0.0	77.24 (65.47–90.39)	128.39 (110.93–160.04)	11.361*
	25.0	11.5±1.5			
	50.0	32.4±1.9			
	75.0	44.7±0.8			
	100.0	63.6±1.2			
	125.0	92.4±1.7			
C.zeylanicum L.	Control	0.0±0.0	71.96 (58.76–86.15)	123.02 (104.78–157.78)	13.858*
	25.0	14.2±1.6			
	50.0	37.9±1.4			
	75.0	51.2±1.9			
	100.0	67.1±0.8			
	125.0	94.4±1.2			

Each value ($\bar{\chi}$ ±SD) represents mean of four values.
*Significant at $P<0.05$ level.

Table 3
Repellent activity of four plant essential oils against *Cx. tritaeniorhynchus*.

Name of the plant	Concentration (mg/cm^2)	% of repellency						
		15 min	30 min	60 min	90 min	120 min	150 min	180 min
Z. officinale Rosc.	1.0	100.0±0.0	100.0±0.0	100.0±0.0	89.6±0.8	77.2±1.7	64.5±0.8	52.4±2.2
	2.5	100.0±0.0	100.0±0.0	100.0±0.0	100.0±0.0	100.0±0.0	86.7±1.2	74.4±1.6
	5.0	100.0±0.0	100.0±0.0	100.0±0.0	100.0±0.0	100.0±0.0	100.0±0.0	88.3±1.8
R. officinalis L.	1.0	100.0±0.0	100.0±0.0	87.3±1.2	79.1±1.2	66.5±1.3	54.3±2.2	43.7±1.2
	2.5	100.0±0.0	100.0±0.0	100.0±0.0	88.2±1.8	78.7±1.5	69.2±1.8	62.6±1.6
	5.0	100.0±0.0	100.0±0.0	100.0±0.0	100.0±0.0	85.3±1.8	77.4±1.4	71.9±2.2
C. citrates Stapf	1.0	100.0±0.0	100.0±0.0	100.0±0.0	86.4±1.2	71.9±1.4	59.7±1.2	47.6±0.8
	2.5	100.0±0.0	100.0±0.0	100.0±0.0	100.0±0.0	84.2±1.2	75.7±1.5	68.9±1.2
	5.0	100.0±0.0	100.0±0.0	100.0±0.0	100.0±0.0	100.0±0.0	88.6±1.3	79.4±1.4
C. zeylanicum L.	1.0	100.0±0.0	100.0±0.0	83.4±1.6	74.6±0.8	59.4±1.2	48.5±1.8	37.2±1.5
	2.5	100.0±0.0	100.0±0.0	100.0±0.0	85.6±1.6	67.8±1.8	62.9±2.2	57.1±1.9
	5.0	100.0±0.0	100.0±0.0	100.0±0.0	100.0±0.0	77.5±1.4	71.3±1.2	64.7±1.2

Values are mean of six replicates±SD.

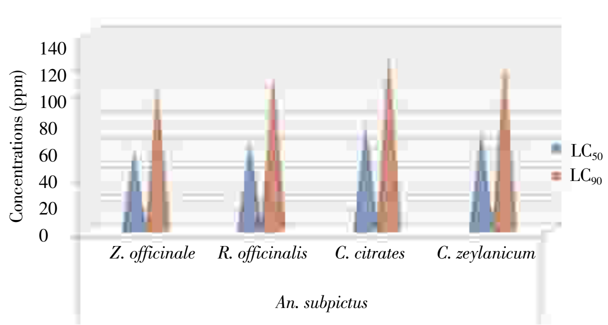

Figure 2. LC_{50} and LC_{90} values of *An. subpictus*.

4. Discussion

Different parts of plants contain a complex of chemicals with unique biological activity[28]. It is thought to be due to toxins and secondary metabolites, which act as attractants or deterrents[29]. This study showed that four essential oils have significant larvicidal as well as repellent activity against two mosquito species. This result is also comparable to earlier reports of Cheng *et al*[30] reported larvicidal effect against *Ae. aegypti* larvae in four compounds derived from the essential oils of *Cinnamon* leaf, including cinnamaldehyde, cinnamyl acetate, eugenol and anethole, with LC_{50} values of 29, 33, 33 and 42 mg/L, respectively. Govindarajan[15] studied that

Table 4

Repellent activity of four plant essential oils against *Anopheles subpictus*.

Name of the plant	Concentration (mg/cm^2)	% of repellency						
		30 min	60 min	60 min	90 min	120 min	150 min	180 min
Zingiber officinale Rosc.	1.0	100.0±0.0	100.0±0.0	100.0±0.0	100.0±0.0	82.8±0.8	76.9±1.9	64.3±0.8
	2.5	100.0±0.0	100.0±0.0	100.0±0.0	100.0±0.0	100.0±0.0	84.3±1.7	78.4±1.2
	5.0	100.0±0.0	100.0±0.0	100.0±0.0	100.0±0.0	100.0±0.0	100.0±0.0	85.6±1.8
Rosmarinus officinalis L.	1.0	100.0±0.0	100.0±0.0	85.6±1.6	77.1±0.8	66.8±1.2	55.6±1.2	47.2±2.2
	2.5	100.0±0.0	100.0±0.0	100.0±0.0	86.7±1.2	79.3±1.8	69.4±1.5	56.7±1.9
	5.0	100.0±0.0	100.0±0.0	100.0±0.0	100.0±0.0	88.5±2.2	77.9±1.8	68.2±1.4
Cymbopogan citrates Stapf.	1.0	100.0±0.0	100.0±0.0	89.7±1.4	81.2±1.6	77.4±2.2	67.4±2.2	56.9±1.5
	2.5	100.0±0.0	100.0±0.0	100.0±0.0	100.0±0.0	84.9±1.8	76.8±1.6	68.2±1.7
	5.0	100.0±0.0	100.0±0.0	100.0±0.0	100.0±0.0	100.0±0.0	87.2±1.2	74.2±1.2
Cinnamomum zeylanicum L.	1.0	100.0±0.0	100.0±0.0	81.2±1.4	69.2±1.8	54.9±1.9	42.3±1.8	33.7±0.8
	2.5	100.0±0.0	100.0±0.0	100.0±0.0	82.8±1.4	71.6±1.5	62.7±1.4	44.3±1.6
	5.0	100.0±0.0	100.0±0.0	100.0±0.0	100.0±0.0	83.5±1.2	72.4±1.2	61.2±1.2

Values are mean of six replicates±SD.

the essential oil from the leaves of *Clausena anisata* exhibit significant larvicidal activity against *Cx. quinquefasciatus*, *Ae. aegypti* and *Anopheles stephensi*, with 24 h LC_{50} values of 140.96, 130.19 and 119.59 ppm, respectively. Traboulsi *et al*[31] reported that the larvicidal activity of essential oils of *Citrus sinensis, Eucalyptus* spp., *Ferrula hermonis, Laurus nobilis*, and *Pinus pinea* against *Cx. pipiens*. LC_{50} values were 60.0, 120.0, 44.0, 117.0, and 75.0 ppm, respectively. The essential oil of *Tagetes minuta*, providing a repellency of 90% protection for 2 h was observed by Tyagi *et al*[32]. Tawatsin *et al*[33] have reported that the essential oils were extracted from 18 plant species, belonging to 11 families, and the oils were then prepared as 10% solution in absolute ethanol with additives evaluated for repellent effects, and the result showed that the night-biting mosquitoes, *Anopheles dirus*, *Cx. quinquefasciatus* and *Ae. albopictus*, were more sensitive to all the essential oils (repellency 4.5-8 h) than was *Ae. aegypti* (repellency 0.3-2.8 h), whereas deet and IR3535 provided excellent repellency against *Ae. aegypti, An. albopictus, An. dirus,* and *Cx. quinquefasciatus* (repellency 6.7-8 h). Tawatsin *et al*[34] have reported repellent activity against *Ae. aegypti, An. dirus*, and *Cx. quinquefasciatus*, which is due to 5% vanillin, which has been added to the essential oil of *Curcuma longa*. Amer and Mehlhorn[35] have reported that the five most effective oils were those of Litsea (*Litsea cubeba*), Cajeput (*Melaleuca leucadendron*), Niaouli (*Melaleuca quinquenervia*), Violet (*Viola odorata*), and Catnip (*Nepeta cataria*), which induced a protection time of 8 h at the maximum and a 100% repellency against *Ae. aegypti*, *An. stephensi*, and *Cx.quinquefasciatus*. The essential oil of *Z. officinalis* showed repellent activity at 4.0 mg/cm^2, which provided 100% protection up to 120 min against *Cx. quinquefasciatus*[36]; the larvicidal activities of plant essential oils. Tiwary *et al*[37] observed the larvicidal activity of linalool rich essential of *Zanthoxylum armatum* against different mosquito species viz., *Cx. quinquefasciatus* (LC_{50}=49 ppm), *Ae. aegypti* (LC_{50}=54 ppm) and *An. stephensi* (LC_{50}=58 ppm). Singh *et al*[38] reported the larvicidal activity of *Ocimum canum* oil against vector mosquitoes, namely, *Ae. aegypti* and *Cx. quinquefasciatus* (LC_{50}=301 ppm) and *An. stephensi* (LC_{50}=234 ppm). The essential oil of *Ipomoea cairica* showed 100% mortality at 170 ppm for *Cx. tritaeniorhynchus* and 120 and 170 ppm for *Ae. aegypti* and *An. stephensi*, respectively[39]. Our study indicate that four essential oils which were distilled from *C. citrates, C. zeylanicum, R. officinalis* and *Z. officinale* show promising larvicidal and repellent agent against *Cx. tritaeniorhynchus* and *An. subpictus*.

Conflict of interest statement

We declare that we have no conflict of interest.

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