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Larvicidal, ovicidal and repellent activities of *Cymbopogan citratus* Stapf (Graminae) essential oil against the filarial mosquito *Culex quinquefasciatus* (Say) (Diptera : Culicidae)

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Abstract. Essential oils extracted by steam distillation from *Cymbopogan citratus* were evaluated for larvicidal, ovicidal and repellent activities against the filarial mosquito *Culex quinquefasciatus*. The larval mortality was observed after 24 hours treatment. The LC_{50} values calculated for the 2nd, 3rd and 4th larval instar were 144.54 ± 2.3, 165.70 ± 1.2 and 184.18 ± 0.8 ppm respectively. Hundred percent ovicidal activity was observed at 300 ppm. Skin repellent test at 1.0, 2.5 and 5.0 mg/cm^2 concentration of *C. citratus* gave 100% protection up to 3.00, 4.00 and 5.00 hours respectively. The total percentage of protection of this essential oil was 49.64% at 1.0 mg/cm^2, 62.19% at 2.5 mg/cm^2 and 74.03% at 5.0 mg/cm^2 for 12 hours.

INTRODUCTION

Mosquitoes are the oldest human enemy and are well known vectors of several diseases causing pathogens. The mosquito *Culex quinquefasciatus* acts as a vector for *Wuchereria bancrofti* responsible for filariasis in India. The study of biologically active materials derived from plant sources can act as larvicides, insect growth regulators, repellents and ovipositional attractants and have deterrent activities as observed by many researchers (Babu & Murugan, 1988; Venkatachalam & Jebanesan, 2001a & b). In recent years, essential oils have received much attention as potentially useful bioactive compounds against insects (Kim *et al.*, 2001). Other essential oils from plants like *Myrtus comunis*, *Origanum syriacum*, *Laventula stoechos* and pure compounds like thymol, carvacrol and α-pinene have been documented for larvicidal activities towards *Culex pipens molestus* (Traboulsi *et al.*, 2002). The present study deals with larvicidal, ovicidal and repellent effects of essential oil of *Cymbopogan citratus* against *Cx. quinquefasciatus*.

MATERIALS AND METHODS

Extraction of essential oil

Whole plants *C. citratus* commonly known as lemongrass were collected from Nilgiris, Western Ghats, Tamilnadu, India. The essential oil was isolated by steam distillation for 6 hours and moisture in the essential oil was removed by anhydrous sodium sulphate and stored at 4°C for further experimentation.

Test organism

The filarial vector, *Cx. quinquefasciatus* were reared in the laboratory, maintained at 27 ± 2°C, 70-80% relative humidity (RH), with a photoperiod of 14 h light and 10 h dark. The larvae were fed with dog biscuits and yeast powder in 3:1 ratio. Adults were provided with 10% sucrose solution and

10% multivitamin syrup and they were periodically blood-fed on restrained rats.

Mosquito larvicidal test

Testing of the plant essential oil *C. citratus* for larvicidal activity was carried out at different concentrations ranging from 100 to 300 ppm in distilled water for 24 h according to standard procedure (WHO, 1981). As the essential oil does not dissolve in water it was first dissolved in ethanol (99.8%). The test medium was prepared by adding 1.0 ml of appropriate dilution of essential oil in ethanol and mixed with 249 ml of water to make up 250 ml of test solution. Twentyfive 2^{nd}, 3^{rd} and 4^{th} instar larvae of *Cx. quinquefasciatus* were collected separately and transferred gently to the test medium and simultaneously a control was maintained with ethanol-water mixture. The larval mortality in both treated and control were recorded after 24 hrs. The LC_{50} value was calculated by Probit analysis (Finney, 1971).

Mosquito ovicidal test

The method of Su & Mulla (1998) was followed for the ovicidal activity. Hundred freshly layed eggs of *Cx. quinquefasciatus* were exposed to five (100 to 300 ppm) concentration of essential oil of *C. citratus* in distilled water. Each concentration was replicated six times. Distilled water mixed with ethanol served as control. The hatch rate was assessed 120 h post treatment by the following formula.

$$\frac{\text{Number of hatched larvae}}{\text{Total number of egg in treated water}} \times 100$$

Repellent activity

The percentage of protection is relation to dose method was used (WHO, 1996). Three-four days old blood-starved female *Cx. quinquefasciatus* mosquito (100) were kept in a net cage (45 x 30 x 45 cm^2). The arms of the test person were cleaned with ethanol and ethanol served as control. After air drying the arms of the test person, only 25 cm^2 dorsal side of the skin on the each arm was exposed and the remaining area being covered by rubber gloves. The essential oil of *C. citratus* was applied at 1.0, 2.5 and 5.0 mg/cm^2 separately (Venkatachalam & Jebanesan, 2001a). The control and treated arms were introduced simultaneously into the cage. The number of bites was counted over 3 minutes every 30 minutes from 18:00 h to 06:00 h. The experiment was conducted five times for each concentration. It was observed that there was no skin irritation from the essential oil tested. The percentage of protection was calculated by using the formula:

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

RESULTS AND DISCUSSION

Cymbopogan citratus essential oil exhibited toxicity to *Cx. quinquefasciatus* larvae. The statistical data are presented in Table 1. The LC_{50} values are 144.54 ± 2.3, 165.70 ± 1.2 and 184.18 ± 0.8 ppm for 2^{nd}, 3^{rd} and 4^{th} instar respectively. Chi-square values were significant at $p<0.05$ level. From the LC_{50} it was evident that higher concentration was required for 3^{rd} and 4^{th} instars. The mean

Table 1. Larvicidal activity of essential oil *C. citratus* against *Cx. quinquefasciatus*

Instar	LC_{50} (ppm)	LC_{90} (ppm)	Regression equation	95% Confidence limit (ppm)		Chi-square (d.f)
				LCL	UCL	
II	144.54 ± 2.3	284.27 ± 1.9	Y = 4.424 + 4.363 X	118.72 ± 0.6	175.96 ± 1.6	7.865* (4)
III	165.70 ± 1.2	318.48 ± 1.3	Y = -5.028 + 4.518X	139.43 ± 2.1	196.93 ± 1.0	8.025* (4)
IV	184.18 ± 0.8	359.01 ± 2.0	Y = -5.017 + 4.422X	152.65 ± 1.9	222.65 ± 2.3	10.267* (4)

Values were based of five concentration and six replication ± SE with 25 larvae in each
* Significant at p < 0.05 level.

percent of egg hatchability of *Cx. quinquefasciatus* is shown in Table 2. Arc-sin transformed data are also presented and regression equation is Y = 6.4785 + 0.2598 X. Hundred percent ovicidal activity was observed at higher concentration of 300 ppm. This study revealed that *C. citratus* had repellency activity against the adult mosquito *Cx. quinquefasciatus*. The results of mean protection in relation to dose of *C. citratus* essential oil are presented in Table 3. Maximum of 100% protection time was obtained at the concentration of 5.0 mg/ cm^2. This is statistically significant (1.0 mg/ cm^2, t = 11.511; 2.5 mg/cm^2, t = 16.705; 5 mg/ cm^2, t = 10.755, $p<0.001$) between treated and control groups.

The results are also comparable with earlier reports of Ansari *et al.* (2005) who observed larvicidal activity of *Pinus longifolia* oil against three vector mosquitoes namely *Ae. aegypti* (LC_{50} – 82.1 ppm), *Cx. quinquefasciatus* (LC_{50} – 85.7 ppm) and *An. stephensi* (LC_{50} – 112.6 ppm). Cavalcanti *et al.* (2004) reported that the larvicidal activity of essential oils of Brazilian plants against *Aedes aegypti* and observed the LC_{50} to range from 60 to 533 ppm. Prajapati *et al.* (2005) reported that the larvicidal activity of different plants essential oil showed varied LC_{95} values against *Cx. quinquefasciatus.* They were *Pimpinella anism* (149 µg/ml), *Zingiber officinalis* (202 µg/ml), *Junipers macropoda* (204 µg/ml), *Cinnamomum zeylanicum* (277 µg/ml), *Curcuma longa* (292 µg/ml), *Cyperus scariosus* (408 µg/ml), *Ocimum basilicum* (315 µg/ml), *Cuminum cyminum* (344 µg/ ml) and *Nigella sativa* (365 µg/ml). Similar results were observed in this present study. Tawastin *et al.* (2001) reported the repellent activity of turmeric, kaffir lime, citronella grass and hairy basil with the addition of 5% vannillin provided protection up to six hours. Fradin & Day (2002) have reported bioinsecticide IR 3535 in the USA tested against laboratory reared *Ae. aegypti* female mosquitoes, and provided protection for an average of 22.9 minutes and also reported that the repellent activity of DEET (N,N-diethyl-3-methylbenzamide) 23.8% ingredient provided protection for an average of 301 min. Although DEET has been claimed to be safe for use against biting insects for over 40 year it may still pose a risk to human health (Osimitz & Grothaus, 1995).

Table 2. Ovicidal activity of *C. citratus* essential oil against *Cx. quinquefasciatus*

Concentration (ppm)	% egg mortality (arc- sin transformed data)	Regression equation
300	100 ± 0.0 (90.00)	
250	88.6 ± 1.3 (70.80)	
200	66.8 ± 0.9 (54.87)	Y = 6.4785 +
150	44.1 ± 1.4 (41.65)	0.2598 X
150	44.1 ± 1.4 (41.65)	
100	22.4 ± 1.6 (28.16)	
Control	4.6 ± 0.4 (12.32)	

Values are mean of six replicates ± SE

Table 3. Repellent activity of *C. citratus* essential oil against *Cx. quinquefasciatus*

Concentration of essential oil (mg/cm^2)	Mean number of bites ± SE		Mean number of 100% protection (hours)	Total % of protection for 12 hours	't' value (df)
	Control	Treated			
1.0	69.50 ± 2.44	35.00 ± 2.49	3.00	49.64	11.511* (5)
2.5	67.00 ± 2.88	25.00 ± 2.21	4.00	62.19	16.705* (5)
5.0	64.66 ± 1.26	17.83 ± 1.79	5.00	74.03	10.755* (5)

Values are mean of six replicates ± SE
*Significant at p <0.001 level.

Therefore, it is necessary to look for and find a better repellent, which could provide a safer and long-lasting protection against arthropod bites. The results of our study showed that essential oils were very effective against mosquito bites. Rajkumar & Jebanesan (2004) studied ovicidal activity of *Moschosma polystachyum* leaf extract against *Cx. quinquefasciatus* and observed 100% egg mortality at 100 ml/l. Insecticidal activity of essential oil, even from the same source, can be inherently variable for many reasons. The chemical composition and broad spectrum of biological activity for essential oils can vary with plant age, the plant tissues, geographical origin of plant, organ used in the distillation process, the type of distillation and the species and age of a targeted pest organism (Chiasson *et al.* 2001). In the present study it was concluded that the essential oil of *C. citratus* exhibited effective larvicidal, ovicidal and repellent properties. Further studies on identification of active compounds for larval control and commercial preparation of repellent products and field trials are needed to recommend the development of ecofriendly chemicals from this plant based oil for mosquito control and protection against the bites of haematophagous insects.

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