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LARVICIDAL ACTIVITY OF CINNAMALDEHYDE AGAINST THE FILARIAL VECTOR *CULEX QUINQUEFASCIATUS*

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ABSTRACT: Mosquitoes are deadly agents being vectors for yellow fever, dengue, chikungunya, Zika virus fever, malaria, filarial fever and Japanese Encephalitis. Conventional pesticides are effective in controlling mosquito larvae but frequent use of these synthetic pesticides has increased serious environmental and health concerns. Alternatively, usage of natural products as eco-friendly agents is appreciated. In the present study, the larvicidal activity of essential oils was screened against the major urban filarial vector, *Culex quinquefasciatus* using different concentrations (50, 100, 200 and 400µg/mL) for 24 hours. The preliminary screening results revealed that cinnamon oil exhibited the highest larvicidal activity by providing cent percent larval mortality at 400µg/mL. Based on GC-MS analysis, cinnamaldehyde a major constituent of cinnamon oil was found to exhibit cent percent larval mortality at 200µg/mL and LC_{50} of 29.24µg/mL after 24 hours of exposure upon further bioassay.

KEYWORDS: *Culex quinquefasciatus*, larvicidal activity, essential oils, cinnamon, cinnamaldehyde.

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1. INTRODUCTION

Vector-borne diseases account for more infectious diseases, causing deaths annually. Among these, mosquitoes represented from the genera *viz*., *Aedes*, *Anopheles* and *Culex* transmit major diseases like yellow fever, dengue, chikungunya, Zika virus fever, malaria, filarial fever and Japanese

Encephalitis [1-3]. *Culex quinquefasciatus* is a pantropical pest and urban vector of *Wuchereria bancrofti* causing lymphatic filariasis [4] and is probably the most abundantly found in towns and cities of the tropical countries [5]. In recent years, use of many synthetic insecticides in mosquito control has been reduced, due to concern for environmental sustainability, harmful effect on human health, their non-biodegradable nature, and increasing insecticide resistance [6]. One of the most effective alternative approaches is to explore the herbal biodiversity as a new weapon against the synthetic insecticides and for sustainable method of vector control. Medicinal and aromatic plants which constitute a major source of natural organic compounds possess antibacterial [7], antifungal [8], antiviral [9], insecticidal [10] and antioxidant properties [11]. Essential oils derived from various medicinal plants exhibits strong mosquito repellent and larvicidal activities [12-17]. Hence, the present study was aimed to evaluate the efficacy of some essential oils against the filarial vector, *Culex quinquefasciatus*.

2. MATERIALS AND METHODS

Plant essential oils

Based on the knowledge and experience of traditional practitioners and literature survey, five essential oils (cinnamon, clove, lemongrass, lime and tulsi) (Table 1) were selected and procured from Government approved trade centers at Chennai, Tamil Nadu, India. The oils were stored in amber coloured bottles at room temperature until further use.

Table 1: Selected plant essential oils used for the study

Common Name	Tamil Vernacular Name	Botanical Name	Family
Cinnamon	Lavangapattai	*Cinnamomum zylanicum*	Lauraceae
Clove	Kirampu	*Syzygium aromaticum*	Myrtaceae
Lemongrass	Karppurappul	*Cymbopogon citratus*	Gramineae
Lime	Narathai	*Citrus aurantifolia*	Rutaceae
Tulsi	Tulsi	*Ocimum sanctum*	Lamiaceae

Mosquito culture

Culex immatures collected at drains in and around Chennai, Tamil Nadu, India were transported to the laboratory in plastic containers and maintained at the Department of Zoology, University of Madras, Guindy Campus, Chennai, Tamil Nadu, India. Immatures on becoming adults were species identified and confirmed and maintained at 27 ±2°C and 70-80% RH with a photoperiod of 14:10 hours light and dark cycles. For continuous maintenance and culture of this vector species, they were reared in a 2 feet cage (2' x 2' x 2') with ovitraps. The egg rafts laid were then transferred to enamel larval trays. Larvae were fed with larval food (dog biscuits and yeast) (3:1) ratio. Larvae on becoming pupae were transferred to another 2 feet cage for adult emergence.

Larvicidal bioassay

For preliminary screening of essential plant oils, larvicidal bioassay was conducted on the third instar larvae of F_1 generation according to the methodology of WHO [18] with minor modifications. Different test concentrations of 50, 100, 200 and 400μg/mL were prepared. The test concentration was prepared by adding 1mL of appropriate dilution of essential oil in Dimethyl sulfoxide (DMSO) and mixed with 249 mL of distilled water. Appropriate volume of DMSO dissolved in distilled water served as the negative control while distilled water alone was used as positive control. Twenty five healthy third instar larvae were released into each glass beaker (500mL) containing the desired test solution. Mortality was recorded after 24 hours of exposure. The larvae were considered dead when they showed no sign of movement when probed by a needle at their respiratory siphon. Further bioassay was carried out against the active major compound isolated with five different concentrations *viz.*, 12.5, 25.0, 50.0, 100.0 and 200.0μg/mL and larval mortality was recorded after 24 hours of exposure. A total of five replicates per trial and a total of three trials for each concentration were carried out.

Statistical analysis

The percent larval mortality was calculated using the formula (A) and corrections for control mortality (5-20%) when necessary was done using formula (B) of Abbott [19]. Statistical analysis of all mortality data of larvicidal activity was subject to probit analysis. Chi-square analysis was performed and the differences were considered as significant at $P \leq 0.05$ level. All statistics was conducted in IBM SPSS Statistics v22 with significance set at 95% confidence [20].

Percent larval mortality (A):

$$\frac{\text{Number of dead larvae}}{\text{Number of larvae introduced}} \text{ X } 100$$

Corrected percentage of control mortality (B):

$$\frac{1 - n \text{ in T after treatment}}{n \text{ in C after treatment}} \text{ X } 100$$

Where, n is the number of larvae, T: treated and C: control.

Gas chromatography mass spectrometry (GC-MS) analysis

Based on the preliminary screening, the potential essential oil was subjected to GC-MS analysis using Shimadzu capillary GC–quadrupole MS system QP 5000 to find out the major constitutes. The relative amount of individual components of the total oil was expressed as the percentage peak area relative to total peak area. Qualitative identification of the different constituents was performed by comparison of their relative retention times and mass spectra with those of authentic reference compounds, or by Retention Indices (RI) and mass spectra.

3. RESULTS AND DISCUSSION

The results for the preliminary screening of essential oils against the larvae of the filarial vector are represented in Figure 1. The results revealed varying degree of activity. The larval mortality increased according to the increasing concentrations of the essential oils tested. Among the five essential oils, cinnamon exhibited highest activity with cent percent mortality followed by tulsi (74.67%), lime (65.33%), lemongrass (56.00%) and clove (42.67%) at the highest concentration. Further, the cinnamon oil exhibiting the highest larvicidal activity was subjected to GC-MS analysis.

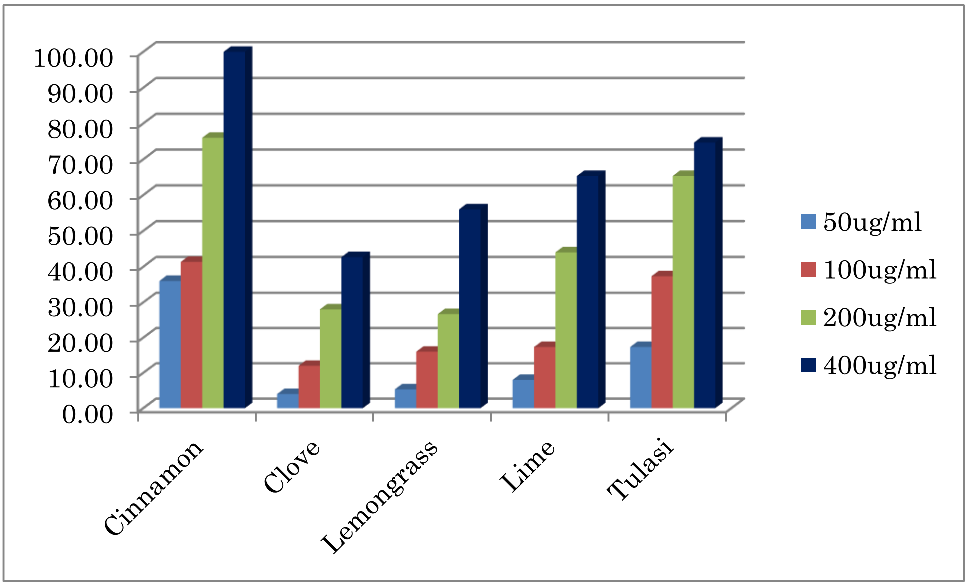

Fig 1: Percent larval mortality of *Culex quinquefasciatus* against essential oils

Initial GC-MS analysis of crude cinnamon oil identified thirty-eight phytochemicals as constituents; of which cinnamaldehyde was the major compound (52.4%) followed by benzaldehyde (12.31%), benzoic acid (8.20%) and benzyl alcohol (2.23%). Remaining chemical compounds were in trace amounts. The GC-MS spectrum of crude cinnamon oil is presented in Figure 2.

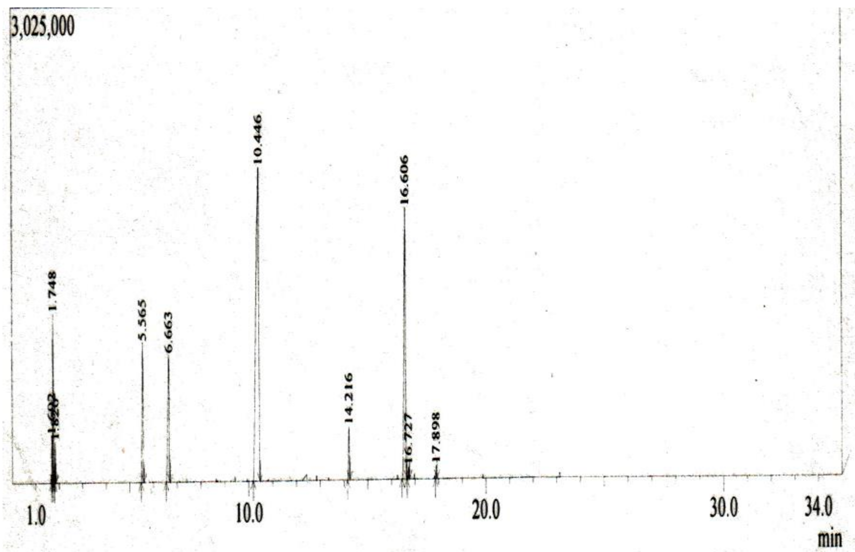

Figure 2: GC-MS spectrum analysis of cinnamon oil

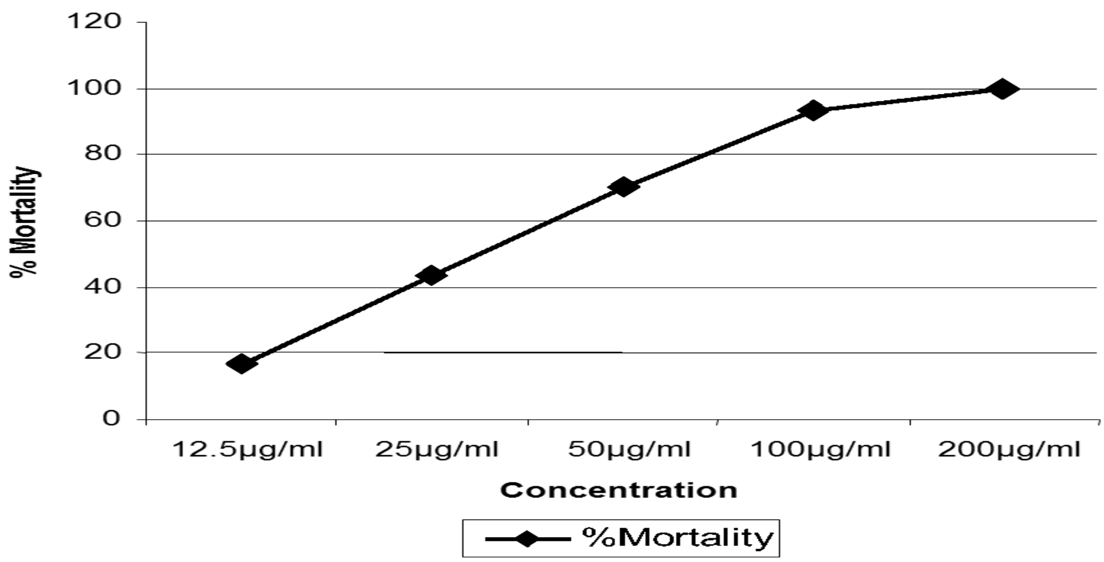

Figure 3: Percent mortality of *Culex quinquefasciatus* larvae by cinnamaldehyde

Table 2: LC_{10}, LC_{50} and LC_{90} values of cinnamaldehyde against *Culex quinquefasciatus*

LC values	Concentration (µg/mL)	95% confidential limit		Heterogeneity X^2
		Lower	Upper	
LC_{10}	10.10	2.86	16.74	7.81*
LC_{50}	29.24	18.07	42.95	
LC_{90}	84.76	54.93	228.99	

*Values are statistically significant at $P \leq 0.05$ level.

Essential oils are reported to have insecticidal properties, *viz.,* ovicidal, larvicidal, growth inhibitory, repellency and antifeedant [21]. With specificity to mosquitoes, larvicidal activity is an effective method to reduce mosquito population before they emerge as adults and botanical insecticides have been widely used for this purpose [22]. The findings of this investigation also indicate the larvicidal properties of the active compound, cinnamaldehyde from cinnamon oil against larvae of *Culex quinquefasciatus*. Cheng *et al.* [23] tested eleven compounds in cinnamon leaf oil against *Aedes aegypti*, of which cinnamaldehyde, cinnamyl acetate, eugenol and anethole exhibited high activity after 24 hours and all the four above mentioned compounds possessed LC_{50} value of less than 50ppm, with cinnamaldehyde showing the strongest activity at an LC_{50} of 29ppm and LC_{90} of 48ppm. Cinnamon oil and its main constituents like cinnamaldehyde and eugenol are grouped as aromatic monoterpenoids (phenylpropanoids) since they are effective insecticides against a variety of mosquito species [23, 24]. The isolated active compound, trans-cinnamaldehyde possess contact and fumigant toxicity against the oak nut weevil, *Mechoris ursulus*, the rice weevil, *Sitophilus oryzae* and the bean weevil, *Callosobruchus chinensis* [25]. Further, cinnamaldehde has been recognized as a very active insecticide for mosquito larva, *Aedes aegypti* and 50% mortality was observed even at a low concentration of 29ppm in 24 hours [23]. Mosquito larvae and pupae breathe through spiracles by coming to the surface of the aquatic habitat frequently and the oil components block the spiracles, resulting in blockage of respiratory siphons (asphyxiation) and death [26]. Rattan [27] studied the mode of action of essential oils on the inhibition of acetylcholinesterase activity, a key enzyme responsible for terminating the nerve impulse transmission through synaptic pathway. The majority of metabolites isolated from plants represent an important tool to replace the synthetic products used today.

4. CONCLUSION

Essential oils from plants may be a potential benefit for mosquito control, since they have a rich source of bioactive compounds that may be biodegradable into nontoxic products and are potentially suitable for use in integrated mosquito control program. It is proved from the present study that plant oils possess larvicidal activity against *Culex quinquefasciatus* and the results have identified additional plant oils as promising larvicides. Moreover, these results could be useful in the search of eco-friendly herbal formulations against the vector mosquitoes.

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CONFLICT OF INTEREST

The authors have no conflict of interest to declare.

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