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Larvicidal Activity of Selected Plant Essential Oil Against Important Vector Mosquitoes: Dengue Vector, *Aedes aegypti* (L.), Malarial Vector, *Anopheles stephensi* (Liston) and Filarial Vector, *Culex quinquefasciatus* (Say) (Diptera: Culicidae)

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Abstract: In this study, three essential oils of plant species (Camphor oil) Cinamomum camphora, (Clove oil) Myrtus caryophyllus and (Eucalyptus oil) Eucalyptus globulus were evaluated for their larvicidal activity of against three vector mosquito larvae Aedes aegypti (L.), Anopheles stephensi (Liston) and Culex quinquefasciatus (Say). Three essential oils were tested at 1000 ppm concentrations at 24 h. Of these, the essential oils of Camphor oil, Clove oil and Eucalyptus oil exhibited relatively high larvicidal effect. However, in this study. The Plant Oil Formulation was tested for its larvicidal activity against 25 numbers of late third instar larvae of Aedes aegypti, Anopheles stephensi and Culex quinquefasciatus. The effect of different concentration of the Plant oil formulation 25, 50, 100, 200 and 400 ppm on the Larvicidal activity against Aedes aegypti LC_{50} 68.18 and LC_{90} 248.37. Larvicidal activity against Anopheles stephensi LC_{50} 56.83 and LC_{90} 208.30. Larvicidal activity against Culex quinquefasciatus LC_{50} 70.80 and LC_{90} 234.15.

Key words: Plant essential oil · *Anopheles stephensi* · *Aedes aegypti* · *Culex quinquefasciatus* · Larvicidal activity

INTRODUCTION

Mosquito-borne diseases are endemic in more than over 100 countries, causing mortality of nearly two million people every year and at least one million children die of such diseases each year, leaving as many as 2100 million people at risk around the world. Mosquitoes constitute a major public health problem as vectors of serious human diseases like malaria, filariasis, japanese encephalitis, dengue fever, chikungunya and yellow fever. Mosquitoes alone transmit disease to more than 700 million people annually [1,2]. In India, seventeen states and six Union Territories were identified to be endemic with about 553 million people exposed to the risk of infection; and of them, about 146 million live in urban and the remaining in rural areas. About 31 million people are estimated to be the carriers of microfilaria and over 23 million suffer from filarial disease manifestations in India [3]. During the past several decades, many synthetic organic insecticides have been developed and effectively used to eliminate mosquitoes. Unfortunately, the management of this disease vector by using synthetic insecticides has failed because the long term harmful effects on non-target organisms and environment [4]. In addition, the continuous and indiscriminate use of conventional chemical insecticides has resulted in the development of physiological resistance [5,6-9]. The use of herbal products is one of the best alternatives for mosquito control. Several experiments have been reported on the larvicidal properties of plant essential oils against *Anopheles* mosquitoes. Essential oils extracted from *Azadirachta indica* [10] and leaves and rhizomes of *Curcuma longa* [8] demonstrated larvicidal activity against *Anopheles gambiae*. Essential oils of *Eucalyptus camaldulensis* [11], *Plectranthus amboinicus* [13], *Zanthoxylum armatum* [14], *Eucalyptus tereticornis* [15] and *Tagetes patula* [16] demonstrated larvicidal activity against *Anopheles stephensi*.

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MATERIAL AND METHODS

Plant Oils: The Plant oils were obtained from the Government recognized (TNGST 030223) Aromatic Oil Stores, TEGRAJ and CO, Chennai-600 003, Tamil Nadu and formulated for the experiment.

Mosquitoes Rearing Technique: The vector mosquitoes taken for the present study as experimental species are *Aedes aegypti* (L.), *Anopheles stephensi* (Liston) and *Culex quinquefasciatus* (Say).

Aedes Aegypti: *Aedes aegypti* colony was maintained at insectary (54 cm x 45 cm x 40 cm) at 27 ± 2°C and 80 ± 2 % Relative humidity with a photoperiod of 12:10 hours light and dark cycles. The egg strips were obtained from Malaria Research Center (MRC) Anna Nagar, Chennai to start the colony. The strips were immersed in dechlorinated tap water for hatching. To obtain the larvae of equal developmental stage, eggs were introduced by adding a stimulant such as ascorbic acid (100 mg/L) to water [17]. This has tended the eclosion process. The emerged larvae were maintained in Petri dishes (10.5 cm diameter) with dechlorinated tap water. Larvae were fed with a diet of yeast and dog biscuits in the ratio of 3: 1. The first instar larvae developed into pupae in about 7-10 days through four stages. The pupae were separated by using a glass dropper into glass Petri dishes and were kept in mosquito net cages (40 cm x 45 cm x 40 cm) for emergence. The newly emerged mosquitoes were provided with 5 % glucose solution soaked in cotton wool, which was placed inside the mosquito net cage for nourishment [18]. After three days of emergence, adults were given a blood meal of pigeon [19]. Glass Petri dishes of 50 mL of tap water lined with filter paper was kept inside the cage for oviposition. The eggs thus obtained were immersed in larval trays containing dechlorinated tap water for hatching.

Anopheles Stephensi: Initially egg strips of *Anopheles stephensi* were obtained from Malaria Research Center (MRC) Anna Nagar, Chennai. They were placed in Petri dishes (10.5 diameter) containing dechlorinated tap water. Larvae were provided with powdered yeast and dog biscuits of 3: 1 ratio. The larval development was completed within 6-8 days; pupae were separated by using glass dropper and were kept inside the mosquito cage for emergence. The cotton soaked in 5 % glucose solution was placed inside the cage for nourishment. After three days of emergence, pigeon blood meal was given to adult mosquitoes. After three days of blood meal, the eggs were laid in the Petri dishes containing tap water.

Culex Quinquefasciatus: The egg rafts of *Culex quinquefasciatus* were obtained from Malaria Research Center (MRC) Anna Nagar, Chennai. The egg rafts were placed in Petri dishes (10.5 diameter) containing aged tap water. Larvae were fed with finely ground mixture of yeast and dog biscuits in 3: 1 ratio. The first instar larvae developed into pupae through four stages in about 8-10 days. The pupae were transferred into mosquito cage for emergence. Blood meal from a pigeon was given to adult mosquitoes after three days of emergence. After 3-4 days of blood feeding for adult mosquitoes, the Petri dishes filled with tap water were placed inside the cage for oviposition. The egg rafts were separated and placed in glass Petri dishes for hatching.

Larvicidal Activity: The Plant Oil Formulation was tested for its larvicidal activity against 25 numbers of late third instar larvae of *Aedes aegypti, Anopheles stephensi* and *Culex quinquefasciatus* by the standard procedure of [20]. The Plant Oil Formulation was volumetrically diluted to 500 mL with dechlorinated water to obtain the test solution of 400, 200, 100, 50 and 25 ppm. Control group larvae were introduced in the container containing only water and DMSO (no plant oil was mixed). For each dose four replicates were maintained. The data were recorded after 24 h of treatment. The LC_{50} was carried out by Probit Analysis [21] and the level of significance was found out by Duncan's Multiple Range Test [22].

RESULT AND DISSCUSSION

Larvicidal Activity Of *Aedes aegypti:* The efficacy of different concentrations of the Plant oil formulation viz. 25,50,100, 200 and 400 ppm on the Larvicidal activity against *Aedes aegypti* was furnished in Table 1, 2.

The results clearly indicated that the highest of 97.20 % larval mortality was observed at 400 ppm concentration of plant oil formulation whereas the lowest mortality of 23.32 % was recorded at the 25 ppm concentration. The larval mortality of 51.75, 68.00 and 84.36 % were observed at 50,100 and 200 ppm concentration respectively. The total mortality of 1.26 % was observed in methanol served as a control. As the concentration of the Plant oil formulation increases the total mortality of *Aedes aegypti*

Table 1: List of plant volatile oils used in the preliminary screening against three different species of mosquitoes

Common Name	Botanical Name	Larvicidal activity (%) of 1000 ppm at 24 hr.
Camphor oil	*Cinamomum camphora*	70
Clove oil	*Myrtus caryophyllus*	100
Eucalyptus oil	*Eucalyptus globulus*	100

Table 2: Larvicidal activity of plant oil formulation against fourth instar larvae of *Aedes aegypti*

Concentration (ppm)	Mortality (%)	LC_{50} (ppm)	LC_{90} (ppm)	Chi-square X^2
25	23.32 ± 1.26	68.18	248.37	18.531
50	51.75 ± 3.46			
100	68. 0 0± 2.84			
200	84.36 ± 6.32			
400	97.2 ± 4.18			
Control	1.26 ± 0.08			

Values represent mean ± S. D of five replications.

Table 3: Larvicidal activity of plant oil formulation against fourth instar larvae of *Anopheles stephensi*

Concentration (ppm)	Mortality (%)	LC_{50} (ppm)	LC_{90} (ppm)	Chi-square X^2
25	26.33 ± 2.46	56.83	208.30	15.703
50	56.48 ± 5.82			
100	70.75 ± 3.35			
200	88.22 ± 6.32			
400	99.00 ± 7.55			
Control	1.26 ± 0.10			

Values represent mean ± S. D of five replications.

Table 4: Larvicidal activity of plant oil formulation against fourth instar larvae of *Culex quinnquefasciatus*

Concentration (ppm)	Mortality (%)	LC_{50} (ppm)	LC_{90} (ppm)	Chi-square X^2
25	22.46 ±1.24	70.80	234.15	16.037
50	52.68 ± 1.82			
100	68.26 ± 2.32			
200	80.75 ± 6.33			
400	99.24 ± 4.26			
Control	1.26 ±0.12			

Values represent mean ± S. D of five replications

was also found to be increased. The LC_{50} and LC_{90} values of the Plant oil formulation 68.18 and 248.37 ppm respectively. The Chi-square value was 18.531 which indicates significant larvicidal activity at 0.05 % level.

Larvicidal Activity Of *Anopheles stephensi:* The consequences of different concentrations of the Plant oil formulation viz. 25,50,100,200 and 400 ppm on the Larvicidal activity against *Aedes aegypti* were depicted in Table 3.

The results revealed that the highest larval mortality of 99.00 % was observed at 400 ppm concentration, whereas the lowest mortality of 26.33 % was noted at 25 ppm concentration. The mortality of 56.48, 70.75 and 88.22% were observed at 50,100 and 200 ppm concentration respectively. In the control, the total mortality of 1.26 % was observed. The results clearly indicated that the larvicidal activity of Plant oil formulation was directly correlated with the concentration of the oil. The 24 h LC_{50} and LC_{90} values of the Plant oil formulation were 56.83 and 208.30 ppm respectively. The Chi-square value was 15.703 and it indicated that the larvicidal activity was significant at 0.05 % level.

Larvicidal Activity Of *Culex quinquefasciatus:* The effect of different concentration of the Plant oil formulation viz. 25,50,100,200 and 400 ppm on the Larvicidal activity against *Culex quinquefasciatus* was presented in Table 4.

The percentage of larval mortality was found to be maximum of 99.24 % at 400 ppm concentration of the plant oil formulation. Whereas, the lowest mortality of 22.46 % was recorded at the 25 ppm concentration. The mortality of 52.68, 68.26 and 80.75 % were observed at 50,100 and 200 ppm concentration respectively. The total mortality of 1.26 % was recorded in methanol, which served as a

control. The increase in the concentration of the Plant oil formulation was found to increase the total mortality of *Culex quinquefasciatus*. The LC_{50} and LC_{90} values of Plant oil formulation 70.80 and 234.15 ppm respectively. The Chi-square value was 16.037 which indicated that the larvicidal activity was significant at 0.05 % level.

Many plant products produce Oviposition, Ovicidal, Larvicidal, Pupicidal and adulticidal effects, most behaving like general toxicants. The differential responses induced by phytochemicals on various species of mosquitoes were influenced by extrinsic and intrinsic factors. A major drawback in the synthetic insecticide application is that they are non-selective and could be harmful to other beneficial organisms, animals and human beings [23]. Further they are not easily biodegradable. But the biopesticides are ecofriendly and do not leave residues in the environment.

As the concentration of the Plant oil formulation increases the total larval mortality of mosquitoes was also found to be increased. In the present study, the results of *Aedes aegypti*, *Anopheles stephensi* and *Culex quinquefasciatus* clearly indicated that the highest larval mortality was observed at 400 ppm concentration of 97.20, 99.00 and 99.24% respectively. Whereas the lowest mortality of 23.32, 26.33 and 22.46 respectively was noted at 25 ppm concentration. Similarly [24] reported that the ethyl acetate extract of *E. prostrata* showed LC_{50} value of 78.28 and LC_{90} value of 360.75ppm against *A. subpictus* and LC_{50} 119.89 and LC_{90} 564.85ppm against *Culex tritaeniorhynchus*. *Eclipta paniculata* were the most active with a LC_{90} of 17.2 mg/L and LC_{50} of 3.3 mg/L against the larvae of *Aedes fluviatilis* [25-26] have reported that the secondary plant metabolite alpha-terthienyl derived from the plant family Asteraceae is among the new class of light activated insecticide. Also, trials under tropical conditions indicate a very high level of activity as a Larvicidal to mosquito. In *N. nucifera* synthesized AgNPs against the larvae of *A. subpictus* (LC_{50} = 0.69 ppm; LC_{90} = 2.15 ppm) and against the larvae of *C. quinquefasciatus* (LC_{50} = 1.10 ppm; LC_{90} = 3.59 ppm), respectively [27].

CONCLUSION

The results of the present investigation proved that the selected plant essential oil can be used effectively to control the larvae of medically important human vector mosquitoes as an important element in Integrated Vector Control Programme. The data obtained in the present investigations are first hand report on mosquito larvae about the oil formulation against mosquito larvae. Further studies will throw more light on the mechanism of essential oil's action/physiological disturbances with mosquito larvae.

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