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Short Communication

Larvicidal activity of essential oil from *Vitex negundo* and *Vitex trifolia* on dengue vector mosquito *Aedes aegypti*

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Abstract

Introduction: The ability of *Vitex trifolia* and *Vitex negundo* essential oils to kill *Aedes aegypti* and *Culex quinquefasciatus* larvae was evaluated. **Methods:** The larvae were treated with their respective essential oils at 50–125 ppm concentration. **Results:** LC_{50} and LC_{90} for *V. trifolia* against *Ae. aegypti* and *C. quinquefasciatus*, and those for *V. negundo* against *Ae. aegypti* were 57.7 ± 0.4, 77.9 ± 0.9 ppm and 55.17 ± 3.14, 78.28 ± 2.23 ppm, and 50.86 ± 0.9, 73.12 ± 1.3 ppm, respectively. Eucalyptol and caryophyllene were the major components in *Vitex trifolia* and *Vitex negundo* essential oil, respectively. **Conclusions:** This study revealed potential larvicidal properties of essential oil from *V. trifolia*.

Keywords: *Aedes aegypti*. *Culex quinquefasciatus*. *Vitex trifolia*. *Vitex negundo*. Essential oil.

Of all arthropods, mosquitoes are the most essential for medical research, because they are vectors for diseases such as malaria, yellow fever, dengue fever, chikungunya fever, filaria, encephalitis, and West Nile virus infection. Mosquito-borne diseases are prevalent in almost all tropical and subtropical countries, as well as many other parts of the world[1]. Although there are several disease vector mosquitoes, only three of them are considered dangerous: *Aedes aegypti*, which transmits the dengue virus, *Culex quinquefasciatus*, which harbors and transmits the West Nile Virus, and finally *Anopheles gambiae*, which transmits deadly malarial parasites[1]. Among the genus *Aedes*, the troublesome species include *Ae. aegypti* and *Ae. Albopictus*, which transmit diseases such as dengue, zika, and chikungunya. In the genus *Culex*, the dominant disease-causing species are *C. quinquefasciatus*, *C. pipiens,* and *C. tritaeniorynchus*, which transmit diseases such as filaria, avian malaria, Japanese encephalitis, and West Nile virus. Since no vaccines or effective drugs have been developed, to combat dengue or other mosquito-borne viral diseases to date, mosquito control remains the primary strategy for controlling mosquito-borne diseases. Control measures rely mainly on chemical insecticides that target mosquitoes. Although synthetic repellents and larvicides are present in the market, they fail to produce significant protection against mosquitoes; synthetic repellents also have side effects**.** Overuse of synthetic insecticides results in increased resistance among mosquito species[1]. One of the most effective alternative approaches to finding a safer, sustainable, and straightforward insecticide for mosquito control, is to explore the floral biodiversity.

Furthermore, the chance of mosquitoes developing resistance towards plant metabolites is very little. Plant-derived insecticides comprise of a cocktail of compounds that may have a synergistic action on killing mosquitoes, unlike conventional pesticides that are based on a single active ingredient[2]. In this context, the present study reports the mosquito larvicidal properties of the essential oils extracted from the leaves of *Vitex trifolia* (*V. trifolia*) and *Vitex negundo* (*V. negundo*), which are shrubs belonging to the family Lamiaceae.

Plant materials used in the study were collected from the herbal garden of SASTRA Deemed University, Thanjavur, Tamil Nadu 613401, India. The collected plants were identified and authenticated with the help of Flora of Tamil Nadu Carnatic Herbarium available in the Rapinet Herbarium, St. Joseph's College[3], Trichy; the specimen numbers are RHT 7317 and RHT

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493 for *V. trifolia*, and *V. negundo*, respectively. The leaves were weighed and collected, and essential oils were extracted by steam distillation using a Clevenger apparatus. The oils were collected in glass vials after 4–5 h of distillation (**Figure 1**). A pinch of anhydrous sodium sulphate was added to remove any residual moisture in the oil and was then stored for one week at −4 °C in airtight vials to avoid evaporation until further trials. A total of 273 g of leaves from both the plants yielded 5% extracted oils. The essential oil was analyzed for its chemical composition using gas chromatography-mass spectrometry (GC-MS). The retention index (RI) of the gas chromatography peaks was compared using a homologous series of n-alkanes (C8–C20) for identifying components of the essential oil. The mass spectra obtained were compared with standard spectra and the NIST 2005 MS library.

The *Aedes* and *Culex* egg rafts were collected from different breeding grounds and area of stagnant water puddles in Thanjavur, Tamil Nadu, India. They were fed with a ratio of brewer's yeast to dog's biscuit of 1:3. After 2–3 days, the eggs hatched and the early stage larvae (3^{rd} instar) were taken for the study. Larvicidal activities of the essential oils were carried out as per the standard protocol of the World Health Organization[4]. A sufficient amount of target oil was stabilized, using 100% acetone, to produce a stock solution. The bioassay was carried out for 20 of the 3^{rd} instar larvae of *Ae. aegypti* and *C. quinquefasciatus*, which were taken in disposable cups (250 mL) containing 100 mL of tap water. From the prepared stock of the oil, different concentrations, ranging from 50–125 ppm, were added to the beakers. The larvicidal activity of *V. trifolia* on *Ae. aegypti* and *C. quinquefasciatus*, and that of

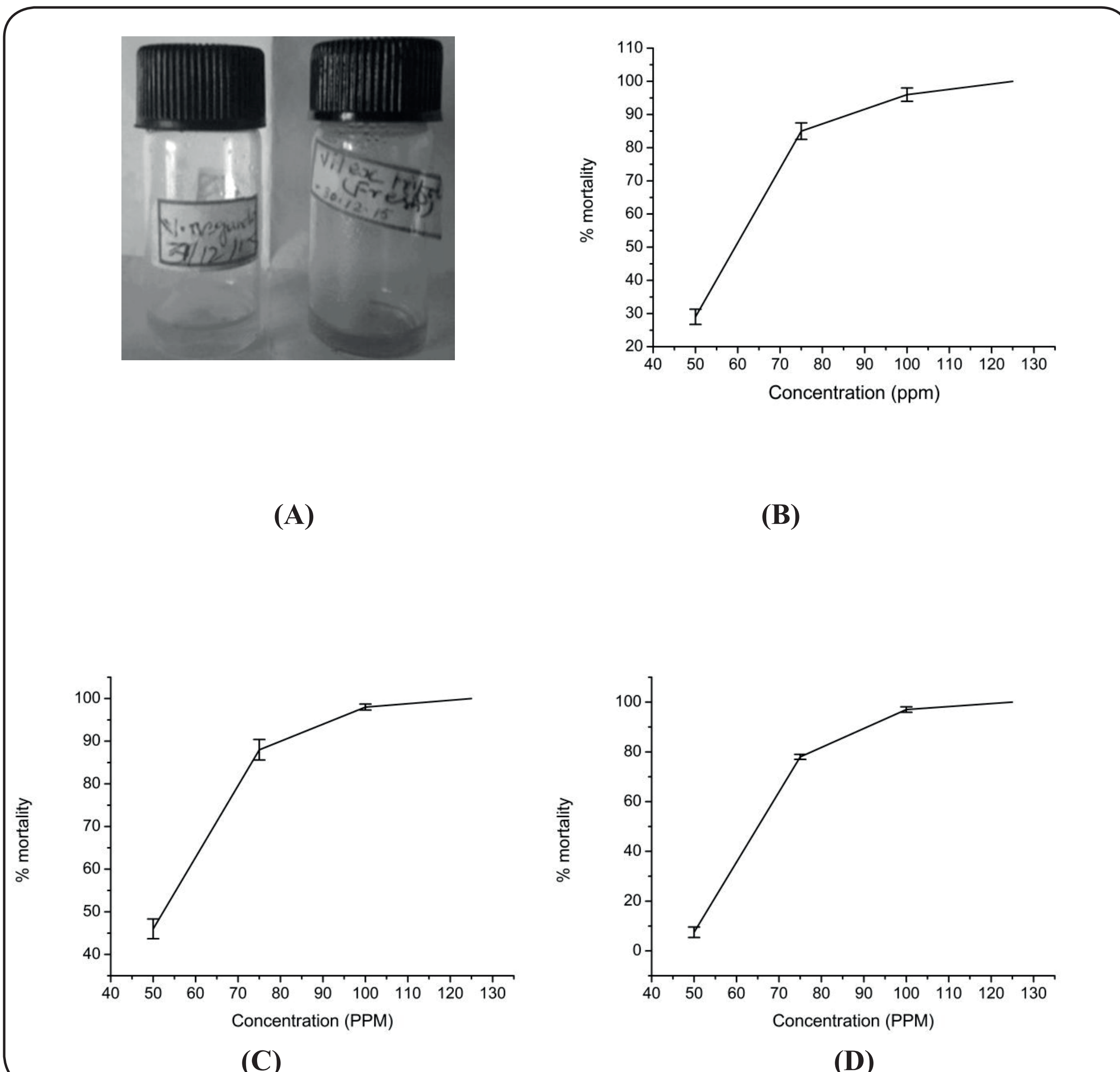

FIGURE 1: (A) Extracted essential oils of *V. negundo* and *V. trifolia*. **(B)** Effect of *V. trifolia* essential oil on 3^{rd} instar larvae of *Aedes aegypti* at concentrations of 50, 75, 100, and 125 ppm. **(C)** Effect of *V. negundo* essential oil on 3^{rd} instar larvae of *Aedes aegypti* at concentrations of 50, 75, 100, and 125 ppm. **(D)** Effect of *V. trifolia* essential oil on 3rd instar larvae of *Culex quinquefasciatus* at concentrations of 50, 75, 100, and 125 ppm.

V. negundo on *Ae. aegypti* were studied. For each experiment, a set of controls, using acetone and untreated larvae in tap water, were also maintained for comparison. The mortality of the larvae were observed over 24 hours post-treatment. Both dead and moribund larvae were classified as non-existent. Each trial was performed in duplicates. The mortality was observed and documented. The lethal concentrations, of 50% (LC_{50}) and 90% (LC_{90}), were calculated as the concentration (ppm) required to kill 50% and 90% of the larvae population respectively.

The mortality data were analyzed statistically[5]. From the regression line, between logarithmic dose and mortality, $LC_{50,}$ and LC_{90} were determined at the 95% confidence interval. The regression line was plotted using Microsoft Excel.

GC-MS analysis of *V. trifolia* showed a total of 89 components (**Figure 2A**). The major component was identified as eucalyptol, a known insect repellent. Sabinen and caryophyllene were identified as the other main components. Regarding *V. negundo*, a total of 60 compounds were found (**Figure 2B**). The principal component of which was found to be caryophyllene, with a peak area of 27.984% and a retention time of 16.48 min.

The bioassay results revealed a significant larvicidal activity ($P<0.05$) of the oils against *Ae. aegypti* and *C. quinquefasciatus* larvae (**Table 1**). Percentage mortalities after the addition of essential oil is shown in **Figures 1A, 1B,** and **1C**. From the figures, it is clear that *Culex quinquefasciatus* is comparatively more susceptible than *Ae. aegypti* to the larvicidal activity of *V. trifolia*. The LC_{50} and LC_{90} values were calculated using probit analysis. The bioassay of the third instar stage of *Aedes* species showed an LC_{50} value of 57.7 ± 0.4 ppm and an LC_{90} value of 77.9 ± 0.9 ppm of oils from *V. trifolia*. The LC_{50} and LC_{90} values of *V. trifolia* on *C. quinquefasciatus* were 55.17 ± 3.14 and 78.28 ± 2.23 ppm respectively. The LC_{50} and LC_{90} values of *V. negundo* on *Ae. aegypti* was 50.86 ± 0.9 and 73.12 ± 1.3 ppm respectively. A concentration of 125 ppm *V. trifolia* oil was required to kill 20 *Ae. aegypti* and *C. quinquefasciatus* larvae.

Similarly, when the susceptibility of *Ae. aegypti* to *V. negundo* was evaluated, 125 ppm concentration was required to kill the larvae. From these trials, it was found out that both *V. negundo* and *V. trifolia* had significant larvicidal activity against *Ae. aegypti* and *C. quinquefasciatus*.

Vitex species are commonly seen on the banks of water bodies like channels, rivers, and ponds. Many plants of this genus were reported to have high therapeutic values[6]. Their dried leaves were said to be burnt to deter mosquitoes. Methanol extracts of the leaves from *Vitex altissima*, *Vitex negundo*, *Vitex trifolia,* and *Vitex peduncularis* were reported to possess potential mosquito larvicidal property against the larvae of *C. quinquefasciatus*. Among all the species of *Vitex*, *V. trifolia* possess the highest ability to control mosquito larvae[7]. Another study by the group analyzed the larvicidal activity of fatty acid methyl esters obtained from the leaf extract of *Vitex altissima*, *Vitex negundo* and *Vitex trifolia*. The study found the highest toxicity effect in the extract of *V. trifolia* against *C. quinquefasciatus* larvae (LC_{50}=9.26 and LC_{90}=21.28 ppm)[8]. In the same study, methyl-p-hydroxybenzoate was separated from the plant extract and studied for its toxic effects on the 4th instar larvae of *C. quinquefasciatus* and *Ae. aegypti*. The compound exhibited a very high killing activity against mosquito larvae of both species at low concentration[9]. However, no studies have explored the mosquito larvicidal properties of the essential oil of the leaves of *V. trifolia*, except a study which reported the oviposition deterrent activity of the oil against adult mosquitoes[10]. Since mosquito larvae and pupa stages can be targeted effectively for mass control measures, unlike adult mosquitoes[11], the present study assessed the efficacy of the essential oils of *V. trifolia* and *V. negundo* against the larvae of *C. quinquefasciatus* and *Ae. aegypti*.

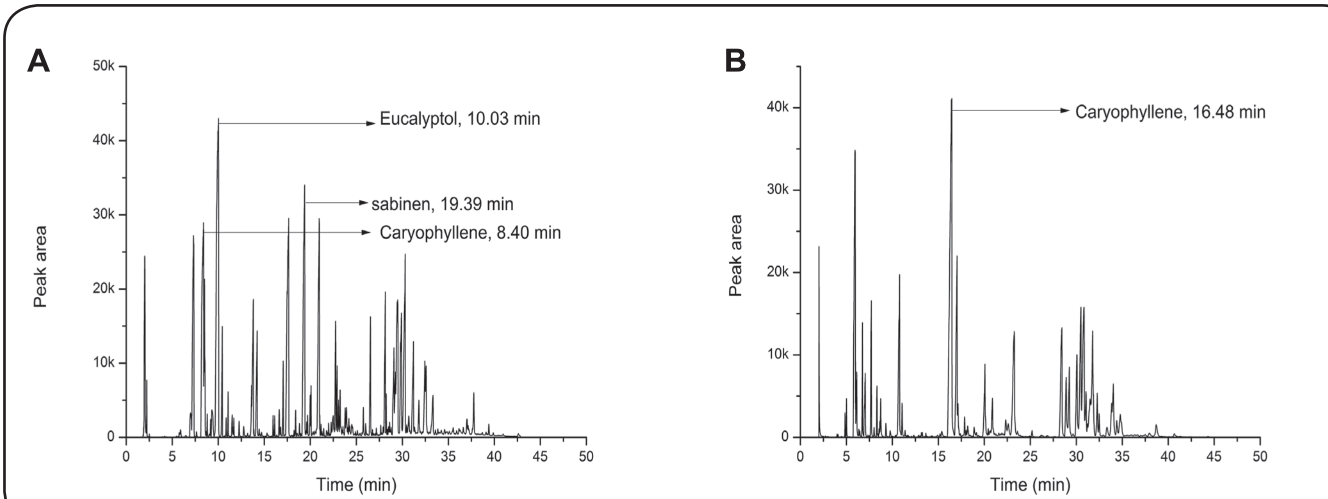

FIGURE 2: (A) GC-MS analysis of Vitex trifolia. In total, 89 components were recorded. The major components were eucalyptol, sabinen, and caryophyllene with respective peak areas of 16.35%, 9.44%, and 8.91%. The retention times were 10.03, 19.39, and 8.40 min. The percentage peak areas were calculated using Turbomass software, version 5.2.0. **(B)** GC-MS analysis for *Vitex negundo*. A total of 60 compounds were found. The primary component was found to be caryophyllene, with a peak area of 27.984% and a retention time of 16.48 min.

TABLE 1: The average mortality (±SE), and LC_{50} and LC_{90} of *Aedes aegypti* and *Culex quinquefasciatus* larvae in the presence of *Vitex trifolia* and *Vitex negundo* leaves essential oil *in vitro*.

Larval stage	Larva species	Essential oil	Percentage mortality at each concentration (PPM) 50	75	100	125	Equation	R^2	LC_{50} (ppm)	LC_{90} (ppm)
3rd instar	*Aedes aegypti*	*Vitex trifolia*	29±2.3	85±2.5	96±2.0	100±0.0	Y= 76.22ln(X) - 259.1	0.873	57.77±0.4	77.91±0.9
3rd instar	*Aedes aegypti*	*Vitex negundo*	46±2.3	88±2.4	98±2.7	100±0.0	Y= 59.08ln(X) - 178.4	0.851	50.86±0.9	73.12±1.3
3rd instar	*Culex quinquefasciatus*	*Vitex trifolia*	7.5±2.1	78±1	97±1.1	100±0.0	Y = 66.54ln(X) - 215.0	0.937	55.17±3.14	78.28±2.23

*p-value <0.05, statistically significant difference in death rate for the concentrations tested using a chi square test.

From the results of the present study (**Table 1**), it is evident that the essential oil of *V. trifolia* and *V. negundo* demonstrated significant larvicidal activity against the third instar stages of *Ae. aegypti*. This study also showed a significant larvicidal action of *Vitex trifolia* against *C. quinquefasciatus* larvae. However, the activity of *V. negundo* on *C. quinquefasciatus* was not carried out because of the lack of sufficient *Culex* eggs during the study period. The percentage of larval mortality increased with increasing concentrations of the oil. Among the oils studied, *V. negundo* showed improved efficacy towards *Ae. aegypti* larvae; The LC_{50} and LC_{90} for this oil on these larvae were 50.86$\pm$0.9 and 73.12$\pm$0.3 ppm respectively. As per the classification established by Cheng et al. (2003), compounds with LC_{50}> 100 mg/mL were considered not active, those with LC_{50}< 100 mg/mL were active, and those with LC_{50}< 50 mg/mL were highly active[12]. Therefore, based on the LC_{50} calculated in the present study, the essential oil of *V. trifolia* and *V. negundo* is considered to be active in controlling mosquito larvae.

The essential oil contains a blend of chemicals that can facilitate an increase or decrease in larvicidal activity, compared with the actions of their isolated constituents[13]. In most cases, the mosquito larvicidal activity of the blend is higher than those of its purified constituent compounds[14,15]. However, the individual compound or synergistic action of the studied oil is not known. A previous study has documented the presence of eucalyptol, a major compound in the essential oil of the leaves of *V. trifolia*. The GC-MS analysis of the purified oil carried out in the present study also confirmed these earlier results. The resultant spectra showed a major peak that corresponded to the eucalyptol compound, which occupies a total of 16.35% of all other compounds present in the oil (**Figure 2A**). Eucalyptol is reported to be present in the essential oil of other plants that exhibited larvicidal activity against mosquitoes[16]. Therefore, eucalyptol may contribute to the high larvicidal activity of this studied oil. However, the exact mechanisms of the oil's larvicidal property needs further study into a more extensive range of mosquito species.

In conclusion, we found from this study that the essential oil of *V. trifolia* possesses potent larvicidal activity. The oil showed 100% mortality against third instar stages of *Ae. aegypti* and *C. quinquefasicatus* larvae at a lower concentration of 125 ppm. The oil could be used separately or in combination with existing larvicides, creating new effective eco-friendly and affordable approaches for effective control of disease-transmitting vector mosquitoes.

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Conflict of interest

The authors declare that they have no conflict of interests.

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