Utilizing larvicidal and pupicidal efficacy of *Eucalyptus* and neem oil against *Aedes* mosquito: An approach for mosquito control

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**Background and Objectives:** Plant-based products can provide safe and biodegradable mosquito control agents. The essential oils have a strong odor due to complex secondary metabolites and exhibit lower density than that of water, which renders them suitable to form a thin layer above the water surface. The present study was designed to evaluate the larvicidal, pupicidal activity of *Eucalyptus* and neem oils against *Aedes aegypti* and *Aedes albopictus*.

**Materials and Methods:** We evaluated the activity of commercially available *Eucalyptus* (*Eucalyptus globulus*) and neem (*Azadirachta indica*) oils against larvae and pupae of *A. aegypti* and *A. albopictus* for their larvicidal and pupicidal activity, stability in different water types, dependence on volume and surface area of the water body, and residual efficacy.

**Results:** *Eucalyptus* oil was found to be more effective against larvae and pupae at lower concentrations, i.e., concentration at which 50% is observed (LC50) for larvae and pupae was 93.3 and 144.5 parts per million (ppm) and concentration at which 90% is observed (LC90) was 707.9 and 741.3 ppm, respectively, while for neem oil, LC50 for larvae and pupae was 7852 and 19,054 ppm and LC90 was 10,092 and 19,952 ppm, respectively. The efficacy of *Eucalyptus* oil depended on surface area rather than volume of water, and the residual efficacy of *Eucalyptus* oil was up to 8 days.

**Conclusions:** *Eucalyptus* oil was more effective against mosquito larvae at lower concentration as compared to neem oil. It can, therefore, be utilized in the community in artificial and small temporary water bodies as an eco-friendly vector control measure in the era of increasing resistance to chemical insecticides.

**Keywords:** *Aedes mosquitoes*, *Eucalyptus* oil, larvicidal, mosquito control, neem oil, pupicidal

**INTRODUCTION**

The mosquito-borne diseases account for >700 million cases annually worldwide and are responsible for >1 death for every 17 persons infected. Around 2.5 billion people are estimated to be at risk of dengue, the most rapidly spreading mosquito-borne disease. The disease incidence of dengue has increased 30-fold in the last 50 years.[1,2] There have been recurrent outbreaks of dengue fever in...
India associated with rapid increase and spread of *Aedes aegypti*, particularly in major towns and cities.[4,6]

The risk of arthropod-borne illnesses is increasing due to climate change and intensifying globalization, tropical regions of the globe being especially more vulnerable.[8] In recent years, increasing insecticide resistance, cross-resistance, toxicity hazards associated with synthetic insecticides, and their rising price have set back the mosquito control programs, which thereby prompts the interest in the use of plant-based products.[7,8] Nature has provided plants with a repertoire of mosquitocidal elements in the extracts from leaves, flowers, and roots of plants and oils.[9,10] The essential oils have a strong odor due to complex secondary metabolites and exhibit lower density than that of water, which renders them suitable to form a thin layer above the water surface.[11,12] These oils interfere with basic behavioral, physiological, biochemical, and metabolic functions of insects.[13] Therefore, essential oils from various plants such as *Litsa salicifolia*, *Ocimum suave*, *Azadirachta indica*, i.e., neem, *Eucalyptus camaldulensis*, and *Curcuma longa*, among others, have been reported to exhibit an effective larvicultural activity against several mosquito species.[14-18] The present study was designed to evaluate the larvicultural, pupicidal activity of *Eucalyptus* and neem oils against *A. aegypti* and *Aedes albopictus*.

**MATERIALS AND METHODS**

Mosquito larvae collection, identification, and maintenance

Mosquito larvae were collected from artificial containers, ditches, pots, and water coolers from different rural and urban areas of Chandigarh, and *Aedes* larvae were identified following morphological keys.[19] Late third and early fourth instar larvae were sifted out for further testing. The larvae were supplemented with nutrition by a mixture of yeast powder and dog biscuit in 1:3 ratio. One-fifth of the collected larvae from each batch were reared up to the adult stage to confirm the species (*A. aegypti* and *A. albopictus*).

Plant oils

The commercial preparations of oils of *Eucalyptus globulus* (Agrawal Pharmaceuticals, New Delhi, India) and neem, i.e., *A. indica* (Brahmastra Ayurvedic Products, Lucknow, Uttar Pradesh, India), were used for the experiments.

Larvicidal and pupicidal bioassays

The larvicultural activity of *Eucalyptus* and neem oil formulations was tested against the late third and early fourth instar larvae (*n* = 20) and pupae (*n* = 20) of *A. aegypti* and *A. albopictus* in 500 mL glass beakers, each containing 200 mL distilled water in the following concentrations: 10 μL (100 parts per million [ppm]), 20 μL (200 ppm), 40 μL (400 ppm), 80 μL (800 ppm), and 100 μL (1000 ppm). In the control group, larvae (*n* = 20) and pupae (*n* = 20) were introduced in the beaker containing only water and dimethyl sulfoxide (no plant oil was used). The larvae were observed at 4, 8, 12, and 24 h and lethal concentrations at which there was 50% (LC50) and 90% (LC90) mortality were calculated. The experiments were carried out in four replicates for each testing concentration of oil formulations. The efficacy and stability of oil formulations were evaluated by testing the larvicultural and pupicidal activity of *Eucalyptus* and neem oils in different water conditions such as tap water and breeding habitat water. For this, the concentration of *Eucalyptus* and neem oils which showed 100% mortality in distilled water was tested on late third instar and early fourth instar larvae and pupae in natural breeding habitat water (*n* = 20 each) and tap water (*n* = 20 each). The experiments were carried out in four replicates.

**Effect of volume versus surface area**

Larvicidal (*n* = 20 each) and pupicidal (*n* = 20 each) experiments with the efficacious concentration of *Eucalyptus* oil as derived from the above experiments were carried out keeping the water volume and surface area constant and changing the alternate parameter. For a constant surface area of 15 inch², different volumes of water, i.e., 100, 200, 400, 800, and 1000 mL, were tested, and for a constant volume of 200 mL, different surface areas were tested, i.e., 4.37, 7.74, 12.19, 17.49, 27.34, and 48.77 inch² were tested. The experiments were carried out in four replicates.

Larvicidal and pupicidal assays in simulated field conditions

*Aedes* larvae predominantly breed in artificial water bodies in a domestic environment; hence, the efficacy of *Eucalyptus* oil was tested in water coolers (30 inch × 30 inch, surface area 900 inch²). For this, the water coolers were filled with 15 L of tap water, to which 6 mL of *Eucalyptus* oil and 80 larvae and 40 pupae were added, and mortality was recorded after 4, 8, 12, and 24 h. The experiments were carried out in four replicates.

**Residual efficacy of *Eucalyptus* oil**

The residual effect of *Eucalyptus* oil formulation showing 100% mortality was carried out against the late third instar and early fourth instar larva up to 240 h (10 days). On the first day, 10 beakers were filled with 200 mL tap water each and the test oil was applied in all the beakers. On each of the subsequent 10 days, 20 freshly collected larvae were introduced in the beakers successively, one beaker each day.
The mortality was calculated after 4, 8, 12, and 24 h. The experiments were carried out in four replicates.

**Statistical analysis**
All experiments were done in four replicates, and the mean values were considered for analysis. The mortality rates of the larvae and pupae in both Eucalyptus and neem oil formulations were corrected using the standard Abbott’s formula in accordance with the results obtained from the negative control. Median LC50 and LC90 were derived using log-probit analysis. Karl Pearson’s correlation coefficient was used to study the association between the radius/surface area of the container and the number of dead larvae/pupae.

**RESULTS**

**Larvicidal and pupicidal bioassays**
*A. aegypti* and *A. albopictus* species were tested for larvicidal and pupicidal action of Eucalyptus and neem oils. The LC50 of Eucalyptus oil for larvae and pupae was found to be 93.3 and 144.5 ppm and LC90 was found to be 707.9 and 741.3 ppm, respectively. For neem oil, there was negligible mortality for the maximum concentrations tested in this set of experiment, i.e., concentration up to 1000 ppm.

A further set of experiments with higher concentrations of neem oil (5000, 10,000, 15,000, and 20,000 ppm) was thus carried out, and the LC50 for larvae and pupae was found to be 7852 and 19,054 ppm and LC90 was found to be 10,092 and 19,952 ppm, respectively. The data of larvicidal and pupicidal activity of neem and Eucalyptus oil against *Aedes* larvae are presented in Table 1.

As Eucalyptus oil was found to be more effective at low concentrations as compared to neem oil, therefore, further characterization was done only for Eucalyptus oil at the concentration which resulted in 100% mortality of larvae and pupae, i.e., 1000 ppm. In different water sources, i.e., tap water and breeding habitat water, 100% mortality was observed with 1000 ppm of Eucalyptus oil.

**Effect of volume versus surface area**
It was observed that Eucalyptus oil formed a clear and even layer over the water surface while neem oil formed droplets or micelles [Figure 1a and b]. Hence, we further tested the effect of water volume and surface area on the efficacy of Eucalyptus oil.

On applying 1000 ppm of Eucalyptus oil in different volumes of water ranging from 100 to 1000 mL, 100% mortality was observed in all the water volumes tested.

### Table 1: Larvicidal and pupicidal activity of eucalyptus and neem oil formulations against larvae of *Aedes* spp.

| Oil formulation | Concentration (ppm) | Nor of larva/pupae dead* | Mortality larva (%) | LC50* (ppm) | LC90* (ppm) |
|-----------------|---------------------|--------------------------|---------------------|-------------|-------------|
| **Larvae**      |                     |                          |                     |             |             |
| Eucalyptus oil  | 100                 | 10.00±0.5                | 50                  | 93.32       | 707.94      |
|                 | 200                 | 15.00±0.81               | 75.00               |             |             |
|                 | 400                 | 16.75±0.5                | 83.75               |             |             |
|                 | 600                 | 17.50±0.5                | 87.50               |             |             |
|                 | 800                 | 18.25±0.5                | 91.25               |             |             |
|                 | 1000                | 20.00±0.0                | 100                 |             |             |
| Neem oil        | 100                 | 0.0±0.0                  | 0                   |             |             |
|                 | 200                 | 0.25±0.5                 | 1.25                |             |             |
|                 | 400                 | 0.25±0.5                 | 1.25                |             |             |
|                 | 600                 | 0.50±0.5                 | 2.50                |             |             |
|                 | 800                 | 0.50±0.5                 | 2.50                |             |             |
|                 | 1000                | 0.75±0.5                 | 3.75                |             |             |
| Higher concentration | 5000             | 5.50±0.5                | 27.5                 | 7852       | 19054      |
|                 | 10,000              | 11.50±0.5               | 57.5                 |             |             |
|                 | 15,000              | 16.50±0.5               | 82.5                 |             |             |
|                 | 20,000              | 20.00±0.0               | 100                  |             |             |
| **Pupae**       |                     |                          |                     |             |             |
| Eucalyptus oil  | 100                 | 9.25±0.5                 | 46.25               | 144.5       | 741.3       |
|                 | 200                 | 12.00±1.41              | 60.00               |             |             |
|                 | 400                 | 14.75±0.5               | 73.75               |             |             |
|                 | 600                 | 16.00±0.0               | 80.00               |             |             |
|                 | 800                 | 18.50±1.0               | 92.50               |             |             |
|                 | 1000                | 20.00±0.0               | 100                 |             |             |
| Neem oil        | 100                 | 0.0±0.0                 | 0                   |             |             |
|                 | 200                 | 0.0±0.0                 | 0                   |             |             |
|                 | 400                 | 0.0±0.0                 | 0                   |             |             |
|                 | 600                 | 0.0±0.0                 | 0                   |             |             |
|                 | 800                 | 0.25±0.5                | 1.25                |             |             |
|                 | 1000                | 0.50±0.5                | 2.50                |             |             |
| Higher concentration | 5000             | 4.50±0.5                | 22.5                 | 10,092     | 19,952     |
|                 | 10,000              | 9.50±0.5                | 47.5                 |             |             |
|                 | 15,000              | 15.50±0.5               | 77.5                 |             |             |
|                 | 20,000              | 20.00±0.0               | 100                  |             |             |

*Values represent mean±SD of four replications; *LC50 and LC90 were calculated using log-probit analysis. SD: Standard deviation
mortality was observed irrespective of the volume, whereas when surface area was changed keeping the volume constant, a decrease in mortality was observed with surfaces areas >15 inch² with 1000 ppm Eucalyptus oil [Table 2]. The dose of Eucalyptus oil to be applied was found to be 66.67 ppm per sq. inch or 6.67 μL/sq. inch. There was a strong negative correlation between radius/surface area of container and number of larvae/pupae dead. As the radius or surface area of container was increased, the number of dead larvae or pupae decreased significantly [Table 3]. Therefore, based on the above findings, it was clear that the efficacy of Eucalyptus oil will not vary depending on the water body, rather it will depend upon the surface area.

**Larvicidal and pupicidal efficacy in simulated field conditions**

The efficacy of Eucalyptus oil in water cooler having surface area of 900 inch² was tested using 60,030 ppm (6.67 × 900 = 6003 μL, approximately 6 mL). At 4 h, 100% mortality of larvae and pupae was observed.

**Residual efficacy of Eucalyptus oil**

On observing up to 240 h, there was 100% mortality up to 192 h, i.e., 8 days. Thereafter, continuous decline in mortality of larvae was recorded. On day 10, i.e., 240 h, none of the larvae introduced in the beaker were killed [Figure 2].

**DISCUSSION**

In the era of increasing insecticide resistance to synthetic chemical pesticides, the present work evaluated the efficacy of environmentally safe options Eucalyptus oil and neem oil to control larvae and pupae of the dengue vector A. aegypti, Meigen. A. aegypti is the primary vector of dengue found throughout the tropical countries and an extremely

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**Table 2: Effect of 1000 ppm eucalyptus oil at different volumes and surface areas of water**

| Volume of water (ml) | Radius/surface area of the container (inch/inch²) | Number dead (n=20)* |
|----------------------|-----------------------------------------------|---------------------|
|                      |                                               | Larva | Pupae |
| 100                  | 15                                            | 20    | 20    |
| 200                  | 15                                            | 20    | 20    |
| 400                  | 15                                            | 20    | 20    |
| 800                  | 15                                            | 20    | 20    |
| 1000                 | 15                                            | 20    | 20    |

| Radius/surface area of the container (inch/inch²) | Volume of water (ml) | Number dead (n=20)* |
|--------------------------------------------------|---------------------|---------------------|
| 1.18/4.37                                        | 200                 | 20                  |
| 1.57/7.74                                        | 200                 | 20                  |
| 1.97/12.19                                       | 200                 | 20                  |
| 2.36/17.49                                       | 200                 | 19.5                |
| 2.95/27.34                                       | 200                 | 19.5                |
| 3.94/48.77                                       | 200                 | 18                  |

*Values represent mean±SD of four replications. SD: Standard deviation

**Table 3: Karl Pearson’s correlation coefficient between radius/surface area and number of larvae/pupae dead**

| Surface area | Pearson’s correlation coefficient | P    | Radius | Pearson’s correlation coefficient | P   |
|--------------|---------------------------------|------|--------|----------------------------------|-----|
| Number larvae dead | -0.946*                         | 0.0043 | Number pupae dead | -0.882*                         | 0.020 |
| Number pupae dead   | -0.961*                         | 0.002  |

*P<0.05
successful species in its capability of adapting to varying climatic conditions.\textsuperscript{[20–23]} The most common and practical anti-larval method to control the mosquito breeding involves temephos, an organophosphate compound, which is being used under the public health programs since the 1980s in potable water; however, at present, the development of resistance to temephos in countries such as Brazil and Thailand poses an alert.\textsuperscript{[23,24]} Until recently, the resistance of \textit{A. aegypti} to temephos was not reported from India; however, recent reports from Andaman and Nicobar Islands, Assam, and Tamil Nadu signal the alarm for India too.\textsuperscript{[25,26]} Mineral oils are also recommended for their larvicidal action; however, they are hazardous to the aquatic fauna present in small water bodies. Therefore, natural plant products may have the advantage of being eco-friendly and were the focus of the present work.

Of the two plant oils tested, \textit{Eucalyptus} oil was found to be a better larvicidal and pupicidal as compared to neem oil at low concentrations. To achieve similar efficacy, more than 20 times higher concentration of neem oil was required. Neem oil was also observed to have an unpleasant pungent odor which enhanced on increasing the dose. Pugazhvendan and Elumali tested the efficacy of three essential oils of plant species \textit{Cinnamomum camphora} (camphor oil), \textit{Myrtus caryophyllus} (clove oil), and \textit{E. globulus} (\textit{Eucalyptus} oil) at 1000 ppm concentrations for their larvicidal activity against larvae of \textit{A. aegypti} (L.), \textit{Culex quinquefasciatus} (Say), and \textit{Anopheles stephensi} (Liston) and found them to exhibit relatively high larvicidal effect. The LC50 and LC90 against \textit{Aedes} were found to be 68.18 and 248.37 ppm, respectively.\textsuperscript{[27]} Another study found \textit{Eucalyptus} oil LC50 and LC90 to be 64 and 80 ppm.\textsuperscript{[28]} Similarly, Medhi \textit{et al.} found 100\% mortality of \textit{A. stephensi} larvae using 160 ppm of \textit{Eucalyptus} oil.\textsuperscript{[29]} This variation in LC50 and LC90 values across studies is interesting to note and may be attributed to the different oil extraction techniques or formulations used by different manufacturers. Neem oil has also been used in earlier studies to evaluate larvicidal activity. In a study, the aqueous extract of the neem showed 87\% mortality at 18\% concentration. None of the studies compared the efficacy of \textit{Eucalyptus} with neem oil.\textsuperscript{[30]}

The larvicidal action of oils is by virtue of the monomolecular film formation on the water surface which reduces the surface tension of the aqueous surface and kills the larvae by interference with the spiracular opening and prevention of tracheal respiration. We found that the efficacy of \textit{Eucalyptus} oil remained same in different volumes of water bodies with same surface area due to its ability to form a uniform layer on the surface of the water, irrespective of the volume. This ability is dependent on the physicochemical properties such as specific gravity, surface tension, and viscosity which determine the nature of the oils to spread horizontally into a smooth and slippery surface. Both \textit{Eucalyptus} oil and neem oil have a specific gravity lower than one which enables them to float over the water surface, with \textit{Eucalyptus} oil having a lower specific gravity (0.87–0.91) as compared to neem oil (0.908–0.934). \textit{Eucalyptus} oil also spreads evenly as it exhibits a very low contact angle as well as lower surface tension, while neem oil has a tendency to form micelles on the surface of water and thereby forming an uneven layer due to its higher viscosity (35.83 cSt at 40°C) as compared to \textit{Eucalyptus} oil (30 cSt at 40°C).\textsuperscript{[30,31]} Therefore, the formation of a uniform oily layer on the surface of water probably enhanced the efficacy of \textit{Eucalyptus} oil at a much lower concentration than neem oil.

The efficacy of \textit{Eucalyptus} oil was also found to be preserved in different water conditions including the natural stagnant breeding habitats with organic matter as well as clean waters used in domestic environments such as in water coolers and artificial containers. We demonstrated its efficacy in water coolers which are the prominent breeding habitat of \textit{Aedes} at a low dose of 6 mL for 900 inch\textsuperscript{2} surface area of water, thereby easing its household application by common public. The residual effect was found to be up to 8 days (196 h) after its first application on the water body; however, after 8 days, the efficacy declined sharply, thereby emphasizing the need to drain or replace the water at the earliest. This finding may be of immense practical importance as stagnant water in unused coolers is a major breeding site for \textit{Aedes}, and there is a tendency of the general public to delay the changing of water; a buffer period of 8 days may enable better compliance to decant the stagnant waters.

The major limitation in the use of \textit{Eucalyptus} oil is the unstandardized preparations by different manufacturers which may alter the effective dose. This can be overcome by further exploration of different products and shortlisting the effective ones for recommendation to the general public in national or local health programs. Second, \textit{Eucalyptus} oil is colorless and has a mildly pungent camphor-like odor, whereas neem oil is greenish brown with a repulsive garlicky odor. This favors the use of \textit{Eucalyptus} oil in water coolers; however, the odor perception will have a subjective variation. The aroma can be further softened by adding rose water to it. Moreover, certain aromas may precipitate asthmatic attacks in sensitive individuals and this caution needs to be pointed out while recommending the use of such oils in households.
CONCLUSIONS

This study evaluated environment-friendly, nontoxic, affordable, and biodegradable mosquitoes control agents. Eucalyptus oil was found to be more effective larvicidal and pupicidal at low concentrations with a residual effect of up to 8 days. We also demonstrated pupicidal activity of Eucalyptus oil which has not been reported in the literature, to the best of our knowledge. Eucalyptus oil can thus be recommended for evaluation in natural field conditions, and future experiments may be carried out to assess the ovicidal effect, epidemiological impact, and cost-effectiveness of this natural oil.

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Conflicts of interest
There are no conflicts of interest.

REFERENCES

1. World Health Organization. Dengue: Guidelines for Diagnosis, Treatment, Prevention and Control – New Edition. WHO/HTM/NTD/DEN/2009.1. Geneva, Switzerland: World Health Organization; 2009.

2. World Health Organization. Guideline for Laboratory and Field Testing of Mosquito Larvicides. WHO/CDS/HOPES/GCDPP/2005. Geneva, Switzerland: World Health Organization; 2005.

3. Pham HV, Doan HT, Phan TT, Minh NN. Ecological factors associated with dengue fever in a central highlands Province, Vietnam. BMC Infect Dis 2011;11:172.

4. Parida MM, Dash PK, Upadhyaya C, Saxena P, Jana AM. Serological and virological investigation on an outbreak of dengue fever in Gwalior, India. Indian J Med Res 2005;121:36‑8.