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Larvicidal potential of *Eucalyptus globulus* oil against *Anopheles stephensi*

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Abstract
Malaria transmitted by *Anopheles stephensi* mosquito is one of the serious infectious diseases among vector borne diseases. Many plant-based extracts/oils have unique larvicidal activity against mosquitoes. This study was designed to determine the toxicity of *Eucalyptus globulus* oil against *An. stephensi* larvae. Different concentrations of *E. globulus* oil @ 50, 60, 70, 80, 90 and 100 ppm were tested against 4th instar larvae of *An. stephensi*. Mortality was recorded after 3, 6, 12, 24 and 48 hrs. LC_{50} and LC_{90} values of toxicity were determined by log- probit technique. Out of these tested concentrations, 90 ppm of *E. globulus* were calculated out to be the effective ones. LC_{50} and LC_{90} values were found to be 46.11 and 69.33 ppm for *E. globulus* oil, against *An. stephensi* larvae after 48hours. *E. globulus* proved to have good potential as a bio-larvicide against *An. stephensi* and could be used as effective and ecofriendly mosquito control agent in future.

Keywords: Malaria, *Anopheles stephensi*, *Eucalyptus globulus* oil, larvicidal potential, phytochemicals

1. Introduction
Mosquitoes are biting two-winged flying insects that suck blood from humans and animals. They transmit a number of diseases, such as malaria, filariasis, dengue, Japanese encephalitis, etc. Malaria is one of the deadliest diseases among these, which occurs due to the infection of parasitic protozoan, *Plasmodium* spp. While the malaria parasite is the true agent of infection, the female *Anopheles* mosquito is the agent of transmission, as males are nectar feeders [1]. *Anopheles stephensi* is an important malaria vector in India, especially of urban areas. According to the latest estimates [2], there were 214 million new cases of malaria in 2015 (ranging from 149–303 million) and estimated 4,38,000 deaths due to malaria (ranging from 2,36, 000–6,35, 000) worldwide. Children under five and pregnant women are more likely to suffer malaria attacks [3]. Mosquitoes often inhabit standing water bodies like ponds, swamps, marshes, paddy fields and man-made reservoirs. They breed in varied habitats and different genera have shown specific habitat and breeding preference like *Anopheles* species are associated with fresh water habitats [4]. The warm tropical climate and living conditions of people in our country make us an easy target for mosquitoes. The increase in environmental modification as a result of urbanization is usually being associated by the increase of breeding sites for mosquitoes which most often lead to increase in the incidence of mosquito borne diseases [5].
Many essential oils extracted from plants have proved to be effective and eco-friendly for mosquito control, as these possess certain chemicals with unique larvicidal activity [6]. The leaves of *Eucalyptus* spp. are rich in the compound cineole, which have excellent larvicidal and repellent properties against mosquito vector [7]. The larvae cannot escape from the breeding sites until they reach to adult stage. But some of these oils generally show degradation after some period due to some external factors such as temperature, light, and accessibility to atmospheric oxygen which are, therefore, needed to be thoroughly studied. Thus, the aim of present study was to test the larvicidal potential of *E. globulus* against malaria spreading *An. stephensi* mosquito.

2. Materials and Methods
2.1 Collection of water samples
Water samples were collected from different types of temporary standing water bodies like

paddy fields, road side ditches, gardens, nurseries, orchards and earthen pots of Ludhiana district by using plastic dippers. From the various types of mosquito larvae present in the collected water samples, *An. stephensi* larvae were identified on the basis of their morphological features by following the standard keys [8].

2.2 Larvicidal assay

Eucalyptus oil (*Eucalyptus globulus* Labillardiere) was obtained from Loba Chemie Private Limited, Mumbai, India. The oil was procured in the pure forms. Different concentrations of *E. globulus* oil @ 50, 60, 70, 80, 90 and 100 ppm were made in 250 ml of de-chlorinated water by mixing the oil in 1ml of dimethyl sulphoxide (DMSO). Twenty five 4th instar *An. stephensi* larvae were exposed to these concentrations in triplicate. A vehicle-control set (having 1ml DMSO mixed in 250 ml of de-chlorinated water) and a control set (having 250 ml de-chlorinated water) were also run simultaneously. All mosquito larvae were adequately fed with mixture of dog biscuits and yeast ground in the ratio of 3:1 (2mg/100ml). These experimental sets were kept in B.O.D. incubator at 26±2 °C. Mortality of larvae after 3, 6, 12, 24 and 48 hours of treatment with different oil concentrations were recorded in oil treated, vehicle-control and control sets.

2.3 Statistical analysis

Data was statistically analyzed by comparing *E. globulus* oil treated sets with their respective control/vehicle-control sets by using ANOVA (one-way) procedure using Statistical Package for Social Sciences (SPSS 20.0). LC_{50} and LC_{90}, values after 48 hrs post-exposure were worked out by log probit technique [9] employing the computer programme POLO [10].

3. Results and Discussion

Exposure of 4th instar larvae of *An. stephensi* to 50 ppm of *E. globulus* oil resulted in 30.67±2.31 per cent mortality after 3 hours. The larval mortality was found to increase with exposure time and after 48 hours 65.33±4.62 per cent mortality was observed. The per cent larval killing was found to increase with increase in concentration of *E. globulus* oil and exposure to 60, 70 and 80 ppm of *E. globulus* oil resulted in 77.33±2.31, 88.00±0.00 and 98.67±2.31 per cent mortality, respectively after 48 hours. Exposure of different concentrations of *E. globulus* oil resulted in killing of larvae within 3 to 48 hours (Table 1).

Table 1: Effect of different concentrations of *Eucalyptus globulus* oil on mortality of 4th instar larvae of *Anopheles stephensi*

Oil concentration (ppm)	Per cent mortality (Mean±S.D) (n=25)					Range of mortality (Within hours)
	3hr	6hr	12hr	24hr	48hr	
50	30.67±2.31^{e} (7-8)	34.67±2.31^{e} (8-9)	44.00±0.00^{e} (11)	52.00±0.00^{e} (13)	65.33±4.62^{d} (15-17)	3-48
60	58.67±2.31^{d} (14-15)	66.67±2.31^{d} (16-17)	69.33±2.31^{d} (17-18)	72.00±0.00^{d} (18)	77.33±2.31^{c} (19-20)	3-48
70	68.00±4.00^{c} (17-18)	72.00±0.00^{c} (18)	74.67±2.31^{c} (18-19)	78.67±2.31^{c} (19-20)	88.00±0.00^{b} (22)	3-48
80	73.33±2.31^{b} (18)	80.00±4.00^{b} (19-21)	84.00±4.00^{b} (20-22)	89.33±2.31^{b} (22-23)	98.67±2.31^{a} (24-25)	3-48
90	78.67±2.31^{a} (19-20)	84.00±4.00^{a} (20-22)	94.67±2.31^{a} (23-24)	100.00±0.00^{a} (25)	-	3-24
0 (control)	0.00±0.00^{f} (0)	0.00±0.00^{f} (0)	0.00±0.00^{f} (0)	0.00±0.00^{f} (0)	0.00±0.00^{e} (0)	-
0 (vehicle-control)	0.00±0.00^{f} (0)	0.00±0.00^{f} (0)	0.00±0.00^{f} (0)	0.00±0.00^{f} (0)	0.00±0.00^{e} (0)	-

- N represents number of larvae taken.
- Figures in parenthesis indicate the range in number of dead larvae from the start of experiment till that period.
- Figures followed with different superscripts indicate significant difference (p<0.05) by using Duncan multiple range test.

However, when larvae were exposed to 90 ppm of *E. globulus* oil, 100% mortality was recorded after 24 hours. During the present study, 90 ppm of *E. globulus* oil was found to be the effective concentration out of the tested ones as this concentration resulted in 100% larval killing within 24 hours or before their conversion to pupae. No mortality was recorded in control and vehicle-control sets. The values for LC_{50} and LC_{90} of *E. globulus* oil after 48 hours against 4th instar larvae of *An. stephensi* were worked out to be 46.11and 69.33 ppm, respectively (Table 2).

Table 2: Toxicity values of *Eucalyptus globulus* oil against 4th instar larvae of *Anopheles stephensi*

Toxicity Value (ppm)	Fiducial limits		Slope	χ^2
	Lower limit (ppm)	Upper limit (ppm)		
LC_{50}=46.11	33.38	52.20	7.233±1.878	1.0017
LC_{90}=69.33	63.04	83.15	7.233±1.878	1.0017

In the recent years the emphasis to control the mosquito populations has shifted from the use of conventional chemicals towards more specific and environmental friendly materials, which are generally of botanical origin [11]. *E. globulus* contains many phytochemicals with larvicidal activity and thus can act as new candidates for safe and effective alternate to chemical insecticides. The main constituents in the eucalyptus oil are 1, 8-cineole, γ – Terpinene, α –Pinene and Globulol. These components are responsible for various morphological, physiological and biochemical changes in the larvae resulting in their killing [11-12-13]. In addition to these effects, *A. vera* has been found to

play a role in neurobehavioral and brain transmitter alterations of the nervous system [14]. As mosquito larvae live in aquatic habitat and feed by taking water inside, the oil suspension in water enters their body through tracheal system. The essential oils have the property to increase the tendency of treated flooding, resulting in chemical toxicity. However, Nathan [15] observed 100% mortality of *An. stephensi* Liston after treatment with 160 ppm of *E. tereticornis* oil. In a recent study, oils of *E. globulus* and *Mentha piperita* were proved to be highly toxic to *Aedes aegypti* larval and pupal stages and this toxicity response was found to be time and concentration dependent [16]. In the present study the larvicidal activity of *E. globulus* was concentration dependent, as with the increase in oil concentration, the mortality rate was also found to increase. The mortality rate was higher during the initial periods of treatment, as larvae were exposed to active components i.e 1, 8 cineole present in Eucalyptus oil which shows devastating effects inside the body of the larvae like disintegration of the midgut epithelium layer, rifts in microvilli, and disappearance or reduction of fat bodies as well as the epithelium layer of the hindgut [13]. Such structures are affected when they come in contact with Eucalyptus oil leading to their death.

4. Conclusion

The results of the present study concluded that testing of *E. globulus* oil is proven to be found effective in small and temporary water collections under natural conditions may lead to promising results for control of malaria spreading vector mosquito, *An. stephensi*. It can complete very well with synthetic chemicals.

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