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Short communication

# The use of *Eucalyptus tereticornis* Sm. (Myrtaceae) oil (leaf extract) as a natural larvicidal agent against the malaria vector *Anopheles stephensi* Liston (Diptera: Culicidae)

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**Abstract**

Secondary metabolites obtained from the indigenous plants with proven mosquito control potential can be used as an alternative to synthetic insecticides under the integrated vector control. The essential oil extract from the forest redgum, *Eucalyptus tereticornis* Sm. (Myrtaceae) was tested against mature and immature mosquito vector *Anopheles stephensi* Liston (Diptera) under laboratory condition. The extract showed strong larvicidal, pupicidal and adulticidal activity. The leaf oil extracts showed high bioactivity at high doses. Results obtained from the laboratory experiment showed that the leaf extracts suppressed the pupal and adult activity of *Anopheles stephensi* at higher doses. In general, first and second instar larvae were more susceptible to all treatments. Clear dose –response relationships were established with the highest dose of 160 ppm plant extract evoking almost 100% mortality. The results obtained suggest that, in addition to their medicinal activities, *E. tereticornis* can also serve as a natural mosquitocide.


*Keywords:* Mosquito vector; Forest redgum; Biology, mortality, larvicide, adulticide; Oviposition deterrence

## 1. Introduction

Mosquitoes are vectors of etiologic agents of malaria, filariasis, and viral disease. *Anopheles stephensi* Liston (Diptera) is the primary vector of malaria in India and other West Asian countries, and improved methods of control are urgently needed (Gilles and Warrell, 1993; Collins and Paskewitz, 1995; Burfield and Reekie, 2005). Recently, botanical and microbial insecticides have been increasingly used for mosquito control because of their efficacy and documented non-toxic effects on non-target organisms (Ascher et al., 1995; Amalraj et al., 2000; Gunasekaran et al., 2004; Senthil Nathan, 2006). The development of Insect Growth Regulators (IGR) has drawn much attention as selective control of insects of agriculture, medical and veterinary importance and has produced mortality due to their high neurotoxic effects (Al-Sharook et al., 1991; Senthil Nathan et al., 2005, 2006a,b).

Recent studies have also stimulated the investigation of insecticidal properties of chemicals derived from plant material and concluded that they are environmentally safe, degradable, and target specific. Indeed, many medicinally important plant extracts were studied for their efficacy to kill larvae of different species of mosquito (Saxena and Sumithra, 1985; Kumar and Dutta, 1987; Chariandy et al., 1999; Markouk et al., 2000; Tare et al., 2004) and other medically important parasites (Abdel-Shafy and Zayed, 2002; Borges et al., 2003; ICMR, 2003).

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The botanical family Myrtaceae is a potential source for mosquito repellent compounds. Leaves of *Eucalyptus tereticornis* Sm. are rich in the compound cineol, (Franich, 1985). *E. tereticornis* has long been recognized for its insecticidal properties; especially its mosquito repellent activity (Watanabe et al., 1993; Corbet et al., 1995; Traboulsi et al., 2005) but has yet to be extensively analyzed. Extracts from the *Eucalyptus* leaves have shown excellent larvicidal and repellent properties against mosquito vectors while at the same time being very eco-friendly (Watanabe et al., 1993). My present investigation demonstrates the efficacy of extracts of *E. tereticornis* in killing larval and adult stages of *A. stephensi* under laboratory conditions.

## 2. Methods

### 2.1. Mosquito culture

*A. stephensi* eggs were collected around the Vivekanandha College Campus, Namakkal District, Tamil Nadu and reared in plastic and enamel trays in tap water. They were maintained in a growth chamber and all the experiments were carried out at $27 \pm 2$ °C, 75–85% relative humidity with 14:10 light and dark photoperiod. Larvae were fed a diet of Brewers yeast, dog biscuits and algae collected from ponds in the ratio of 3 (Brewers yeast): 1 (dog biscuits): 1 (algae). Pupae were transferred from the trays to a cup containing tap water and placed in screened cages ($23 \times 23 \times 32$ cm) where adults emerged. Adults of *A. stephensi* were reared in $30 \times 30 \times 30$ cm glass cages. Adults were continuously provided with 10% sucrose solution in a jar with a cotton wick. On day five post-emergence, adult females were deprived of sugar for 12 h then provided with a mouse placed in resting cages overnight for blood feeding. Adult mosquitoes were maintained at the same environmental condition as larvae.

### 2.2. Plant extracts

*E. tereticornis* leaves (mature) were collected from five trees approximately about 16–18 months old in natural forests of Kolli hills, Namakkal District, Tamil Nadu, India. The leaves were picked out during the morning. The essential oils were isolated by steam-distillation using a Clavenger apparatus, dried over anhydrous sodium sulphate, and stored in amber-colored vials at 5 °C until required for further work.

### 2.3. Bioassays and larval mortality

First to fourth instar larvae and pupa were exposed to test concentrations of 10, 20, 40, 80 and 160 ppm of the oil in distilled water for 24 h according to standard WHO procedure (1981). As the essential oil does not dissolve in water it was first dissolved in ethanol (99.0%). The test medium (250 ml glass beaker) was prepared by adding 1 ml of appropriate dilution of essential oil in ethanol and mixed with 249 ml of water to make up 250 ml of test solution (Dharmagadda et al., 2005). The larvae were fed dry yeast powder on the water surface (50 mg/l). Ethanol served as a control (1%). A minimum of 20 larvae/concentration was used for all the experiments. The dead larvae were counted after every hour, and percentage mortality is reported from the average for the five replicates taken. The lethal concentrations ($LC_{50}$ and $LC_{90}$) were calculated using Probit analysis (Finney, 1971). The percentage mortality was calculated by using the formula (1) and corrections for mortality when necessary were done by using Abbot's (1925) formula.

$$\text{Percentage of mortality} = \frac{\text{Number of dead larvae}}{\text{Number of larvae introduced}} \times 100 \quad (1)$$

### 2.4. Adulticidal assay

*A. stephensi* adults (10 individuals) were exposed to filter paper (90 mm, Advantec Toyo, Japan) treated with concentrations of 10, 20, 40, 80 and 160 ppm *E. tereticornis* oil extract. The paper was kept inside the beaker. Control insects were exposed only to ethanol treated paper and muslin cloth. A mortality count was taken after 24 h. The experiment was repeated five times.

### 2.5. Oviposition assay

During the tests, the groups of females (10 individuals fed on mice blood) were kept separate for 48 h in cage measuring $25 \times 25 \times 30$ cm. The cage contained six glass jars with 10, 20, 40, 80 and 160 ppm concentrations of the *E. tereticornis*. Ethanol was used as the control (five replicates). Ten gravid females were given a choice between treated and control jars. After the eggs were counted, the oviposition deterrence index (ODI) (Hwang et al., 1982) was calculated by using the formula (2):

$$\text{ODI} = \frac{\text{Nt} - \text{Ns}}{\text{Nt} + \text{Ns}} \times 100 \quad (2)$$

where, Nt = total number of egg rafts in test solution, Ns = total number of egg rafts in control.

### 2.6. Statistical analysis

The lethal concentration was calculated by using Probit analysis (Finney, 1971). Mortality was corrected by using Abbot's (1925) formula. Data from mortality and oviposition deterrence were analyzed with ANOVA of arcsine square root transformed percentages. Differences between means were considered significant at $P \leqslant 0.05$ (SAS Institute, 2001). In the Tukey's test, descending order was used. The highest different values from average, detected by statistical testing were marked with letter "a", the next text lower with "b" and continued accordingly (Snedecor and Cochran, 1989).

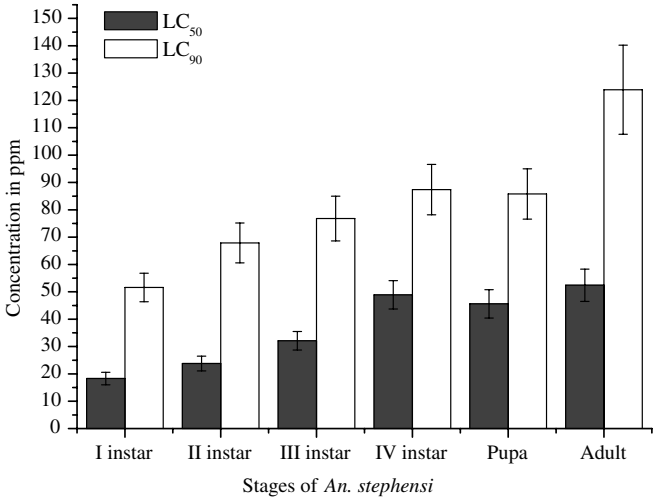


Fig. 1. Lethal concentration 50 ($LC_{50}$) and 90 ($LC_{90}$) of *E. tereticornis* against larval, pupal and adult of *A. stephensi* (values are mean of five replicates with ±(SEM) standard error).

## 3. Results

Exposure of *E. tereticornis* extract in mosquito larval diet reduced larval survivability and increased mortality in all larval instars. The $LC_{50}$ and $LC_{90}$ values of *E. tereticornis* oil against the malaria vector are shown in Fig. 1. First (18.3 and 51.6 ppm for $LC_{50}$ and $LC_{90}$, respectively) and second instar (23.8 and 63.9 ppm for $LC_{50}$ and $LC_{90}$, respectively) larvae were more susceptible with least $LC_{50}$ values (Fig. 1).

The 160 ppm concentration of leaf extract oil killed more than 95% of first instars, ($F = 24.9212$; df = 4; $P < .0001$), 94% of second instars ($F = 24.9521$; df = 4; $P < .0001$), 92% of third instar ($F = 24.9394$; df = 4; $P < .0001$), 90% of fourth instars ($F = 24.9542$; df = 4; $P < .0001$) and 88% of pupae ($F = 24.9412$; df = 4; $P < .0001$) (Figs. 2 and 3). The same trend was also observed on percentage mortality of adult of *A. stephensi* (Fig. 3).

At higher concentrations (80 and 160 ppm), the larvae showed irregular movement for some time and then died

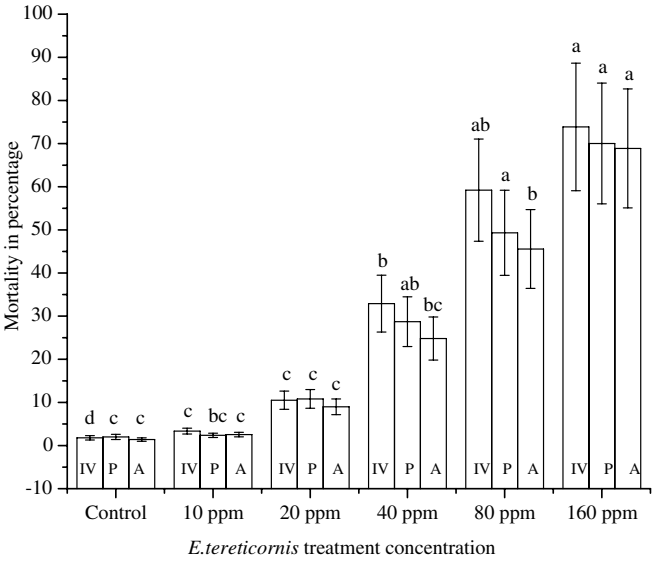


Fig. 3. Percentage of fourth instar, pupae and adult mortality of *A. stephensi* after treatment with *E. tereticornis*. Means (±(SEM) standard error) followed by the same letters within same mosquito stage of indicate no significant difference ($P \leqslant 0.05$) in a Tukey test (IV, P, and A indicate fourth instar, pupae and adult respectively).

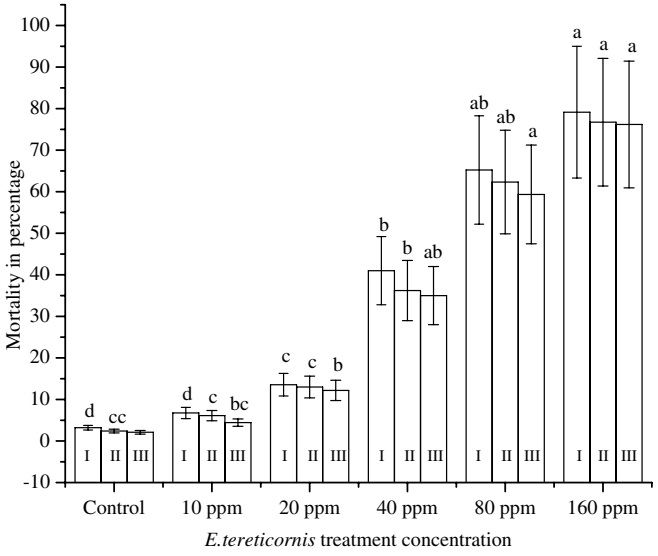


Fig. 2. Percentage of first to third instar larval mortality of *A. stephensi* after treatment with *E. tereticornis*. Means (±(SEM) standard error) followed by the same letters within same instar of indicate no significant difference ($P \leqslant 0.05$) in a Tukey test (I, II, and III indicate first, second and third instar respectively).

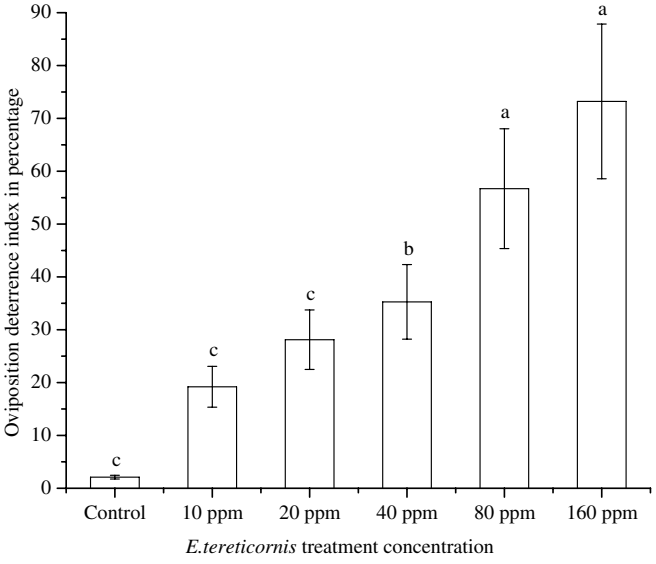


Fig. 4. Ovipositional deterrence index (ODI) of *A. stephensi* after the treatment with *E. tereticornis*. Means (±(SEM) standard error) followed by the same letters within bars of indicate of no significant difference ($P \leqslant 0.05$) in a Tukey test.

at the bottom of the beakers. No pupal or adult emergence was observed among the treatments as almost 100% mortality occurred within 24 h. A significant difference was observed between higher concentrations (80 and 160 ppm) and lower concentrations (20 and 40 ppm). The effect on larval, pupal and adult mortality was concentration-dependent. There was a gradual decrease in oviposition when female *A. stephensi* were treated with *E. tereticornis* oil extract in lower dose treatments but after treatment with more than 80 ppm, the effect was more pronounced (Fig. 4). Maximum oviposition deterrence was observed in 160 ppm and it was significantly different from other lower concentration (except 80 ppm) treatments ($F = 24.9205$; df = 4; $P < .0001$).

## 4. Discussion

Results on the larval mortality and oviposition deterrence of the 80 ppm extract on the adult *A. stephensi* reported in the present study, confirm their potential for control of the mosquito populations. These properties can be exploited to protect the immediate environment of the user or the user from the bites of harmful insects, which may be vectors of disease.

It was also observed that first and second instar larvae were more susceptible to the leaf extract (Fig. 2). Exposure of *A. stephensi* larvae to sub-lethal doses of neem extracts in the laboratory prolonged larval development, reduced pupal weight, high oviposition deterrence and high mortality (Su and Mulla, 1999; Wandscheer et al., 2004; Traboulsi et al., 2005). The direct and indirect contribution of such effects to treatment efficacy through reduced larval feeding and fitness need to be properly understood in order to improve the use of botanical insecticides for management of *A. stephensi*. These and other naturally occurring insecticides may play a more prominent role in mosquito control programs in the future (Amalraj et al., 2000; Gunasekaran et al., 2004; Wandscheer et al., 2004).

After *E. tereticornis* treatment at a higher dose, the larvae died immediately before the pupal stage. The larvae become abnormal and irregular in movement. The larvicidal mode of action of essential oils was investigated by Corbet et al. (1995) who noted the susceptibility of mosquito larvae and pupae to surface materials entering their tracheal system, observing that essential oils increased the tendency to tracheal flooding and chemical toxicity.

However, Senthil Nathan et al. (2005) considered pure limonoids of neem seed, testing for biological, larvicidal, pupicidal, adulticidal, and antiovipositional activity, *A. stephensi*. Larval mortality was dose-dependent with the highest dose of 1 ppm azadirachtin evoking almost 100% mortality, affecting pupicidal and adulticidal activity and significantly decreased fecundity and longevity of *A. stephensi*. The treatment completely inhibited the larval, pupal and adult developments. The limonoids also interfered with oviposition, egg hatchability, and exhibited a growth inhibiting effect against larvae and good mortality effect against adults of *A. stephensi*. The same results were also obtained when *Melia azedarach* L. leaf and seed extracts were used (Senthil Nathan et al., 2006a).

Although the botanical insecticides are the lesser of many hazards in terms of general pesticide toxicities, they are toxins nonetheless. All toxins used in pest control pose some hazards to the user and also to the aquatic environment (Kreutzweiser, 1997). Hence this research is mainly focused on finding newer insecticides which will be more effective, biodegradable and also easily available at low cost.

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