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Implication of *Eucalyptus Tereticornis* Oil for the Larvicidal Activity of the Malarial Vector Anopheles *Stephensi Liston*

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Abstract **– The properties of Eucalyptus extract can be exploited to protect the subject from the bites of harmful insects, which may be vectors of disease. This article is been inked in order to formulate eucalyptus oil as pesticide which could be detrimental for mosquitoes & not for untargeted organism. *Eucalyptus tereticornis* Sm. (Myrtaceae) was tested against mature and immature mosquito vector Anopheles stephensi Liston (Diptera) under laboratory condition. The extract showed strong effect on larvicidal, pupicidal and adulticidal activity. At 165ppm the mosquitoes showed irregular movement and after few minutes mosquitoes dies. As results obtained suggest that, in addition to their medicinal activities, *E. tereticornis* can also serve as a natural mosquitocide.**

Keywords **– Mosquitoes Vector, Eucalyptus, Natural Pesticide etc.**

I. INTRODUCTION

Mosquitoes are the vector of Infectious disease known as Malaria. Malarial parasites belong to the genus PLASMODIUM (Phylum Apicomplexn). There are several variants of Species of plasmodium that affect the human being. Malarial infestation in Human being via P.Falciperem, P.Malaria, P.Oval and P.Vivax is being studied. Since few years, the mosquitoes were controlled by the Insecticide which affected the Non-targeted Organism. So to prevent the utilization toxic insecticide, Botanical insecticide has been used for the people's welfare. The development of Insect growth Regulator (IGR) has drawn much attention as selective control of Mosquitoes which have increased mortality rate, being highly Neurotoxic. (Al-Sharook *et al.,* 1991; Senthil Nathan *et al.,* 2005, 2006a, b).Recent Studies have also revealed the insecticidal properties of chemicals derived from plants and concluded that they are safe for humans & eco-friendly. Targetting specific malarial species, numerous medicinally essential plants extract were studied for their potential to treat mosquito's larva. (Saxena and Sumithra, 1985; Kumar and Dutta, 1987; Chariandy *et al.,* 1999; Markouk *et al.,* 2000; Tare *et al.,* 2004).

The botanical family Myrtaceae is a potential source for mosquito repellent compounds. Leaves of Eucalyptus tereticornis Sm. are rich in cineol, (Franich, 1985). E. tereticornis has been identified for its insecticidal properties; especially for its repellent activity against mosquitos' larva. (Watanabe et al., 1993; Corbet et al., 1995; Traboulsiet al., 2005) but yet to be broadly investigated. The Eucalyptus leaves have shown excellent larvicidal and repellent properties against mosquito vectors as well as it is eco-friendly (Watanabe et al.,1993). My present research demonstrates the efficacy of extracts of E. tereticornis in killing larval and adult mosquitoes of A. stephensi under laboratory conditions.

II. METHODOLOGY

A) Culturing of Mosquitoes -

The eggs of A. stephensi were collected from abandoned shower facility at Naraina College campus, Kanpur district, Uttar Pradesh and were cultivated in plastic and trays of tap water, stagnant water of fountain; they were kept in mosquito's breeder in laboratory. They were maintained in the breeder and all the experiments were carried out at 26 ± 3℃, 70–80% relative humidity with 14:10 light and dark photoperiod. Larvae were fed on Brewers yeast, biscuits and algae collected from fountain in the ratio of 3 (Brewer's yeast): 1 (biscuits): 1 (algae). Pupae of mosquitoes were transfer to the tray which was covered by a frame of (25x25x34) with adults in same frame. The adult A. Stephesi was reared in glass cage of (30x30x30). The adult were providing with 15%of sucrose solution in the cage with cotton wick. Later the adults female were deprived of sugar for 13hr then mouse was placed in cage for overnight feeding. Similar ambience was maintained for adult as well as for Larvae in laboratory.

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Fig. 1. Illustration of Culture (primary stage) of mosquito and larval development

B) Leaf Extract –

E. tereticornis leaves (mature) were collected from trees about 18-20 months found generally in suburbs of Kanpur, Uttar Pradesh. The oil was extracted using steam distillation process using Clavenger apparatus, dried over anhydrous sodium sulphate, and stored in vials at 5℃ until required for further work.

Fig. 2. Eucalyptus tereticornis Sm. (Myrtaceae)

C) Bio-Assay & Mortality –

Instar larvae and pupa were exposed to test concentrations of 15, 25, 45, 85 and 175 ppm of the oil in distilled water for a day, according to standard WHO procedure (1981) the essential oil is insoluble in water and therefore it was 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 (LC50 and LC90) 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.

Percentage of mortality —

$$Mortality\ Percentage = \frac{No.of larava\ eliminated\ from\ laraval\ Population}{Total\ No.Larava} X100$$

D) Adulticidal assay

A. stephensi adults (10 individuals) were exposed to filter paper (Blotting Paper) treated with concentrations of 15, 25, 45, 85 and 165 ppm E. tereticornis oil extract. The paper was kept inside the beaker and Control insects were exposed only to ethanol treated paper and muslin cloth. A mortality count was taken after 22 h-23h. The experiment was repeated five times.

E) 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× 25 ×30 cm. The cage contained six glass jars with 15, 25, 45, 85 and 165 ppm concentrations of the E.tereticornis. Ethanol was used as the control (five replicates).Ten 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):

$$ODI = \frac{Nt - Ns}{Nt + Ns}$$

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

F) 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 were analyzed with ANOVA of transformed percentages. Differences between means were considered significant at P 6 0.05 (SAS Institute, 2001). The descending order was used. The highest different values from average, detected by statistical testing were marked as "α", and lower value as ''β" and continued accordingly (Snedecor and Cochran, 1989).

G) Result:-

E. tereticornis extract in mosquito larval diet reduced larval durability and increased mortality in all larval. The LC50 and LC90 values of E. tereticornis oil against the malaria vector are shown in Fig. 1. First (19.6 and 52.8 ppm for LC50 and LC90, respectively) and second instars (25.6 and 61.0 ppm for LC50 and LC90, respectively) larvae were more susceptible with least LC50 values. The 165 ppm concentration of leaf extract oil killed more than 90% of first set of Mosquitoes, At higher concentrations (85 and 165 ppm), the larvae Showed sporadic movement for some time and then died 165 ppm and it was different from other lower concentration (except 85 ppm) treatments 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 symptom was

difference when observed between higher concentrations (85 and 165 ppm) and lower concentrations (25 and 45 ppm). The effect on larval, pupal and adult mortality was concentration- dependent. There was a gradual decrease in ovipositor.

Fig. 3. Illustration of crippled mosquitoes post leaf oil treatment

III. Conclusion

The leaf oil exhibited high larvicidal activity 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 on larvicidal, pupicidal and adulticidal activity showed asynchronous movement and after few minutes they all died.

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