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Larvicidal activity of selected essential oils against *Aedes aegypti* (Insecta: Diptera: Culicidae)

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Abstract
Mosquitoes are pestiferous insects, which are responsible for the transmission of various diseases. The problems caused because of mosquitoes are worldwide concern due to their inherent ability to act as intermediate host or vectors for several parasitic and viral diseases of human being and domesticated animals. Natural botanicals have provided numerous sources of phytochemicals utilized in the development of effective mosquito control agents. Biologically active plants show great promise for their potential efficiency as larvicides. The use of botanical derivatives in mosquito larvae, as an alternative to synthetic insecticides offers more eco-friendly method of insect control than the use of synthetic chemicals. The present study is focused on the larvicidal effect of essential oils against the third instar larvae of *Aedes aegypti*. Orange oil was found to be the potent larvicide against *Aedes aegypti* exhibiting promising mortality at LC_{50} of 52.27 ppm, followed by other plant oils.

Keywords: larvicidal activity, essential oil, mosquito, *Aedes aegypti*

Introduction
Mosquitoes are pestiferous insects, which are responsible for the transmission of various diseases. WHO has declared the mosquito as a "Public Enemy Number One" because mosquitoes are responsible for the transmission of various dreadful diseases (WHO, 1996) [28]. Mosquitoes are a large group, which contain over 3200 species in 34 genera. In the modern classification of Culicidae, they are grouped in three sub families: Anophelinae, Culicinae and Toxorhynchitinae (Knight and Stone, 1977) [14]. Mosquitoes are known for their nuisance from ages past but they have received attention only recently when a few species were considered as potential vectors of diseases. Malaria, Filaria, Japanese Encephalitis (JE), Dengue and Dengue Haemorrhagic Fever (DHF) are the major mosquito-borne diseases in India. Recent large-scale outbreak of the mosquito-borne viral disease, Chikungunya, has caused manifold loss in terms of morbidity and economic loss (John William, 2007) [13].

Long before the advent of synthetic insecticides, plant and their derivatives were used to kill pests of agriculture, veterinary and public health. Insecticidal activity of plant-derived compounds such as nicotine, rotenoids and pyrethroids have been evaluated and a few of these compounds have been exploited commercially (Jacobson and Crosby, 1971; Medappa, 2003) [12, 15].

In view of growing concern about safety of chemical based repellents, interest is revived in oils extracted from plants as repellent for mosquitoes. Traditional repellents not only provide protection against mosquito bites, but also curtail malaria transmission.

Biologically active plants show great promise for their potential efficiency as larvicides. The use of botanical derivatives in mosquito larvae, as an alternative to synthetic insecticides offers more ecofriendly method of insect control than the use of synthetic chemicals (Sukumar *et al*., 1991) [22]. Nearly 200 species of medically important arthropods have gained resistance to many contemporary pesticides (WHO, 1993) [27]. More potent and also more expensive chemical compounds that replace the less toxic ones have also failed due to resistance gained by vectors.

The plant world comprises a rich storehouse of biochemicals that could be tapped and used as pesticides. The toxic constituents present in the plants represent the secondary metabolites and have only an insignificant role in primary physiological process that synthesizes them (Venkatachalam, 2001) [25]. The phytochemicals of the plants serve as huge storage of compounds that have biological action (Howard *et al*., 2007) [10]. Phytochemicals such as alkaloids, saponins, and tannins are known to possess medicinal and pesticidal properties (Azmathullah *et al*., 2011) [3].

Plant extracts and essential oils possess larvicidal activity against various mosquito species (Berenbaum, 1989; El Hag *et al.*, 1999, 2001; Omolo *et al.*, 2004) [4, 6, 7, 19]. To contain pesticide resistance as well as to protect the environment from the adverse effects of pesticides is today's burning issue (Mehrotra, 1993) [16]. All these factors led to the search for safer and more compatible alternatives among which natural products are of prime importance.

Materials and Methods
The experimental species
The vector mosquito selected for the present study as experimental species is *Aedes ageypti*. *Aedes* immatures were collected from natural breeding habitats such as cisterns, water tanks inside and outside houses, barrels and discarded tyres in New Washermanpet, Anna Nagar West, Perambur and

Korattur area in Chennai. Materials used for collection included a dipper of 200 ml capacity with 7.5 cm in diameter and 5.0 cm in depth, filter/strainer with 9.5 cm diameter, and glass dropper with 5 ml capacity, flash light and plastic containers of 2.0 litres capacity. Immatures collected were transported to the laboratory in plastic containers. In the laboratory, the mosquito larvae were transferred to enamel larval trays (27.0 cm x 30.0 cm) and maintained until emergence. The larvae were given larval food (dog biscuits and yeast in the ratio 3:1) until emergence. Adult mosquitoes emerged were identified using mosquito identification key. Target species were segregated and colonized continuously for many generations under disease and pollution free conditions in mosquito culture laboratory.

Plant Oils

The plant oils were obtained from the government recognized (TNGST 030223) Aromatic Oil Stores, TEGRAJ & Co, No. 14, Naniappa Naicken Street, Chennai, Tamil Nadu.

The bioactivity of ten plant oils, Cedar wood (*Cedrus atlantica*), Mentha oil (*Mentha piperita*), Clove (*Myrtus caryophyllum*), Geranium oil *(Pelargonium graveolen)* Lemon grass (*Cymbopogon flexuosus*), Orange (*Citrus sinensis*), Nutmeg (*Myristica fragrans*), Palmarosa (*Cymbopogon martinii*), Pine (*Pinus radiata*) and Tulsi (Ocimum *sanctum*) were tested at 125 ppm, 250 ppm, 500 ppm and 1000 ppm concentrations against the third instar larvae of *Aedes aegypti*.

Preparation of plant oil stock solution

One per cent stock solution was prepared by dissolving 1ml of plant oil in 99ml of distilled water in a standard flask, from the stock solution 1000 ppm, 500 ppm, 250 ppm, 125 ppm and 62.5 ppm concentration were prepared (WHO, 1996) [28].

Methods

Larvicidal Activity

Larvicidal is generally used for the process of killing mosquitoes by applying natural agents or commercial products designed to control the larvae of aquatic habitats. Twenty number of the late 3rd instar larvae of *Aedes aegypti* were kept in 500ml of glass container, containing 249ml of dechlorinated tap water and 1ml of stock solution were added. Three replicates were set up for each concentration. Tween 80 used as an emulsifier. Control was set up with Tween 80. After 24 hours, the percent mortality was calculated by using the formula,

No. of larva dead

$$\text{Percent Mortality} = \frac{\text{No. of larva dead}}{\text{No. of larva Introduced}} \text{ X } 100$$

However, when the control mortality ranged from 5-20 percent, the observed percentage mortality was corrected by Abbott's formula (1925).

$$\text{Corrected Percent Mortality} = \frac{\text{\% test mortality - \% control mortality}}{\text{100 - \% control mortality}} \text{ X } 100$$

Mean and standard deviation of larval mortality and percent larval mortality was calculated. LC_{50} and LC_{90} values with lower and upper confidence limits were calculated by Probit analysis.

Statistical Software

SPSS version 21 statistical package (IBM SPSS Statistics, 2012) was used for statistical analysis.

Results

Ten plant oils were screened for the bioefficacy against the larvae *Aedes aegypti.* Cedarwood oil, Clove oil, Lemongrass oil, Mentha oil, Pine oil, Palmarosa oil, Tulsi oil, Nutmeg oil, Orange oil and Geranium oil gave promising results. The results of the larvicidal activity of the selected plant oils are given in Tables 1 and 2.

Table 1: Larvicidal activity of some plant oils of different concentrations on *Aedes aegypti* at 24h

S. No	Concentration (ppm)	Name of the plant oils (% Mortality)				
		Cedarwood oil	Palmarosa oil	Pine oil	Clove oil	Lemongrass oil
1	62.5ppm	23.3±2.9	65.0±5.0	26.7±2.9	21.7±2.9	21.7±2.9
2	125ppm	36.7±2.9	88.3±2.9	33.3±2.9	38.3±2.9	38.3±2.9
3	250ppm	48.3±2.9	100.0±0.0	41.7±2.9	56.7±2.9	58.3±2.9
4	500ppm	58.3±2.9	100.0±0.0	51.7±2.9	83.3±2.9	76.7±2.9
5	1000ppm	81.7±2.9	100.0±0.0	66.7±2.9	100.0±0.0	93.3±2.9
LC_{50} Value (ppm)		436.13	62.79	575.62	247.68	306.24
LC_{90} Value (ppm)		1077.75	113.80	1453.49	515.73	736.67

(Mean ± S.D) (n=20)

Table 2: Larvicidal activity of some plant oils of different concentrations on *Aedes aegypti* at 24h

S. No	Concentration (ppm)	Name of the plant oils (% Mortality)				
		Tulsi oil	Mentha oil	Orange oil	Nutmeg oil	Geranium oil
1	62.5ppm	38.3±2.9	46.7±2.9	78.3±2.9	66.7±5.8	21.7±2.9
2	125ppm	88.3±2.9	61.7±2.9	100.0±0.0	100.0±0.0	31.7±2.9
3	250ppm	98.3±2.9	78.3±2.9	100.0±0.0	100.0±0.0	58.3±2.9
4	500ppm	100.0±0.0	100.0±0.0	100.0±0.0	100.0±0.0	73.3±2.9
5	1000ppm	100.0±0.0	100.0±0.0	100.0±0.0	100.0±0.0	93.3±2.9
LC_{50} Value (ppm)		80.79	120.96	52.27	56.34	325.95
LC_{90} Value (ppm)		140.61	270.99	69.06	74.70	756.65

(Mean ± S.D)

Discussion

Mosquitoes play an important role in the transmission of several vector-borne dreadful diseases affecting mankind (WHO, 1996) [28]. Despite many attempts to successfully eradicate them, mosquitoes still co-exist with man and animals transmitting dreaded diseases like malaria, filariasis and dengue etc. (Service, 1983) [20]. The annual incidence of this disease in the country ranges between 60,000 and 75,000 cases with fatal ending among the age group 35-40. The WHO (1982) [26] felt the resistance in vectors was probably the "biggest single obstacle in the struggle against vector borne diseases". The problem of mosquitoes is worldwide concern because of their inherent ability to act as intermediate host or vectors for several parasitic and viral diseases of human being and domesticated animals.

Natural botanicals have provided numerous sources of phytochemicals utilized in the development of effective mosquito control agents. Plant materials is not only an effective mosquito control agents, but also is promising to be environmentally safe (Choochote *et al.,* 1999) [5]. Botanical derivatives in mosquito control especially for mosquito larvae, as an alternative to synthetic insecticides offer a more environmentally friendly method of control (Irungu and Mwangi, 1995) [11]. Various plant species have been exploited to control the mosquito population throughout the world (Zebitz, 1984; 1986; Tare and Sharma, 1991; Sharma and Goel, 1994; Muthukrishnan *et al*., 1997; Mwangi and Mukiama, 1998) [29, 30, 18]. The bioactive organic chemical contents may serve as insecticides, antifeedants, repellents etc. They are less toxic, easily biodegradable and do not have any adverse effects on the target organisms.

As the concentration of the plant oil increases the total larval mortality of the mosquitoes was also found to be increased. In the present study, when the *Aedes aegypti* larvae were examined after 24hrs, LC_{50} and LC_{90} values of the plant oils are presented in table (1&2). The highest larval morality was observed in Orange oil with the LC_{50} of 52.27 ppm, followed by Nutmeg oil with 56.34 ppm, Palmarosa oil with 62.79 ppm, Tulsi oil with 80.79 ppm, Mentha oil with 120.96 ppm, Clove oil with 247.68, Lemon grass oil with 306.24 ppm, Geranium oil with 325.95 ppm, Cedar wood oil with 436.13 ppm and Pine oil with 575.62 ppm respectively.

Focusing on mosquito reduction efforts on the larval stage has the advantage of controlling the vector prior to dispersal or acquisition of the disease and interrupting the life cycle before it can cause harm (Hardin and Jackson, 2009). Larviciding is a successful method of reducing mosquito population in their breeding places before they emerge into adults (Tiwary *et al*., 2007) [24]. The advantage of targeting larvae is that they cannot escape from their breeding sites until the adult stage. The control of mosquito at the larval stage is necessary and efficient in integrated mosquito management since during the immature stage, mosquitoes are relatively immobile (Elimam *et al*., 2009) [8] and mosquitoes in the larval stage are attractive targets for insecticides because mosquitoes breed in water and thus, it is easy to deal with them in this habitat.

The present study has identified more plant oils showing larvicidal activity against *Ae. aegypti* and the obtained results suggest that the plant oils are promising as larvicides against *Ae. aegypti* larvae. Orange oil was found to be the potent larvicide against *Aedes aegypti* providing promising mortality at LC_{50} of 52.27 ppm, followed by other plant oils. Moreover, these results could be useful in the search for newer, more selective and biodegradable larvicidal natural compounds.

Conclusion

An attempt has been made to evaluate the role of selected essential oils in *Aedes aegypti* larvicidal activity. The results reported here open the possibility for further investigations of efficacy on their larvicidal properties of natural oil products.

The results of the present study would be useful in promoting research aiming at the development of new agent for mosquito control based on bioactive chemical compounds from indigenous oil sources.

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