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Larvicidal Efficacy of Plant Oils Against the Dengue Vector *Aedes aegypti* (L.) (Diptera: Culicidae)

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Abstract: The bioactivity of ten plant oils, Cedar wood (*Cedrus atlantica*), Citronella (*Cymbopogon nardus*), Clove (*Myrtus caryophyllum*), *Eucalyptus* (*Eucalyptus globulus*), Lemon grass (*Cymbopogon flexuosus*), Orange (*Citrus sinensis*), Nutmeg (*Myristica fragrans*), Palmarosa (*Cymbopogon martinii*), Pine (*Pinus radiata*) and Tulsi (*Ocimum sanctum*) were tested at 125, 250, 500 and 1000 ppm concentrations against the third instar larvae of *Aedes aegypti*. Larval mortality was observed after 24 hours. Among the plant oils tested, orange oil exhibited highest larvicidal activity with LC_{50} of 85.93, followed by palmarosa with 88.78, tulsi with 92.48 and nutmeg oil with 93.62 ppm.

Key words: Vector mosquitoes · Essential plant oils · Larvicide

INTRODUCTION

Mosquito vectors of Dengue and Dengue haemorrhagic fever (DHF) belong to the genera *Aedes*. *Aedes aegypti* is the major vector in urban areas. The major obstacle in mosquito control program is the development of the resistance to conventional insecticides. The WHO expert committee [1] felt the resistance in vectors was probably the "biggest single obstacle in the struggle against vector-borne diseases". Most of the mosquito control programs target the larval stage in their breeding sites [2, 3]. Synthetic organic insecticides used to control mosquitoes have produced an ill effect on environment, non-targets organisms being affected and most mosquito species becoming physiologically resistant [4]. In recent times, the use of eco-friendly, biodegradable insecticides from plants to control insect vectors of diseases is gaining importance [5] and botanicals have been found to be effective [6].

Essential oils of many plants have shown larivicidal activity against various mosquito species [7-20]. Ansari *et al.* [21] studied the larvicidal action of *Dalbergia sissoo* oil against *Anopheles stephensi, Aedes aegypti* and *Culex quinquefasciatus*. Essential oil of *Ocimum sanctum* showed larvicidal activity against *Cx. quinquefasciatus*, *Ae. aegytpi* and *An. stephensi* [22]. Neem oil showed larvicidal activity against *Cx. quinquefasciatus* [23]. Carvalho *et al.* [14] reported the larvicidal activity of the essential oil from *Lippia sidoides* against *Ae. aegypti*. Cheng *et al*. [24] reported the bioactivity of fourteen essential plant oils against the yellow fever mosquito larvae of *Ae. aegypti* and all essential oils screened was found to be effective. The essential oil of *Ipomoea cairica* was found to be more toxic to the larvae of *Cx. tritaeniorhynchus* followed by *Ae. aegypti* and *An. stephensi* [25]. Dharmagadda *et al*. [26] reported that larvae of *An. stephensi*, *Cx. quinquefasciatus* and *Ae. aegypti* exposed to essential oil of *Tagetes patula*, *Ae. aegypti* was the most sensitive, followed by *An. stephensi* and *Cx. quinquefasciatus*. Therefore, the present work was carried out to study the larvicidal efficacy of plant oils, Cedar wood (*Cedrus atlantica*), Citronella (*Cymbopogon nardus*), Clove (*Myrtus caryophyllum*), *Eucalyptus* (*Eucalyptus globulus*), Lemon grass (*Cymbopogon flexuosus*), Orange (*Citrus sinensis*), Nutmeg (*Myristica fragrans*), Palmarosa (*Cymbopogon martinii*), Pine (*Pinus radiata*) and Tulsi (*Ocimum sanctum*) against the dengue vector, *Ae. aegypti* (L.).

MATERIALS AND METHODS

Plant oils *viz*., Cedar wood, Citronella, Clove, *Eucalyptus*, Lemon grass, Orange, Nutmeg, Palmarosa, Pine and Tulsi were obtained from Government recognized aromatic oil store, Chennai, Tamil Nadu, India. Tests were carried out against laboratory reared vector mosquito, *Ae. aegypti*. Cyclic generation of *Ae. aegypti* was

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maintained at 25-29°C and 80-90% R.H. in insectarium. Larvae were fed larval food (powdered dog biscuit and yeast in the ratio 3:1) and adult mosquitoes on 10 per cent glucose solution. Adult female mosquitoes were periodically blood-fed on restrained albino mice for egg production. Bioassay for the larvicidal activity was carried out using WHO [27] procedure with slight modifications. A series of concentrations, 125, 250, 500 and 1000 ppm were prepared. Twenty early third instar larvae were introduced in 250 ml beaker containing 200 ml of water with each concentration. Control was prepared by the addition of Tween 80 to water. A total of three trials were carried out with three replicates per trial. Mortality was recorded after 24 hours. When the control mortality ranged from 5-20 per cent, the observed percentage mortality was corrected using Abbott's formula [28]. SPSS 11.5 version was used for determination of LC_{50} and LC_{90} values [29]. The data obtained was subjected to analysis of variance and results with $P<0.05$ were considered to be statistically significant.

RESULTS AND DISCUSSION

All plant oils tested at various concentrations were found be effective against the larvae of *Aedes aegypti*. No larval mortality was observed in control (Table 1). The LC_{50} and LC_{90} values of the plant oils are presented in Table 2. Among the plant oils tested, orange exhibited the highest larvicidal activity with LC_{50} of 85.93, followed by palmarosa with 88.78, tulsi with 92.48 and nutmeg with 93.62 ppm.

The results of the present study were comparable with the LC_{50} values of previous reports. Cavalcanti *et al*. [15] screened essential oils of Brazilian plants *viz*., *Ocimum gratissimum* (60 ppm), *Lippia sidoides* (63 ppm), *Ocimum americanum* (67 ppm), *Cymbopogon citrates* (69 ppm), *Hyptis suaveolens* (261 ppm), *Alpinia zerumbet* (313 ppm), *Syzygium jambolana* (433 ppm), *Citrus limonia* (519 ppm) and *Citrus sinensis* (538 ppm) and found their LC_{50} values to be effective against the larvae of *Ae. aegypti*. Essential oils of eleven local plants were

Table 1: Larvicidal efficacy of plant oils against *Aedes aegypti* larvae

			Concentration (ppm) Mean larval mortality ±S.D.			
Plant oils	Scientific Name	Plant Family	125	250	500	1000
Cedar wood	*Cedrus atlantica*	Pinaceae	8.00 ± 0.00^{b} (40.0)	8.33 ± 0.57^{b} (41.6)	8.66 ± 0.57^{b} (43.3)	9.00 ± 0.00^{b} (45.0)
Citronella	*Cymbopogon nardus*	Poaceae	6.00 ± 0.00^{bc} (30.0)	6.00 ± 0.00^{bc} (30.0)	6.66 ± 0.57^{c} (33.3)	6.66 ± 0.57^{c} (33.3)
Clove	*Myrtus caryophyllum*	Myrtaceae	15.00 ± 0.00^{bc} (75.0)	15.66 ± 0.57^{cd} (78.3)	16.33 ± 0.57^{d} (81.6)	17.66 ± 0.57^{e} (88.3)
Eucalyptus	*Eucalyptus globulus*	Myrtaceae	15.66 ± 0.57^{d} (78.3)	18.33 ± 0.57^{e} (91.6)	20.00 ± 0.00^{f} (100.0)	20.00 ± 0.00^{f} (100.0)
Lemon grass	*Cymbopogon flexuosus*	Poaceae	14.66 ± 0.57^{d} (73.3)	15.66 ± 0.57^{e} (78.3)	16.66 ± 0.57^{f} (83.3)	17.66 ± 0.57^{g} (88.3)
Nutmeg	*Myristica fragrans*	Myristicaceae	16.66 ± 0.57^{b} (83.3)	19.33 ± 0.57^{c} (96.6)	20.00 ± 0.00^{c} (100.0)	20.00 ± 0.00^{c} (100.0)
Orange	*Citrus sinensis*	Rutaceae	18.00 ± 0.00^{b} (90.0)	19.33 ± 0.57^{c} (96.6)	20.00 ± 0.00^{c} (100.0)	20.00 ± 0.00^{c} (100.0)
Palmarosa	*Cymbopogon martinii*	Poaceae	17.66 ± 0.57^{b} (88.3)	19.33 ± 0.57^{c} (96.6)	20.00 ± 0.00^{c} (100.0)	20.00 ± 0.00^{c} (100.0)
Pine	*Pinus radiata*	Pinaceae	13.33 ± 0.57^{b} (66.6)	14.66 ± 0.57^{c} (73.3)	16.33 ± 0.57^{d} (81.6)	17.66 ± 0.57^{e} (88.3)
Tulsi	*Ocimum sanctum*	Lamiaceae	17.66 ± 0.57^{bc} (88.3)	19.00 ± 0.00^{c} (95.0)	19.33 ± 0.57^{c} (96.6)	20.00 ± 0.00^{c} (100.0)
Control			0.00 ± 0.00^{a}	0.00 ± 0.00^{a}	0.00 ± 0.00^{a}	0.00 ± 0.00^{a}

Values are mean of three replicates of three trials ±standard deviation and figures in parentheses denote per cent larval mortality. ANOVA followed by Duncan mortality range test (DMRT) performed. Different superscripts in the column indicate significant difference at $P<0.05$ levels

Table 2: Probit analysis of plant oils against *Aedes aegypti* larvae

Plant oils	Scientific Name	Plant Family	LC_{50} (ppm)	LC_{90} (ppm)
Cedar wood	*Cedrus atlantica*	Pinaceae	947.09	2625.10
Citronella	*Cymbopogon nardus*	Poaceae	1374.05	3247.01
Clove	*Myrtus caryophyllum*	Myrtaceae	135.20	771.65
Eucalyptus	*Eucalyptus globulus*	Myrtaceae	106.21	198.76
Lemon grass	*Cymbopogon flexuosus*	Poaceae	138.36	759.69
Nutmeg	*Myristica fragrans*	Myristicaceae	93.62	164.07
Orange	*Citrus sinensis*	Rutaceae	85.93	153.26
Palmarosa	*Cymbopogon martinii*	Poaceae	87.88	156.05
Pine	*Pinus radiata*	Pinaceae	182.28	784.42
Tulsi	*Ocimum sanctum*	Lamiaceae	92.48	232.18

evaluated for larvicidal activity against *Ae. aegypti* early fourth instar larvae and their LC_{50} and LC_{90} values ranged from 63.7 to 162.9 ppm with *Ocimum lamiifolium* and *Chenopodium ambrosioides* exhibiting highest larvicidal effects with LC_{50} of 47.3 and LC_{90} 97.9 ppm [30]. Essential oil from *Mentha piperita* showed larvicidal action against *Ae. aegypti* with LC_{50} and LC_{90} values of 111.9 and 395.1ppm after 24 hour exposure [31]. Govindarajan [32] studied the larvicidal activity of essential oils from four plants and the highest activity was observed in essential oil from *Zingiber officinale* against *Cx. tritaeniorhynchus* and *An. subpictus* with LC_{50} and LC_{90} values as 98.83, 57.98 and 186.55, 104.23 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 [33]. Larviciding is a successful method of reducing mosquito population in their breeding places before they emerge into adults [34]. 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 [35] 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.

Essential oils of many plants were observed to have mosquito larvicidal property [36] and have received attention as potentially controlling vectors of mosquito-borne disease [37]. Therefore, the use of plant oils in insect/mosquito control is an alternative pest control method for minimizing the noxious effects of some pesticidal compounds on the environment [38]. 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. Moreover, these results could be useful in the search for newer, more selective and biodegradable larvicidal natural compounds.

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