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Preliminary screening of plant essential oils against larvae of *Culex quinquefasciatus* Say (Diptera: Culicidae)

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Preliminary screenings of 22 plant essential oils were tested for mortality of the mosquito larvae *Culex quinquefasciatus* under laboratory conditions. Percent (%) mortality of the mosquito larvae were obtained for each essential oil. At different exposure periods, viz. 1, 3, 6, 12 and 24 h among the 22 plant oils tested, eight oils viz., aniseed, calamus, cinnamon, clove, lemon, orange, thyme, and tulsi oils gave promising results on larvicidal activity. For larvicidal screening bioassay, the mortality was recorded at different exposure periods viz., 1, 3, 6, 12 and 24 h, and it was found that larval mortality increased when exposure time increased. The clove oil was found to be the most effective treatment. In the preliminary screening, clove oil gave 100% mortality at all exposure periods. Vetiver oil recorded 36.2, 61.2, 76.2, 87.5 and 100% mortality in 1, 3, 6, 12 and 24 h, respectively. Results of this study show that the essential oils may be a potent source of natural larvicides.

Key words: Screening, essential oils, *Culex quinquefasciatus,* Larvicidal.

INTRODUCTION

Culex quinquefasciatus, a domestic mosquito mainly found in urban areas is a vector of human filariasis in India. *C. quinquefasciatus* acts as a vector of *Wucherreria bancrafti, Brugia malayi* and *Brugia timori,* which are responsible for lymphatic filariasis, a prevalent disease in India. Filariasis is an endemic disease in many parts of India especially in Kerala, Mysore, Tamil Nadu, Andhra Pradesh and Maharashtra states. Lymphatic filariasis infects 80 million people annually, of which 30 million cases exist in chronic infection. In India, 45 million cases of lymphatic filariasis have been recorded (Bowers et al., 1995).

The plant world comprises a rich storehouse of phytochemicals which are widely used as alternatives to synthetic insecticides. Secondary metabolites obtained from plants have shown proven mosquito control potential. *Chloroxylons wietenia* DC. (Rutaceae) is a medicinal as well as an aromatic tree of dry deciduous forests. It is commonly known as East Indian Satin Wood. The potential mosquitocidal activity of *C. wietenia*, is widely known in tribal areas of South India, where it is common for people in these forest areas to hang leaf garlands in their houses to eradicate mosquitoes and other insects (Ansari and Razdan, 1995).

Pyrethrin based products have been widely used to protect people from mosquito bites through their repellent and killing effects. Many other material products of botanical origin especially, essential oils hold significant promise in insect vector management (Skinner and Johnson, 1980). Kiso and Hikino (1991) have reported that the compounds vinblastine and vincristine obtained from *Catharanthus roseus* inhibited cell division in

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Table 1. Plant essential oils selected for the bioassay experiments.

Botanical name	Family	Common name
Pimpinella anisum	Apiaceae	Aniseed
Citrus bergamia	Rutaceae	Bergamot
Acorus calamus	Aeraceae	Calamus
Cinnamomum camphora	Lauraceae	Camphor
Cedrus atlantica	Pinaceae	Cedarwood
Cinnamomum veerum	Lauraceae	Cinnamon
Cybopogon nardus	Poaceae	Citronella
Myrtus caryophyllus	Myrtaceae	Clove
Eucalyptus globules	Myrtaceae	Eucalyptus
Pelargonium graveollens	Geraniaceae	Geranium
Citrus limon	Rutaceae	Lemon
Cybopogon flexuosus	Poaceae	Lemongrass
Citrus aurantifolia	Rutaceae	Lime
Gaultheria frgagrantissima	Acanthaceae	Luchi
Myritica fragrans	Myristiceae	Nutmeg
Citrus sinensis	Rutaceae	Orange
Cybopogon martini	Lamiaceae	Palmarosa
Pinus radiate	Pinaceae	Pine
Rosmarinus officinalis	Lamiaceae	Rose mary
Thymus vulgaris	Labiatae	Thyme
Ocimum sanctum	Lamiaceae	Tulsi
Vetiveria zizanioides	Poaceae	Vetiver

fertilized mosquito eggs. Tulsi (*Ocimum sanctum*) is known to have various insectidal properties (Saxena et al., 1979). Extracts of *Vitex* species and *Ocimum* species leaves have been traditionally known as natural insecticides. Indian farmers have been using various plant extracts as insecticides and pesticides (Ghosh, 2000).

Many studies have shown the bioactivity of essential oils against mosquitoes as growth inhibitors and/or larvicides, adulticides, repellents, or oviposition deterrents (Sukumar et al., 1991; Carvalho et al., 2003; Cavalcanti et al., 2004; Ansari et al., 2005). Essential oils from a large number of plants, including *Ocimum* spp, *Cymbopogon* spp), *Eucalyptus maculate citriodon Pelargonium citrosum*, *Artemisia vulgaris*, *Lantana camara*, *Mentha piperita*, *Vitex rotundifolia*, *Curcuma* spp. (Pitasawat et al., 2003), *Conyza newii*, *Plectranthus marrubioides*, *Tetradenia riparia*, *Tarchonanthus camphoratus*, *Lippia javanica* and *Lippia ukambensis* (Omolo et al., 2004), have been demonstrated to exhibit good repellent properties against vector mosquitoes. Paula et al. (2003) reported the chemical composition, toxicity and mosquito repellency of *Ocimum selloi* oil. *Ocimum* essential oils have been traditionally used to kill or repel insects, and also to flavour food products.

In the present investigation, the essential oils were selected in order to find out a new mosquitocidal compound against filarial vector mosquito *C. quinquefasciatus*. The plant essential oil is largely cultivated throughout India and in all tropical countries.

MATERIALS AND METHODS

Plant essential oils

The plant essential oils were obtained from Tegraj and Co (P) Ltd, Chennai,Tamil Nadu. Based on various biological effects on larvae, the 22 essential oils were selected (Table 1).

Maintenance of larvae

The larvae of laboratory stock culture were reared in plastic troughs (size 18 cm diameter x 19 cm height) and were maintained at 27 ± 1°C, 75 to 85% RH, under 14:10 L/D photoperiod cycles. The larvae were fed with dog biscuits and yeast at 3:1 ratio (per weight). Water was changed alternate days. The breeding medium was regularly checked and dead larvae were removed at sight and the troughs in which larvae were maintained were kept closed with muslin cloth to prevent contamination through foreign mosquitoes.

Screening assay

The selected plant oils were screened for larval toxicity by following standard procedure as follows: In the preliminary screening a single dose (500 ppm) of each oil was tested. Twenty fourth instar larvae of *C. quinquefasciatus* were kept in a 500 ml glass beaker containing 249 ml of dechlorinated water and 1.0 ml of desired plant

Table 2. Preliminary screening of plant essential oils against fourth instar larvae of *Culex quinquefasciatus* at 500 ppm in different exposure periods.

Essential oil	Larval mortality (%)				
	1 h	**3 h**	**6 h**	**12 h**	**24 h**
Aniseed	91.2 ± 0.6[g]	100 ± 0.0[j]	100 ± 0.0[m]	100 ± 0.0[k]	100 ± 0.0[j]
Bergamot	11.2 ± 0.2[bc]	18.7 ± 0.7[bc]	30.0 ± 0.5[gh]	45.0 ± 0.4[e]	61.2± 0.8[efg]
Calamus	21.2 ± 0.2[de]	32.5 ± 0.2[e]	43.7 ± 0.3[j]	66.2 ± 0.4[f]	100 ± 0.0[j]
Camphor	0[a]	2.25 ± 0.0[a]	7.5 ± 0.2[bc]	20.0 ± 0.9[bc]	38.7± 0.4[c]
Cedarwood	18.7 ± 0.4[c]	30.0±0.2[e]	40.0 ±0.3[j]	58.7 ± 0.6[f]	68.7 ±0.8[hi]
Cinnamon	47.5 ± 0.8[g]	83.7± 0.5[a]	100 ± 0.0[m]	100 ± 0.0[k]	100 ±0.0[j]
Citronella	8.75 ± 0.3[c]	20.0 ± 0.5[cd]	35.0 ± 0.4[hi]	52.5 ± 0.6[f]	93.7± 0.4[j]
Clove	100 ± 0.0[h]	100 ± 0.0[i]	100 ± 0.0[m]	100 ± 0.0[k]	100 ± 0.0[j]
Eucalyptus	0[a]	3.7 ± 0.2[a]	12.5 ± 0.5[cd]	26.2 ± 0.4[c]	38.8 ± 0.4[c]
Geranium	0[a]	2.5±0.4[a]	15.0 ± 0.5[de]	25.0 ± 0.4[c]	36.2 ± 0.7[c]
Lemon	23.7 ± 0.2[c]	40.0 ± 0.5[f]	53.7 ± 0.7[k]	86.2 ± 0.8[i]	100 ± 0.0[j]
Lemongrass	0[a]	1.2 ± 0.3[a]	3.75 ± 0.4[ab]	16.2 ± 0.6[b]	28.7± 0.5[b]
Lime	13.7 ± 0.4[bc]	22.5 ± 0.2[b]	38.7 ± 0.6[ij]	57.5 ± 0.6[f]	67. ± 0.6[g]
Luchi	5.0 ± 0.4[c]	21.2 ± 0.4[d]	33.7 ± 0.7hi	58.75 ± 0.6[f]	73.7 ± 1.1[i]
Nutmeg	10 ± 0.8[b]	15.0 ± 0.9[bcd]	30.0 ± 0.9[gh]	40.0 ± 0.8[d]	56.2 ± 0.2[def]
Orange	28.7 ± 0.2[cd]	43.7 ± 0.8[f]	58.7 ± 0.2[k]	75.0 ± 0.4[h]	100 ± 0.0[j]
Palmarosa	5.0 ± 0.8[bc]	12.5 ± 0.9b[cd]	28.7 ± 0.9[gh]	37.5 ± 0.8[d]	52.5 ± 0.2[d]
Pine	2.7 ± 0.2[ab]	11.2 ± 0.2[b]	21.2 ± 0.4[ef]	35.0 ± 0.7[d]	55.0 ± 0.7[de]
Rose mary	1.2 ± 0.4[b]	13.7 ± 0.7[c]	25.0 ± 0.4f[g]	36.2 ± 0.7[d]	82.5 ± 0.5[fg]
Thyme	42.5 ± 0.2[f]	57.5 ± 0.6[g]	68.7 ± 0.2l	80.0 ± 0.4[hi]	100 ± 0[j]
Tulsi	47.5 ± 0.2[ef]	68.7 ± 0.6i	92.5 ± 0.6[m]	100 ± 0.0[k]	100 ± 0[j]
Vetiver	36.2 ± 0.4[ef]	61.2 ± 0.6[g]	76.2 ± 0.8[m]	87.5 ± 0.6[j]	100 ± 0[j]
Reference pesticide (temephos 1 ppm)	40.0 ± 0.8[ef]	65.0 ± 0.4[hi]	100 ± 0.0[m]	100 ± 0[k]	100 ± 0[j]
Control (Tween 80 0.01%)	0[a]	0[a]	0[a]	0[a]	0[a]

Each value (mean ± S.E.) represents mean of four replicates, Mean values followed by same letters in a column are not significantly different at *P*<0.05 level (Tukey's test).

essential oil concentrations. The larvae were collected by dropper, placed onto filter paper strips and immediately transferred to the beaker containing test solutions. Five replicates for each concentration were maintained. In the control, 1.0 ml of Tween 80 (0.01%) in 249 ml of dechlorinated water was added. Mortality of larvae was recorded after 1, 3, 6, 12 and 24 h of treatment. While recording the percentage mortalities for each concentration, the moribund and dead larvae in five replicates were combined. It has been described that dead larvae are those that cannot be induced to move when they are probed with a needle in the siphon or the cervical region; moribund larvae are those incapable of rising to the surface (Hasan and Deo, 1994).

Statistical analysis

The larvicidal mortality was corrected by Abbott's formula (Abbott, 1925). Percentages of mortality were determined and transformed to arcsine square root values for analysis of variance (ANOVA). Tukey's test ($P < 0.05$) was used to analyze for significant differences among the test essential oils against mosquitoes.

RESULTS AND DISCUSSION

All the 22 plant oils were screened against the larvae of *C. quinquefasciatus* for larvicidal activity. The results are given in Table 2. Among the 22 plant oils tested, eight oils viz., aniseed, calamus, cinnamon, clove, lemon, orange, thyme, and tulsi oils gave promising results on larvicidal activity. For the larvicidal screening bioassay, the mortality was recorded at different exposure periods viz., 1, 3, 6, 12 and 24 h, and it was found that larval mortality increased when exposure time increased. The clove oil was found to be the most effective treatment. In the preliminary screening, clove oil gave 100 percent mortality at all exposure periods.

Vetiver oil recorded 36.2, 61.2, 76.2, 87.5 and 100% mortality in 1, 3, 6, 12 and 24 h, respectively. The lowest mortality of 28.7% was recorded at 500 ppm concentration in lemongrass oil after 24 h exposure. In the temephos treatment, larval mortality of 40, 65 and 100% was recorded at 1 ppm concentration in 1, 3 and 6 h treatments.

Vector control by means of chemicals is creating many unwanted effects including emergence of pesticide resistance in vector mosquitoes (Chandra et al., 1998). Botanical insecticides may serve as suitable alternatives to synthetic insecticides in future as they are relatively safe, biodegradable and are readily available in many areas of the world (Prabakar and Jebanesan, 2004). Interest in the development of natural products has been revived during the last two decades. Plants are considered as rich source of bioactive chemicals and they may be an alternative source of mosquito control agents. Natural products of plant origin with insecticidal properties have been tried in the recent past for control of variety of insect pests and vectors (Wink, 1993). More than 2000 plant species have been reported to possess chemicals with pest control properties (Ahmed et al., 1984) and among them about 344 species of plants have been known to possess some degree of activity against the mosquitoes (Sukumar et al., 1991).

Though several plants from different families have been reported for insecticidal activity only a few botanicals like neem based insecticides have moved from the laboratory to field use, which might be due to the light and heat stability of neem compounds compared to synthetic insecticides (Green et al., 1991). In the present study, 22 essential oils registered larvicidal, pupicidal, adulticidal, ovicidal, oviposition deterrent, growth regulator regulating and histopathological effects. Plenty of literature is available with regard to bioefficacy of volatile oils against vector mosquitoes.

The most successful method to minimize the incidence of mosquito borne diseases is by means of eradicating the mosquito vector through systematic treatment of their breeding places with the help of larvicides (Cetin et al., 2004). Chemical pesticides that are applied in water bodies are not only killing mosquito larvae but also killing non-target organisms. In addition chemical pesticides select for resistance in mosquitoes. As an alternative to chemical pesticides volatile oils are largely studied against *C. quinquefasciatus* mosquito. In the present study, the larvicidal activity was tested at 500 ppm concentration. Among the 22 essential oils, eight oils registered high larvicidal activity. The larval mortality was found to be directly proportional to the exposure period. Clove oil was found to be the most toxic to the larvae since it registered 100% mortality from 1 h treatment period onwards.

The present investigation revealed that the essential oils possess remarkable larvicidal activity against the tested mosquito *C. quinquefasciatus*. The control of selected mosquito by affecting their survival by essential oils was found evident. Further purification and characterization of the bioactive compound of effective oils are underway in our laboratory.

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