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Ovicidal and oviposition response activities of plant volatile oils against *Culex quinquefasciatus* say.

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Abstract
The ovicidal and oviposition response of plant volatile oils was evaluated under laboratory conditions against *Culex quinquefasciatus* Say. Ten oils viz., aniseed, calamus, cinnamon oil, citronella, clove oil, lemon, orange, thyme, tulsi and vertiver were tested for ovicidal and oviposition response assays at five different concentrations viz., 12.5, 25, 50, 100 and 200 ppm concentrations. Among the ten oils clove, aniseed and cinnamon oils registered highest (100 ± 0.0 %) ovicidal activity at 200 ppm. Lemon (97.5 ± 0.5%) and tulsi (91.2 ± 0.6 %) oils were also highly effective against *C. quinquefasciatus* eggs at 200 ppm. Maximum oviposition response activity (100 ± 0.0%) was obtained in clove oil. The results indicate that both ovicidal and oviposition activity were dose dependent. It can be inferred from the investigation that the plant volatile oils are an effective agent for ovicidal and oviposition response against *C. quinquefasciatus* and further studies on identification of active compounds and field trials are needed to recommend the development of eco-friendly chemicals of botanical origin.

Keywords: Ovicidal, Oviposition response, Volatile oils, Dose response assay, *Culex quinquefasciatus*

1. Introduction

Mosquitoes are insect vectors that transmit deadly diseases like malaria, Japanese encephalitis, yellow fever, dengue and filariasis [1]. Mosquito borne diseases are still a major problem in the world, particularly in tropical and subtropical regions and WHO has declared the mosquitoes as "Public Enemy Number One", [2]. A major portion of the National Health budget is spent for the control of these diseases. Sustainable reduction in vector mosquito population and mosquito borne diseases can be achieved by judicious use of various chemical control measures [3]. Synthetic insecticides are used by the community because they are practical in use and rapid in action. However, the use of synthetic insecticides has led to environmental pollution, and contamination. On the contrary, there are reports from many countries about the occurrence of insecticide resistance. Most of the available synthetic insecticides kill only adult mosquitoes, and only a few kill mosquito larvae [4, 5, 6].

In this context, screening of natural products has received the attention of researchers around the world, but seems to be particularly important for public health in developing countries. Since many diseases transmitted by insects (e.g., malaria, dengue, yellow fever, leishmaniasis and Chagas disease) are endemic in developing countries, the search for insecticides and repellents of botanical origin has been driven by the need to find new products that are effective, but also safer and cheaper than current products [7]. In recent decades, research on the interactions between plants and insects has revealed the potential use of plant metabolites or allelochemicals for this purpose [8]. It is known that some chemical constituents of essential oils have insecticidal properties [9].

In some studies, essential oils obtained from commercial sources have been used. Specific compounds isolated from plant extracts or essential oils have been tested for fumigation purposes [10].The ovicidal activity of neem products (azadirachtin) has been evaluated against *C. pipiens* in Egypt [11, 12, 13]. Ovicidal effects of neem extracts against mosquito eggs [14, 15] have also been investigated in some parts of the world. Zebitz [16] exposed *Culex* egg rafts to water treated with neem leaves extracts and reported remarkable reduction in hatchability. Oviposition is one of the most important events in the life cycle of mosquitoes. If oviposition is prevented, the mosquito life cycle is disrupted and population growth reduced [17]. Botanical insecticides may serve as suitable alternatives to synthetic insecticides in future as they are relatively safe, biodegradable and readily available in many areas of the world [18]. 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 may be an alternative source of mosquito control agents. Thus, the environmental friendly and biodegradable natural insecticides of plants origin have been receiving attention as an alternative green measure for controlling arthropods of public health importance [19].

Plant essential oils are natural volatile substances obtained from a variety of plants and have been recognized as an important natural resource of insecticides [20]. Research on the use of plant essential oils to control mosquitoes has increased in recent years. This is especially true for the use of natural products based on plant essential oils (EOs) as larval mortality has been observed at different concentrations [21, 22]. A wide number of medicinal plants have not had their mosquitocidal potential assessed. Thus, the key objective of this study was to investigate the ovicidal and oviposition response of volatile oils from ten Indian herbal plants against filaria vector mosquito, *Culex quinquefasciatus* Say.

2. Material & Methods

2.1 Volatile oils

The plant volatile oils (VOs) used in this study was procured from Tegraj & Co (P) Ltd, India (commercial producers of plant VOs and aromatic substances). The ten volatile oils selected for study were Aniseed (*Pimpinella anisum* Linn.), Calamus (*Acorus calamus*), Cinnamon (*Cinnamomum verum*), Citronella (*Cymbopogon nardus*), Clove (*Myrtus caryophyllus*), Lemon (*Citrus limon*), Orange (*Citrus sinensis*), Thyme (*Thymus vulgaris*), Tulsi (*Ocimum sanctum*) and Vetiver (*Vetiveria zizanioides*).

2.2 Test Insect

The test organism *C. quinquefasciatus*, was reared continuously from several generations in the Entomology Research Institute, Loyola College, Chennai-34, India. They were free of exposure to pathogens and insecticides and maintained at 25±2 °C and 60-80% relative humidity. The larvae were fed on dog biscuits and yeast powder in a ratio 3:2 until moulting to become pupae, which were then transferred into a mosquito cage. The pupae were transferred from culture trays to glass beakers containing tap water and placed in screened cages (45 x 45 x 45 cm), where adults emerged. The cage was made up of metal frames and covered with a muslin cloth. The emergent adults were fed with 10% glucose solution dipped in a piece of cotton [21].

2.3 Ovicidal activity

Ovitraps were kept in the cages after 2 days of blood meal given to the female mosquitoes. Egg rafts were obtained from the ovitraps. Freshly laid egg rafts of 0 to 3 h were collected between 4: 00 A.M and 7: 00 A. M. The egg rafts were carefully removed from the piece of filter paper with a brush. The eggs of same age group (3 h old) were exposed for 4 days to different concentrations of test solutions. Based on the preliminary results, five doses viz., 12.5, 25, 50, 100 and 200 ppm were used for dose response bioassay test. In each treatment, 100 eggs were used for each treatments and four replicates maintained. Hatching rate was calculated on the basis of non–hatchability of eggs according to Sahgal and Pillai [23]. The control mortality, if any was corrected by using Abbott formula [24].

$$\text{Percent egg hatchability} = \frac{\text{Number of eggs hatched}}{\text{Number of eggs released}} \times 100$$

$$\text{Corrected ovicidal activity (\%)} = \frac{\text{Larvae hatched in control (\%)} - \text{larvae hatched in treatment (\%)}}{100 - \text{Larvae hatched in control (\%)}} \times 100$$

2.4 Oviposition response activity

Effect of volatile oils (VOs) on the oviposition response of gravid female mosquito *C. quinquefasciatus* was determined. Based on the preliminary results five doses viz., 12.5, 25, 50, 100 and 200 ppm were used for dose response bioassay. Twenty gravid females were introduced into colony cages. Enamel bowls containing 250 ml of dechlorinated water with different desired concentrations of plant VOs were placed in different cages (45 x45 x 45 cm) and a bowl with dechlorinated water and Tween80 (0.01%) served as control. The number of egg rafts found in treatment and control were recorded. The position of the bowl was interchanged during different replicates so as to nullify any effect of position on oviposition. Each set of experiment was replicated 4 times. The percentage of Oviposition response (% OR) for each concentration was calculated (Xue *et al.,* 2001) as follows [17]:

$$\text{Percent Oviposition deterrent (OD)} = \frac{NC - NT}{NC} \times 100$$

NC=No. of eggs in control
NT=No. of eggs in treatment

2.5 Data analysis

Statistical analysis was performed using SPSS software package, version 15. The values were analyzed by one way analysis of variance (ANOVA) followed by Duncan's multiple range test [25]. All the results were expressed as mean ± SE of four replicates in each treatment. *P*-Values 0.05 were considered as significant.

3. Results

3.1 Ovicidal Activity

Ten oils viz., aniseed, calamus, cinnamon oil, citronella, clove oil, lemon, orange, thyme, tulsi and vetiver were tested for ovicidal activity at five different concentrations viz., 12.5, 25, 50, 100 and 200 ppm. Among the effective oils clove, aniseed and cinnamon oils registered 100 percent ovicidal activity at 200 ppm. Lemon (97.5 ± 0.5%) and tulsi (91.2 ± 0.6%) oils were also highly effective against *C. quinquefasciatus* eggs at 200 ppm. In all treatments the ovicidal activity was concentration dependent. The lowest ovicidal activity was recorded in calamus oil (77.8 ± 0.2%) at 200 ppm (Table 1).

Table 1: Ovicidal activity of volatile oils against *C. quinquefasciatus*

Essential oils	% Ovicidal activity (mean ± S.E.)				
	12.5 ppm	**25 ppm**	**50 ppm**	**100 ppm**	**200 ppm**
Aniseed	45.2 ± 0.4^{d}	55.0 ± 0.4^{d}	66.5 ± 0.4^{de}	80.0 ± 0.4^{ef}	100 ± 0.0^{d}
Calamus	23.7 ± 0.4^{bc}	33.7 ± 0.4^{cd}	45.0 ± 0.7^{b}	60.0 ± 0.4^{bc}	77.8 ± 0.2^{b}
Cinnamon	36.5 ± 0.4^{d}	47.8 ± 0.64^{e}	60.0 ± 0.4^{cde}	77.5 ± 0.50^{e}	100 ± 0.0^{d}
Citronella	30.0 ± 0.7^{cd}	41.2 ± 0.2^{de}	55.0 ± 0.4^{bcd}	67.5 ± 0.6^{cd}	83.75 ± 0.4^{b}
Clove	46.2 ± 0.4^{e}	62.5 ± 0.2^{ef}	78.75 ± 1.3^{g}	92.5 ± 0.6^{g}	100 ± 0.0^{d}
Lemon	40.0 ± 0.4^{de}	57.5 ± 0.6^{de}	76.25 ± 0.4^{fg}	87.5 ± 0.5^{fg}	97.5 ± 0.5^{cd}
Orange	16.2 ± 1.1^{ab}	36.2 ± 1.1^{cd}	51.25 ± 0.8^{ab}	57.5 ± 0.8^{b}	81.25 ± 0.7^{b}
Thyme	32.5 ± 1.1^{cd}	42.5 ± 1.1^{de}	51.25 ± 1.0^{ab}	68.7 ± 0.7^{d}	82.4 ± 0.4^{b}
Tulsi	36.2 ± 0.4^{d}	45.0 ± 0.4^{e}	53.7 ± 0.4^{ab}	73.5 ± 0.2^{de}	91.2 ± 0.6^{c}
Vetiver	35.0 ± 0.4^{d}	51.2 ± 0.4^{ab}	68.5 ± 0.6^{ef}	82.5 ± 0.8^{ef}	96.2 ± 0.4^{cd}
Control (Tween 80 0.001%)	5.1 ± 0.0^{a}				

Each value represent mean of four replicates, Mean values followed by same letters in a column are not significantly different at $P<0.05$ level (DMRT).

3.2 Oviposition response activity

The dose dependent oviposition activity of seven effective volatile oils (VOs) against *C. quinquefasciatus* is presented in Table 2. It is clear that percent oviposition response activity was maximum in clove oil. The activity was concentration dependent. The oviposition activity of clove oil was recorded as (46.3 ± 0.2, 63.6 ± 0.2, 77.1 ± 0.4, 88.4 ± 0.4 and 100 ± 0.0%) at 12.5, 25, 50,100 and 200 ppm respectively. Aniseed oil also registered 100 ± 0.0% oviposition deterrent activity at 200 ppm concentration. Cinnamon (96.8 ± 0.2%) and Tulsi (94.7 ± 0.2%) oils were registered. At the highest dose, more than 95% oviposition response activity was found at 200 ppm concentration. Among the seven oils, Citronella was the least effective (7.7 ± 0.2%).

Table 2: Oviposition response of effective volatile oils against gravid mosquito

Essential oils	% Oviposition (mean ± S.E.)				
	12.5 ppm	**25 ppm**	**50 ppm**	**100 ppm**	**200 ppm**
Aniseed	36.7 ± 0.7^{b}	55.5 ± 0.7^{ab}	70.1 ± 0.7^{ab}	85.0 ± 0.4^{ab}	100 ± 0.0^{a}
Calamus	26.8 ± 0.6^{de}	37.9 ± 0.2^{c}	46.0 ± 0.2^{c}	64.7 ± 0.4^{c}	84.4 ± 0.2^{b}
Cinnamon	35.1 ± 0.5^{b}	58.0 ± 0.4^{ab}	72.4 ± 0.6^{a}	84.9 ± 0.2^{ab}	96.8 ± 0.2^{a}
Citronella	7.70 ± 0.2^{d}	19.0 ± 0.1^{d}	31.1 ± 0.4^{d}	46.2 ± 0.2^{d}	79.6 ± 0.2^{b}
Clove	46.3 ± 0.2^{a}	63.6 ± 0.2^{a}	77.1 ± 0.4^{a}	88.4 ± 0.4^{a}	100 ± 0.0^{a}
Tulsi	39.7 ± 0.4^{ab}	48.5 ± 0.5^{abc}	58.0 ± 0.2^{bc}	77.1 ± 0.4^{abc}	94.7 ± 0.2^{a}
Vetiver	28.4 ± 0.4^{c}	41.2 ± 0.3^{bc}	52.7 ± 0.4^{c}	73.2 ± 0.2^{bc}	84.4 ± 0.5^{b}
Control (Tween 80 0.001%)	0.0 ± 0.0^{a}				

Each value represent mean of four replicates, Mean values followed by same letters in a column are not significantly different at $P<0.05$ level (DMRT).

4. Discussion

In the present study volatile oils registered ovicidal and oviposition response effects. Plenty of literature is available with regard to bioefficacy of volatile oils against vector mosquitoes. Different volatile oils showed differential toxicity against different life stages of mosquito. All essential oils used in this study reduced the egg hatching in mosquito eggs. However clove oil was toxic to all life stages and also showed different kinds of activity. It is evident from the present data that the exposure of mosquito eggs to the plant oils elicits egg mortality. In oviposition response test, the volatile oil of clove oil greatly reduced the number of eggs deposited by gravid *C. quinquefasciatus*. At the highest concentration (200 ppm), egg laying was reduced to 100%. It can be concluded that dose of 200 ppm could be used for achieving the desired level of control of this mosquito by ovicidal action and reduced egg laying.

Sahal and Pillai [23] have reported the ovicidal activity of permethrin and deltamethrin against the mosquitoes *Aedes aegypti, C. quinquefasciatus* and *Anopheles stephensi.* Ovicidal effects of the seed extract of *Atriplex canescens* was reported against *C. quinquefasciatus* [26]. Su and Mulla [27] reported the ovicidal activity of neem product, azadirachtin, against the mosquitoes *C. tarsails* and *C. quinquefasciatus. Solanum trilobatum*, a thorny shrub that is widely spread in India, has been screened for its ovicidal activity against *C.* mosquitoes [28]. Differences in susceptibility to ovicides are due to differential rates of uptake, penetration through the chorine, and conversion to active inhibitor, detoxification and failure of the toxicant to reach the target. Grosscurt [29] observed that the efficacy to act on the embryo inside the egg shell depends on efficient penetration of the insecticides, which in turn is influenced by the exposure period; the same trend was observed in the present study also. The current study clearly indicated that the ovicidal activity of the plant oils against the egg rafts of *C. quinquefasciatus* may depend upon three key factors viz., dose of the plant oils, age of egg rafts and period of exposure.

Essential oil of *Cinnamomum zeylanicum* showed oviposition deterrent and repellent activities and the essential oils of *Zingiber officinale* and *Rosmarinus officinalis* also showed both ovicidal and repellent activities against *Anopheles stephensi, Ae. aegypti* and *C. quinquefasciatus* [30]. Tawatsin *et al.*, [31] reported that essential oils extracted from 18 Thai plant species belonging to 11 families exhibited oviposition deterrent activity against *Ae. aegypti* with various degree of repellency ranging from 16.6 to 94.7%. The essential oils of peppermint (*Mentha piperita*), basil (*Ocimum basilicum*), rosemary (*Rosmarinus officinalis*), citronella (*Cymbopogon nardus*) and celery seed (*Apium graveolens*) exhibited oviposition deterrent activity against the dengue fever vector; *Ae. aegypti* [32].

In the present study clove oil showed prominent ovicidal and oviposition response activity, this might be due to the volatile compounds present in the oils. Su and Mulla [27] reported that the neem products containing azadirachtin showed oviposition deterrent activity against *C. tarsalis* and *C. quinquefasciatus.* Senthil Nathan [33] has stated that the essential oils obtained from *Eucalyptus tereticornis* Sm. (Myrtaceae) showed strong oviposition deterrent activity against *Anopheles stephensi* Liston. The essential oil derived from clove was effective against *Ae. aegypti* and the clove, lemongrass and citronella oil were effective against *C. quinquefasciatus*. Govindarajan *et al.* [34] have reported that ovicidal activity of crude extract of *Ervatamia coronaria* exerted 100% mortality at 250, 200 and 150 ppm against *C. quinquefasciatus*, *Ae. aegypti* and *An. stephensi*, respectively. While crude extract of *Caesalpinia pulcherrima* exerted 100% mortality at 375, 300 and 225 ppm for *C. quinquefasciatus*, *Ae. aegypti* and *An. stephensi*, respectively.

Elango *et al.* [35] reported effective oviposition repellency (88.26-96.93%) of the leaf acetone, ethyl acetate and methanol extracts of *Aegle marmelos, Andrographis lineata* and *Cocculus hirsutus* against *An. subpictus* at relatively high dose (500 ppm.) while the lowest repellency (47.14-71.09%) was recorded at 31.25 ppm. Singh and Mittal [36] reported that the seed extract of *Solanum nigrum* greatly reduced the number of eggs deposited by gravid *An. stephensi.* At the highest dose (0.5%) egg lying was reduced up to 99%. Phukerd and Soonwera [37] reported that the oviposition deterrent activity at three different concentrations (1%, 5% and 10%) of *Curcuma zedoaria* essential oil 76.0±52.3, 18.7±16.9 and 9.2±17.3 eggs and essential oil from *Boesenbergia rotunda* lower property with 162.0±34.1, 52.0±13.5 and 59.2±13.7 eggs per cup. From the present study it can be concluded that the essential oils from clove, lemongrass and citronella exhibited effective oviposition deterrent effect against *C. quinquefasciatus*. Further studies on identification of active compounds and field trials are needed to recommend the development of eco-friendly chemicals of botanical origin.

5. Conclusion
In conclusion, the use of herbal volatile oil products that reduces the eggs of adult mosquito. Though oviposition response was observed at higher doses, lower doses greatly exhibit the repellent potential of gravid female mosquito *C. quinquefasciatus*. These results are very promising in developing new, effective and inexpensive approaches to control *C. quinquefasciatus*, and thus filarial vector. Studies on mode of action and compound with biocides under field conditions are in progress.

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