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Mosquito knock-down and adulticidal activities of essential oils by vaporizer, impregnated filter paper and aerosol methods

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Abstract

Essential oils from 12 medicinal plants were evaluated by three different bioassay methods (Vaporizer, Filter paper and Aerosol) for Knock-down and adulticidal efficacy on the filarial vector mosquito, *Culex quinquefasciatus*. Based on screening results the effective plants were selected for investigating Knock-down and adulticidal potential against adult female of the laboratory-reared mosquito species, *Cx. quinquefasciatus*. In vaporizer bioassay method four different doses (1.25, 2.5, 5 and 10%) were used. Four different doses (0.625, 1.25, 2.5 and 10%) were used both filter paper (cm²) and aerosol (cm³) bioassay methods. Five essential oils (calamus, camphor, citronella, clove and eucalyptus) were identified as potential treatments in vaporizer bioassay. The result showed that the knock down time decreased with increased concentration in clove oil treatment; the Knock-down time (KT₅₀ = 46.1 ± 0.1, 38.5 ± 0.1, 30.7 ± 0.2, and 20.1 ± 0.1 minutes) was recorded at 1.25, 2.5, 5 and 10% /cm³ respectively. In filter paper method nine essential oils were identified as potential treatments. After 1 hr exposure period clove oil recorded the lowest median Knock-down time (KT₅₀) which was calculated as 9.15 ± 0.1min/cm². Followed by citronella (KT₅₀ = 11.4 ± 0.1 min) and eucalyptus (KT₅₀ = 11.4 ± 0.1min) oils since they recorded lower median Knock-down time. All the twelve essential oils were identified as potential treatments in aerosol activity. The lethal time decreased when the concentration increased. At 5 % concentration the median lethal time (LT₅₀) for clove oil was calculated as (LT₅₀ = 3.80 ± 0.1minutes). The Cinnamon oil was effective which recorded (LT₅₀ = 1.99 mins) as median lethal time. Camphor (LT₅₀ = 19.6 ± 0.1 min) oil were found to be less toxic by aerosol method. These results suggest that clove oil and cinnamon oil have the potential to be used as a eco-friendly approach for the control of the major important filaria vector *Cx. quinquefasciatus* under laboratory condition.

Keywords: Essential oils, Knock-down, Adulticidal, Vaporizer, Filter paper, Aerosol, *Culex quinquefasciatus*.

1. Introduction

Culex quinquefasciatus, a domestic mosquito mainly found in urban areas, is a vector of human filariasis in India. *Cx. quinquefasciatus* acts as a vector of *Wuchereria bancrofti*, *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. Controls of such serious diseases are becoming increasingly difficult because of the high rate of reproduction and development of resistance to insecticides in mosquitoes^[1]. Chemical control of vector mosquitoes is remaining as a main component of integrated vector management^[2, 3]. However, factors including the development of resistance to insecticides are leading to a rise in morbidity and mortality due to malaria and other vector-borne infections. Resistance has been reported to every chemical class of insecticide used in vector control programs, including microbial insecticides and insect growth regulators^[4].

The search for alternative pesticides and control measures that pose no risk or posing minimal risk to human health and the environment is of great interest from the preventive medicine point of view^[5]. Pyrethrin-based mosquito liquid formulations are widely used in many countries, especially in the house hold of rural population. In this context botanical pesticides revived during recent years, because of the deleterious effects of synthetic insecticides, including lack of selectivity, impact on the environment and the emergence and spread of pest resistance. The naturally occurring pesticides appear to have a promising role in the development of future commercial pesticides for safety of the environment and public health^[6].

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Applying mosquito and in many circumstances it is the only way to avoid mosquito bites [7].

Mosquito coil is one of the most common household insecticide products used for personal protection against mosquitoes in Asian countries [8,9]. Mulla *et al.* have pointed out that a large quantity of aerosols and mosquito coils are sold in the market In Thailand, followed by liquid sprays and vaporizers [9]. All of the mosquito coils registered and sold in Thailand contain solely synthetic pyrethroids, for example, d-allethrin, d-transallethrin and transfluthrin as active ingredients. These coils provide a high degree of protection from mosquitoes [8,9]. However, many people still dislike smell of the mosquito coils containing synthetic pyrethroids when they are burned, and these people also feel that the coils may be harmful for their health. It is true that mosquito coils containing synthetic pyrethroids and other organophosphorus compounds cause many side effects such as breathing problem, eye irritation, headache, asthma, itching and sneezing to the users [10].

Evaluation of essential oils against mosquitoes and isolation, identification and development of natural products from them are under the focus of numerous research programmes around the globe. So far only few insecticides of plant origin have reached the market [11]. There is a renewed interest in plant essential oils products as sources of new insect controlling agents, because they may be biodegradable to nontoxic compounds, thus minimizing the accumulation of harmful residues, leading them to be more environmentally friendly compared to synthetic compounds [12]. The Knock down and killing effect of the plant oils on the adult *Culex quinquefasciatus* were studied and the KT_{50} values of the plant oils were noticed [13]. *Eucalyptus tereticornis* (Myrtaceae) has long been recognized for its insecticidal properties; especially its mosquito repellent activity [14] but has yet to be extensively analyzed. Research on the use of plant-derived chemicals to control mosquitoes and other insects has increased in recent years. This is especially true for the use of natural products based on plant essential oils (EOs) as insecticides and repellents [13, 15, 16, 17].

The present study was undertaken to evaluate the bio-efficacy of some volatile oils against *Culex quinquefasciatus* adult stage of the lymphatic filarial vector mosquito in different bioassay methods.

2. Material & Methods

2.1 Selection of botanical essential oils

For the present study, botanical essential oils procured from Tegraj & Co (P) Ltd, India (A commercial producer of plant

essential oils and aromatic substances) were used. The following essential oils (EOs) were selected: Aniseed (*Pimpinella anisum* Linn.), Calamus (*Acorus calamus* L.), Camphor (*Cinnamomum camphora* Linn.), Cinnamon (*Cinnamomum verum* J.S.Presl), Citronella (*Cymbopogon nardus* Linn.), Clove (*Myrtus caryophyllus* Linn.), Eucalyptus (*Eucalyptus globulus* Labill.), Geranium (*Pelargonium graveollens* L.), Lemongrass (*Cymbopogon flexuosus* J.F.Watson), Luchi (*Gaultheria fragrantissima* Cham), Pine (*Pinus radiata* D.Don) and Tulsi (*Ocimum sanctum* Linn.). The essential oils were stored in the refrigerator until used for bioassay experiments.

2.2 Insect culture and conditions

The test organism *Cx. 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 $25 \pm 2^\circ\text{C}$ 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, pupae was transferred into a mosquito cage. The pupae were transferred from culture trays to glass beakers containing tap water and placed in screened cages (45 x45 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 [18].

2.3 Vaporizing bioassay

The knockdown activity was studied by following the modified method of Jaswanth [19]. The experiments were conducted in Peet Grundy Chamber (1m³). Fifty adult female mosquitoes (3-5 day old) were released into the chamber. The plant essential oils were allowed to evaporate by using vaporizing equipment. Based on the preliminary screening the oils were tested over a range of doses viz., 1.25, 2.5, 5 and 10% /cm³. The numbers of female adult mosquitoes knocked-down were recorded at 5 min intervals up to 1h or till complete knock down. The knocked-down mosquitoes were collected and placed in a recovery jar provided with 10% sugar solution to monitor mortality / recovery at 24 h period. The temperature and humidity of the chamber was maintained at $25 \pm 2^\circ\text{C}$ and 60-80% R.H. respectively. The Knock-down time (KT_{50}) were recorded from the average of three replicates. KT_{50} were calculated using probit analysis [20]. A reference control (Prallethrin 1.60%) was also used for comparison. Control was performed by exposing the mosquitoes to the vapors of deodorized kerosene (DOK).

$$\text{Knocked-down (\%)} = \frac{\text{No - of adults Knocked-down (per unit time)}}{\text{No. of adults released}} \times 100$$

2.4 Impregnated Filter paper bioassay

The knock-down activity was performed according to WHO protocol [21]. Based on the preliminary screening results the selected essential oil was prepared in 2 ml of acetone and four different doses viz., 0.625, 1.25, 2.5 and 5% /cm² were

prepared in 2 ml of acetone and applied on Whatman no.1 filter papers (size 12 x15 cm²), control papers were treated with 2 ml of acetone alone and placed in exposure tubes. 3-5 days old sugar fed mosquitoes (In each tube, 20 adult mosquitoes) were exposed on treated paper for one hour and

knocked down and live mosquitoes were recorded at every 5 minute intervals. After one-hour exposure mosquitoes were transferred into recovery test tubes for 24 hour mortality observation. The knock-down time was recorded from the average of three replicates and KT_{50} were calculated using Probit analysis [20]. Deltamethrin (0.05%) was used as a reference control for comparison.

2.5 Aerosol bioassay

Aerosol bioassay was performed according to Umerie [22]. 3-5 days old 50 female mosquitoes were introduced into Peet Grundy Chamber (1 m³). Based on the preliminary results four doses viz, 0.625, 1.25, 2.5 and 5%/cm³ were tested. Aerosol sample was sprayed inside the cage as aerosol repellent/adulticide. Adult mortality was recorded at 5 minutes interval up to 30 minutes. A reference control

(Allethrin 0.25%) was used for comparison. A set of control was maintained in which vapor of deodorized kerosene (DOK) was used. The lethal time (LT_{50}) was recorded from the average of three replicates. LT_{50} were calculated from percentage mortality data using Probit analysis [20].

2.6 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 (DMRT) Duncan [23]. The median Knock down time (KT_{50}) and lethal time (LT_{50}) was calculated by Profit analysis [20]. A *p* value of <0.05 was considered to represent Significant differences. The corrected percent mortality was calculated by using Abbott's formula [24].

$$\text{Corrected mortality (\%)} = \frac{\text{Mortality in control (\%)} - \text{Mortality in treatment (\%)}}{100 - \text{Mortality in control (\%)}} \times 100$$

3. Results

3.1 Vaporizing assay (KT_{50})

Table1 show the median Knock-down time and (KT_{50}) of five effective oils viz., calamus, and camphor, citronella, clove and eucalyptus oils against *Cx. quinquefasciatus* adults at four concentrations. The result showed that the knock down time decreased with increased concentration in

clove oil treatment; the KT_{50} was recorded as 46.1 ± 0.1 , 38.5 ± 0.1 , 30.7 ± 0.2 , and 20.1 ± 0.1 minutes at 1.25, 2.5, 5 and 10% /cm³ concentration respectively. Calamus oil was the least effective treatment at 1.25, 2.5, 5 and 10 %/cm³ concentration and recorded 69.3 ± 0.6 , 64.7 ± 0.3 , 55.8 ± 0.1 and 52.3 ± 0.6 min as KT_{50} respectively.

Table 1: Knock down time (KT_{50}) of effective volatile oils at different concentrations (%/cm³) against female adult *Cx. quinquefasciatus* as determined by Vaporizing assay

Essential oils	KT ₅₀ (Mean ± SE) min			
	1.25%	2.5%	5%	10%
Calamus	69.3 ± 0.6	64.7 ± 0.3	55.8 ± 0.1	52.3 ± 0.6
Camphor	56.2 ± 0.2	50.6 ± 0.6	42.7 ± 0.2	30.7 ± 0.1
Citronella	60.1 ± 0.1	44.3 ± 0.4	32.3 ± 0.3	26.7 ± 0.2
Clove	46.1 ± 0.1	38.5 ± 0.1	30.7 ± 0.2	20.1 ± 0.1
Eucalyptus	52.1 ± 0.3	48.1 ± 0.2	35.0 ± 0.2	27.7 ± 0.1
Reference control (Prallethrin 1.60%)	28.5 ± 0.1			

KT_{50} = Knock down times required to kill 50% of the population exposed
 Mean number of each replicates 3; Mosquitoes were exposed for 1 hr

3.2 Filter paper assay (KT_{50})

Nine essential oils were identified as potential treatments in Knock-down activity in test tube method. Table 2 present the median time (KT_{50}) of the nine essential oils against *Cx. quinquefasciatus* adults at four concentrations. After 1 hr exposure period at 5% clove oil recorded the lowest median time (KT_{50}) which was calculated as 9.15 ± 0.1 min. The result

showed that in clove oil treatment; the KT_{50} was recorded as 21.1 ± 0.1 , 19.7 ± 0.2 , 13.1 ± 0.2 , and 9.15 ± 0.1 minutes at 0.625, 1.25, 2.5 and 5% /cm² concentration respectively. Clove oil was most effective followed by citronella and eucalyptus oils were also effective since they recorded lower median lethal time. Tulsi and calamus oils were the least Knock-down activity treatments.

Table 2: Knock down time (KT₅₀) of effective volatile oils at different concentrations (%/cm²) against female adult *Cx. quinquefasciatus* as determined by filter paper assay

Essential oil	KT ₅₀ (Mean ± SE) min			
	0.625%	1.25%	2.5%	5%
Calamus	58.0± 0.5	45.9± 0.4	37.2± 0.2	31.9± 0.2
Camphor	41.8± 0.3	29.6± 0.1	27.9± 0.1	16.8± 0.2
Cinnamon	48.9± 0.3	34.3± 0.3	24.0± 0.2	17.6± 0.1
Citronella	33.6± 0.2	26.3± 0.1	15.7± 0.1	11.4± 0.2
Clove	21.8± 0.1	19.7± 0.2	13.1± 0.2	9.15± 0.1
Eucalyptus	45.5± 0.3	29.4± 0.1	17.8± 0.1	14.5± 0.1
Lemongrass	42.4± 0.3	34.9± 0.2	31.4± 0.1	24.4± 0.2
Pine	53.2± 0.3	38.7± 0.2	34.2± 0.2	26.0± 0.2
Tulsi	63.4± 0.4	49.9± 0.4	37.8± 0.2	33.3± 0.3
Deltamethrin (0.05%)	11.55 ± 0.2			

KT₅₀ = Knock down times required to kill 50% of the population exposed
Mean number of each replicates 3; Mosquitoes were exposed for 1 hr

3.3 Aerosol assay (LT₅₀)

All the twelve essential oils were found effective treatments in aerosol bioassay method from the preliminary screening. These twelve oils were tested at four different concentrations viz., 0.625, 1, 25, 2.5, and 5 % /cm³. The results clearly indicated that cinnamon oil killed 50 % population within 1.99±0.1minutes at 5% concentration

which was comparatively very low median lethal time. The lethal time decreased when the concentration increased. Next to cinnamon oil lemongrass was effective which recorded 3.77±0.1 minutes as median lethal time. Camphor (LT₅₀ = 19.6±0.1 min) and pine (LT₅₀ = 8.57±0.1 min) oils were found to be less toxic by aerosol method (Table 3).

Table 3: Lethal time (LT₅₀) of effective plant oils at different doses (%/cm³) against female adult of *Culex quinquefasciatus*

Essential oil	LT ₅₀ (Mean ± SE) min			
	0.625%	1.25%	2.5%	5%
Aniseed	17.9 ± 0.1	13.8 ± 0.2	7.02 ± 0.1	6.85 ± 0.0
Calamus	19.6± 0.1	16.8 ± 0.1	10.0 ± 0.2	6.42 ± 0.1
Camphor	27.6± 0.2	26.0 ± 0.2	20.4 ± 0.2	19.6 ± 0.1
Cinnamon	17.1± 0.1	11.6 ± 0.2	7.33 ± 0.1	1.99 ± 0.2
Citronella	23.3± 0.2	18.2 ± 0.1	8.90 ± 0.2	4.84 ± 0.2
Clove	20.7± 0.3	11.7 ± 0.1	5.28 ± 0.6	3.80 ± 0.1
Eucalyptus	21.0± 0.2	18.3 ± 0.1	13.8 ± 0.1	6.88 ± 0.0
Geranium	24.0± 0.1	20.5 ± 0.0	13.4 ± 0.6	7.18 ± 0.2
Lemongrass	15.4± 0.2	9.62 ± 0.1	4.53 ± 0.0	3.77 ± 0.1
Luchi	22.3± 0.1	17.1 ± 0.1	8.82 ± 1.0	4.46 ± 0.0
Pine	22.9± 0.2	19.8 ± 0.1	17.6 ± 0.2	8.57 ± 0.1
Tulsi	11.3± 0.1	6.72 ± 0.0	6.72 ± 0.1	5.07 ± 0.0
Reference Insecticide (Allethrin 0.25%)	2.23 ± 0.6			

LT₅₀ =Lethal times required to kill 50% of the population exposed
Mean number of each replicates 3; Mosquitoes were exposed for 30 minutes

4. Discussion

In the present study 12 essential oils registered Knock-down and adulticidal effects. The literature is available with regard to bioefficacy of volatile oils against vector mosquitoes. These findings are comparable to those of Vartak and Sharma who reported the knock-down effect of terpenoids of volatile oils against *A. aegypti* adult females [25]. Rajkumar reported that the plant *Moschosma polystachyum* Linn commonly known as Thulasi thondu which grows as a shrub in South India is a mosquitocidal plant which is widely used by tribals in Tamil Nadu state of India as fumigant [26]. *Eucalyptus tereticornis* (Myrtaceae) has long been recognized for its insecticidal properties; especially its mosquito repellent activity but has yet to be extensively analyzed. Barnard considered the effect of concentration (5 %, 10 %, 25 %, 50 %, 75 %, and 100 %) and particular

combinations of essential oils (geranium Bourbon, cedarwood, clove, peppermint, and thyme) on repellency to *Aedes aegypti* and *Anopheles albimanus* on human skin. None of the oils were effective at 5% or 10% concentration; cedarwood oil (botanical origin not specified) was ineffective at repelling mosquitoes altogether and *Ae. aegypti* was only repelled by high concentrations of peppermint oil. Thyme and clove oils were found to be the most effective mosquito repellents giving 90–210 min of protection according to the oil concentration applied to the skin [27].

Rajkumar and Jabanesan reported the knock down and killing effects of *Solanum erianthum* D.Don. Leaf extracts against *Culex quinquefasciatus* [28]. Tawatsin *et al.* (2002) studied the efficacy of nine plants namely greater galangale

(*Alpinia galanga*), fingerroot (*Boesenbergia pandurata*), turmeric (*Curcuma longa*), cardamom (*Elettaria cardamomum*), neem (*Azadirachta indica*), Siamese cassia (*Cassia siamea*), citronella grass (*Cymbopogon nardus*), eucalyptus (*Eucalyptus citriodora*) and Siam weed (*Eupatorium odoratum*) in reducing human-mosquito contact when used in mosquito coils [29]. Xue *et al.* evaluated the toxicity of 16 commercial insect repellents (6 botanicals and 10 synthetic organic products) in spray formulations were evaluated in the laboratory for adult knock down and mortality of laboratory reared female *Ae. aegypti*, *Ae. albopictus*, and *An. quadrimaculatus*. All tested products produced significant post treatment knockdown effect and 24 h mortality in all three mosquito species [30].

Repellent properties of several EO appear to be associated with the presence of monoterpenoids and sesquiterpenes. Monoterpenes such as α -pinene, limonene, terpinolene, citronellol, citronellal, camphor and thymol are common constituents of a number of EO as presenting mosquito repellent activity [12]. Senthil Nathan *et al.* studied the adulticidal activity methanol extracts of leaves from the Indian white cedar *Dysoxylum malabaricum* Bedd. (Meliaceae) against *Anopheles stephensi* Liston (Diptera) mosquitoes under laboratory conditions [31]. Choi *et al.* [32] investigated the essential oil of *Lavandula officinalis* as well as other oils such as *Eucalyptus globulus*, *Rosmarinus officinalis* and *T. vulgaris* for their individual repellent activities against *Culex pipiens*. Chaiyasit *et al.* [33] reported the adulticidal activity of essential oils derived from five plant species, celery (*Apium graveolens*), caraway (*Carum carvi*), zedoary (*Curcuma zedoaria*), long pepper (*Piper longum*), and Chinese star anise (*Illicium verum*), against *Aedes aegypti*. Jeyabalan *et al.* [34] have reported the adulticidal effect of *Pelargonium citrosa* on *Anopheles stephensi*, with LC₅₀ and LC₉₀ values as 1.56% and 5.22% respectively.

Prajapati *et al.* [35] have studied 10 essential oils viz., *Cinnamomum zeylanicum*, *Cuminum cyminum*, *Cyperus scariosus*, *Curcuma longa*, *Juniperus macropoda*, *Ocimum basilicum*, *Rosmarinus officinalis*, *Nigella sativa*, *Pimpinella anisum*, and *Zingiber officinale* for adulticidal activity against three mosquito species; *Anopheles stephensi*, *Aedes aegypti* and *Culex quinquefasciatus*. Omoloa *et al.* [36] have reported the fumigant toxicity of essential oils from 15 species of African plants against *Anopheles gambiae* in the laboratory. They reported that oils of 6 plant species viz., *Tarsonanthus camphoratus*, *Lippia javanica*, *Plectranthus marruboides*, *Tetradenia riparia*, *Lippia ukambensis* and *Conyza newii* were found to be relatively more toxic, with *C. newii* and *P. marruboides* showing the highest potency. Dua *et al.* [37] reported the adulticidal activity of the essential oil of *Lantana camara* against different mosquitoes species on 0.208 mg/cm² impregnated papers. The knockdown times (KDT₅₀ and KDT₉₀) values of the essential oil against *Ae. aegypti*, *Cx. quinquefasciatus*, *Anopheles culicifacies*, *Anopheles fluviatilis*, and *An. stephensi* were 20, 18, 15, 12, and 14 min and 35, 28, 25, 18, and 23 min respectively and percent mortality of 93.3%, 95.2%, 100%, 100%, and 100% respectively.

In the present study the volatile oils showed variation in

adulticidal activity of essential oils when tested by different methods. Aniseed, geranium and luchi oils recorded 100 per cent adulticidal activity at 10 per cent concentration only in aerosol method. Thyme oil showed 100 per cent adulticidal activity only in test tube method. The reason for this differential adulticidal activity of essential oils in different methods should be studied. However clove oil was toxic in all bioassays and also showed different kinds of activity. It is evident from the present data that the exposure of adult mosquito to the plant oils elicits Knock down and adulticidal effects. The current study clearly indicated that the adulticidal activity of the plant oils against the female adult of *Culex quinquefasciatus* may depend upon three key factors viz., concentrations, plant oils and exposure periods.

5. Conclusions

The use of herbal volatile oil products that reduces biting of adult mosquito activity at the time of biting period of adult can be used as an eco-friendly personal protection measure on vector mosquito. The selection of natural products limits the environmental impacts of pesticides and it may prevent the evolution of resistance in adult mosquitoes. In addition to addressing the needs for preventing human vector contact and vector borne diseases, natural products may be utilized as mosquito insecticides and may be recommend as one of the useful tool to promote vector-borne diseases control programme.

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