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Larvicidal and knockdown effects of some essential oils against *Culex quinquefasciatus* Say, *Aedes aegypti* (L.) and *Anopheles stephensi* (Liston)

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ABSTRACT

Efficacy of 25 essential oils was screened against filarial vector, *Culex quinquefasciatus*, for their larvicidal and knockdown effects in a preliminary study. Of these, 8 oils viz. calamus oil, cinnamon oil, citronella oil, clove oil, eucalyptus oil, lemon oil, mentha oil and orange oil exhibited 100% larvicidal activity at 1000 ppm and 100% knockdown effect at 10% concentration. These 8 oils were screened further against *Cx. quinquefasciatus*, *Aedes aegypti* and *Anopheles stephensi* for their larvicial and knockdown effects at different concentrations. Mentha oil was the most promising against *An. stephensi* and *Ae. aegypti* recording LC_{50} and LC_{90} values of 39.74 and 115.67 ppm and 46.23 and 165.36 ppm, respectively for larvicidal activity. Calamus oil was the most effective against *Cx. quinquefasciatus* with LC_{50} and LC_{90} values of 40.40, and 140.07 ppm, respectively for larvicidal activity. Orange oil showed the most potent knockdown effect with the KT_{50} and KT_{95} values of 27.44, 26.22 and 29.91 and 70.81, 65.33 and 68.57 min, against *An. stephensi*, *Cx. quinquefasciatus* and *Ae. aegypti*, respectively. The results clearly indicated that mentha oil and calamus oil were the most promising larvicides and orange oil had potent knockdown effect against the tested mosquito species. These oils could be used to develop a new formulation to control mosquitoes.

Keywords: Larvicidal; Knockdown; Essential Oils; *Aedes aegypti*; *Anopheles stephensi*; *Culex quinquefasciatus*

1. INTRODUCTION

Man has suffered from the activities of mosquito since time immemorial and it is ranked as man's most important insect pest. Mosquitoes belonging to the genera *Anopheles*, *Culex* and *Aedes* are the vectors for the pathogens of different diseases such as malaria, filariasis, Japanese encephalitis, dengue and dengue haemorrhagic fever, epidemic polyarthritis, yellow fever and chikungunya [1-3]. These diseases devastate Indian economy every year [4]. Tropical areas are more vulnerable to parasitic diseases and the risk of contracting arthropod-borne illnesses is increased due to climate change and intensifying globalization [5]. Worldwide, mosquitoes transmit diseases to more than 700 million people annually and are responsible for 1 death for every 17 people currently alive [6]. Malaria results from an infection by a protozoan carried by *Anopheles stephensi.* About 2.5 billion people are at risk, more than 500 million people become seriously ill with malaria every year, and more than one million people die due to malaria [7]. *Culex quinquefasciatus* is responsible for the transmission of lymphatic Filariasis caused by *Wuchereria bancrofti*. Lymphatic filariasis, disease affecting the arms, legs and genitals, is much prevalent in India. Lymphatic filariasis infects 80 million people annually of which 30 million cases exist in chronic infection. There are 45 million cases of Lymphatic filariasis in India alone [8].

Essential oils play an important role in controlling several mosquito species. In general, essential oils from plants have been considered important natural resources to act as insecticides [9,10]. Larvicidal activity of essential oils from *Blumea mollis* [11] and *Zingifer officinalis* [12] has been reported against *Cx. quinquefasciatus.* Larvicidal activity of essential oils from *Melaleuca leucadendron*, *Litsea cubeba* and *Litsea salicifolia* [13], *Ocimum suave* and *O. kilimandscharicum* [14] have been reported against *Anopheles arabiensis*, *A. gambiae* and *Cx. quinqefasciatus.* Larvicidal activity of essential oils form *Zanthoxylum armatum* [15] and *Ocimum canum* [16] have been reported against *Cx. Quinquefasciatus*, *Ae.*

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aegypti and *An. stephensi*. Essential oils derived from various plants not only exhibit inhibitory activity against bacteria, fungi and termites but also show strong mosquito repellent and larvicidal activities [17].

The present study was aimed to assess the larvicidal and knockdown activities of the essential oils from various plants against *C. quinquefasciatus*, *Aedes aegypti* and *Anopheles stephensi*.

2. MATERIALS AND METHODS

Rearing of Mosquitoes

***Anopheles stephensi*:** Egg strips of *Anopheles stephensi* were obtained from Malaria Research Center (MRC), Chennai. They were placed in Petri dishes (10.5 diameter) containing dechlorinated tap water for hatching. After hatching of larvae, they were provided with powdered yeast and dog biscuits in 3:1 ratio. After attaining pupae, they were separated and kept inside the mosquito cages for emergence. The cotton soaked in 5% glucose solution was placed inside the cage for nourishment. After three days of emergence, pigeon blood meal was given to adult mosquitoes. After three days of blood meal, the eggs were laid in the Petri dishes containing tap water.

***Culex quinquefasciatus*:** The egg rafts of *Culex quinquefasciatus* were obtained from Vector Control Research Center (VCRC), Puducherry. The egg rafts were placed in Petri dishes (10.5 diameter) containing tap water. Larvae were fed with finely ground mixture of yeast and dog biscuits in 3:1 ratio. The first instar larvae developed into pupae through four stages in about 8 - 10 days. The pupae were transferred into mosquito cage for emergence. Blood meal from a pigeon was given to adult mosquitoes after three days of emergence. After 3 - 4 days of blood feeding for adult mosquitoes, the Petri dishes filled with tap water were placed inside the cage for oviposition. The egg rafts were separated and placed in glass Petri dishes for hatching.

***Aedes aegypti*:** *Aedes aegypti* colony was maintained at insectary (54 cm × 45 cm × 40 cm) at 27˚C ± 2˚C and 80% ± 2% Relative humidity with a photoperiod of 12:10 hours light and dark cycles. The egg strips were obtained from Vector Control Research Center (VCRC), Puducherry to start the colony. The strips were immersed in dechlorinated tap water for hatching. To obtain the larvae of equal developmental stage, eggs were introduced by adding a stimulant such as ascorbic acid (100 mg/L) to water [18]. This started the eclosion process. The emerged larvae were maintained in Petri dishes (10.5 cm diameter) with dechlorinated tap water. Larvae were fed with a diet of yeast and dog biscuits in the ratio of 3:1. The first instar larvae developed into pupae in about 7 - 10 days through four stages. The pupae were separated by using a glass dropper into glass Petri dishes and were kept in mosquito net cages (40 cm × 45 cm × 40 cm) for emergence. The newly emerged mosquitoes were provided with 5% glucose solution soaked in cotton wool, which was placed inside the mosquito net cage for nourishment [19]. After three days of emergence, adults were given a blood meal of pigeon [20]. Glass Petri dishes of 50 mL of tap water lined with filter paper were kept inside the cage for oviposition. The eggs thus obtained were immersed in larval trays containing dechlorinated tap water for hatching.

Larvicidal Activity: For larvicidal activity, lab reared third instar larvae of the respective mosquito species were used for the present investigation. Four replicates were done with 25 larvae per replicate for each concentration [21]. For preliminary screening 1000 ppm of the respective oils were used against *Cx. quinquefasciatus*; the selected active oils were screened at 100, 75, 50 and 25 ppm against *Cx. quinquefasciatus*, *Ae. aegypti* and *An. stephensi*. The data were obtained for larvicidal activity and corrected percentage of larval mortality were calculated using Abbott's formula [22].

Knockdown Effect: The experiments were conducted in a Peet Grandy Chamber of one cubic meter size. One hundred, two days old female mosquitoes were released into the chamber for each study. The Plant Oil Formulation was allowed to vapourize by using the vapourizer equipment [4]. The number of mosquitoes knocked down was recorded at periodic intervals of five minutes till complete knockdown. The maximum exposure period was 60 minutes. 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 were maintained at 28˚C ± 2˚C and 50% - 70% respectively. The data obtained for knockdown were subjected to Finney's method of Probit Analysis to assess the KT_{50} and KT_{95} values and they were drawn from four replicates.

Plant Oils: Some of the plant oils were extracted in the lab and some oils were purchased commercially from the authorized dealers in Chennai, Tamil Nadu, India.

3. RESULTS

Preliminary Screening: In the preliminary screening 25 essential oils were screened against *Cx. quinquefasciatus* in order to select effective oils on the basis of larvicidal and knock down effects for further study against *Cx. quinquefasciatus*, *Ae. aegypti and An. stephensi*. It was observed that eight oils *viz*. calamus oil, cinnamon oil, citronella oil, clove oil, eucalyptus oil, lemon oil, mentha oil and orange oil showed 100% larvicidal and knockdown activities. Besides these, geranium, lime, tulsi and palmarosa oils showed more than 92% larvicidal and knockdown activities. Among the 25 oils vetiver oil exhibited the least larvicidal and knockdown effects (**Table 1**).

Table 1. Preliminary screening of selected essential oils against *Culex quinquefasciatus.*

	Common Name	Botanical Name	Larvicidal Activity of 1000 ppm at 24 h (%)	Knockdown Effect at 10% in 1 h (%)
1.	Aniseed oil	*Pimpinella anisum*	80	67
2.	Bergamot oil	*Citrus bergamia*	64	59
3.	Calamus oil	*Acorus calamus*	100	100
4.	Camphor oil	*Cinnamomum camphora*	60	81
5.	Cedarwood oil	*Cedrus atlantica*	76	89
6.	Cinnamon oil	*Cinnamomum veerum*	100	100
7.	Citronella oil	*Cymbopogon nardus*	100	100
8.	Clove oil	*Myrtus caryophyllus*	100	100
9.	Eucalyptus oil	*Eucalyptus globulus*	100	100
10.	Geranium oil	*Pelargonium graveolens*	92	95
11.	Lavender oil	*Lavandula angustifolia*	68	68
12.	Lemon oil	*Citrus limon* (*medica*)	100	100
13.	Lemongrass oil	*Cymbopogon flexuosus*	84	87
14.	Lime oil	*Citrus aurantifolia* (*acida*)	96	97
15.	Mentha oil	*Mentha piperita*	100	100
16.	Nutmeg oil	*Myristica fragrans*	72	67
17.	Orange oil	*Citrus sinensis*	100	100
18.	Palmarosa oil	*Cymbopogon martinii*	92	93
19.	Patchouli oil	*Pogostemon cablin*	68	81
20.	Pine oil	*Pinus radiate*	64	86
21.	Rose mary oil	*Rosmarinus officinalis*	80	89
22.	Thyme oil	*Thymus vulgaris*	68	61
23.	Tulsi oil	*Ocimum sanctum*	94	98
24.	Vetiver oil	*Vetiveria zizanioides*	52	53
25.	Wintergreen oil	*Gaultheria frgagrantissima*	76	57

Mean of 4 replicates with 25 larvae per replicate (n = 100).

Larvicidal Activity: The oils which exhibited cent percent larvicidal and knockdown effects were evaluated against *Cx. quinquefasciatus*, *Ae. aegypti* and *An. Stephensi* for their larvicidal and knowdown activities. Among the oils tested against *An. stephensi*, the most promising oils were mentha, clove and calamus oils which recorded low LC_{50} and LC_{90} values of 39.74, 39.80, 40.79 ppm and 115.67, 149.81 and 126.05 ppm, respectively with 95% confidence lower limits of 30.64, 20.98 and 31.14 ppm and upper limits of 50.21, 52,10 and 51.94 ppm, respectively for larvicidal activity. Eucalyptus oil showed the least larvicidal activity with LC_{50} and LC_{90} values of 68.45 and 247.18 ppm, respectively (**Table 2**).

Among the oils tested against *Cx. quinquifasciatus*, the most promising oils were calamus, mentha and lemon oils which recorded LC_{50} and LC_{90} values of 40.40, 42.25, 43.79 ppm and 140.07, 132.41, 146.94 ppm, respectively with 95% confidence lower limits of 30.04, 32.25 and 33.07 ppm and upper limits of 52.26, 53.89 and 56.30 ppm, respectively for larvidial activity. Citronella oil showed least larvicidal activity with LC_{50} and LC_{90} values of 91.23 and 327.00 ppm, respectively (**Table 3**).

Among the oils against *Ae. aegypti*, Mentha, citronella and clove oils showed the most potent larvicidal activity and recorded LC_{50} and LC_{90} values of 46.23, 47.21 and 50, 54 ppm and 165.36, 181.70 and 177.52 ppm, respectively with 95% confidence lower limits of 34.64, 34.54 and 38.27 ppm and upper limits of 60.07, 62.49 and 65.55 ppm, respectively. Eucalyptus oil showed the least effective larvicidal activity with LC_{50} and LC_{90} values of 80.93 and 339.01 ppm, respectively (**Table 4**).

The results of the present investigation clearly indicated that mentha oil was the most promising one to con-

Table 2. Larvicidal activity of the selected plant oils against *Anopheles stephensi*—24 h exposure.

Plant Oils	LC_{50} (ppm)	95% Confidence limit		LC_{90} (ppm)
		Lower	Upper	
Calamus oil	40.79	31.14	51.94	126.05
Cinnamon oil	55.06	41.77	71.79	197.91
Citronella oil	47.61	-	-	195.79
Clove oil	39.80	20.98	52.10	149.81
Eucalyptus oil	68.45	52.41	90.12	247.18
Lemon oil	62.78	46.42	84.95	274.38
Mentha oil	39.74	30.64	50.21	115.67
Orange oil	45.93	33.87	60.31	176.83

Each experiment is replicated with four times with 25 larvae for each replicate (n = 100). Probit analysis was employed for larvicidal activity.

Table 3. Larvicidal activity of the selected plant oils against *Culex quinquefasciatus*—24 h exposure.

Plant Oils	LC_{50} (ppm)	95% Confidence limit		LC_{90} (ppm)
		Lower	Upper	
Calamus oil	40.40	30.04	52.26	140.07
Cinnamon oil	67.16	49.95	91.10	289.00
Citronella oil	91.23	-	-	327.0
Clove oil	58.92	45.99	75.23	181.48
Eucalyptus oil	64.64	47.75	88.29	294.00
Lemon oil	43.79	33.07	56.30	146.94
Mentha oil	42.25	32.25	53.89	132.41
Orange oil	63.25	21.37	119.22	216.14

Each experiment is replicated with four times with 25 larvae for each replicate (n = 100). Probit analysis was employed for larvicidal activity.

Table 4. Larvicidal activity of the selected plant oils against *Aedes aegypti*—24 h exposure.

Plant Oils	LC_{50} (ppm)	95% Confidence limit		LC_{90} (ppm)
		Lower	Upper	
Calamus oil	63.25	21.25	69.02	216.14
Cinnamon oil	58.67	44.94	76.23	203.33
Citronella oil	47.21	34.54	62.49	181.70
Clove oil	50.54	38.27	65.55	177.52
Eucalyptus oil	80.93	60.84	111.07	339.01
Lemon oil	61.69	42.61	87.49	367.67
Mentha oil	46.23	34.64	60.07	165.36
Orange oil	54.97	41.43	22.07	204.86

Each experiment is replicated with four times with 25 larvae for each replicate (n = 100). Probit analysis was employed for larvicidal activity.

trol the larvae of all the mosquito species. Calamus oil was able to control *An. stephensi* and *Cx. quinquefasciatus* larvae. Citronella oil had the potential to control *Ae. aegypti* larvae only.

Knockdown Activity: Eight effective oils selected from preliminary screening were evaluated for their knockdown effect (expressed in minutes) against *An. stephensi*, *Cx. quinquefasciatus* and *Ae. aegypti*. Orange oil was the most promising one showing KT_{50} and KT_{95} values of 27.44 and 70.81 min, respectively against *An.*

stephensi with the 95% fiducial lower limit of 20.36 min and upper limit of 34.99 min. The remaining oils showed good knockdown activity with KT_{50} values ranging from 30.53 to 33.92 min and the KT_{95} values ranging from 65.59 to 91.19 (**Table 5**).

Orange oil was the most potent one against *Cx. quinquefasciatus* showing KT_{50} value of 26.22 min and KT_{95} value of 65.33 min with 95% fiducial lower limit of 20.67 min and upper limit of 31.90 min. The remaining oils also had notable knockdown activity with KT_{50} values ranging from 30.37 to 32.70 min and KT_{95} values ranging from 60.93 to 76.42 min (**Table 6**).

Orange oil was the most promising one against *Ae. aegypti* with KT_{50} value of 29.91 min and KT_{95} value of 68.57 min with 95% fiducial lower limit of 20.83 min and upper limit of 40.13 min. Rest of the oils had also exhibited good knockdown activity with KT_{50} values ranging from 30.32 to 32.54 min and KT_{95} values ranged from 62.96 to 76.25 min against *Ae. aegypti* (**Table 7**).

From the results of knockdown activity, it was inferred that orange oil was the most promising one against all the three mosquito species tested.

4. DISCUSSION

Using essential oils to control mosquitoes is a better and environmentally safe option than the use of synthetic chemical pesticides. In the preliminary investigation, calamus oil, cinnamon oil, citronella oil, clove oil, eucalyptus oil, lemon oil, mentha oil and orange oil showed 100% larvicidal and knockdown activities against *Cx. quinquefasciatus*. Amer and Mehlhorn [23] reported that camphor, thyme, lemon, cedorwood, cinnamon, eucalyptus, menthe, citronella, geranium, lemongrass and some other oils showed larvicidal activity against *Ae. aegypti*.

In the present study, lemon oil recorded LC_{50} value of 43.79 ppm against *Cx. quinquefasciatus* after 24 h exposure for larvicidal activity. Similar observation was

Table 5. Knockdown effect of the selected plant oils against the adult *Anopheles stephensi.*

Plant Oils	KT_{50} (Minutes)	95% Fiducial Limits		KT_{95} (Minutes)
		Lower	Upper	
Calamus oil	30.53	20.78	42.16	72.17
Cinnamon oil	33.92	22.07	52.96	76.69
Citronella oil	33.76	23.96	49.09	91.19
Clove oil	30.94	23.14	40.31	77.04
Eucalyptus oil	33.38	26.26	36.78	64.61
Lemon oil	32.05	23.58	42.06	70.22
Mentha oil	31.10	23.44	39.48	65.59
Orange oil	27.44	20.36	34.99	70.81

Probit analysis was employed for knockdown activity; Time duration 60 minutes. Four replicates with 25 adult mosquito per replicate (n = 100).

Table 6. Knockdown effect of the selected plant oils against the adult *Culex quinquefasciatus.*

Plant Oils	KT_{50} (Minutes)	95% Fiducial Limits		KT_{95} (Minutes)
		Lower	Upper	
Calamus oil	31.52	24.55	39.15	66.09
Cinnamon oil	30.37	21.82	39.91	67.96
Citronella oil	32.70	23.41	44.69	74.97
Clove oil	31.23	24.22	39.21	71.28
Eucalyptus oil	31.34	21.36	41.93	60.93
Lemon oil	31.18	24.69	38.05	63.61
Mentha oil	30.64	24.82	37.22	76.42
Orange oil	26.22	20.67	31.90	65.33

Probit analysis was employed for knockdown activity; Time duration 60 minutes. Four replicates with 25 adult mosquito per replicate (n = 100).

Table 7. Knockdown effect of the selected plant oils against the adult *Aedes aegypti*.

Plant Oils	KT_{50} (Minutes)	95% Fiducial Limits		KT_{95} (Minutes)
		Lower	Upper	
Calamus oil	31.92	23.14	42.92	76.25
Cinnamon oil	32.54	25.23	41.42	75.31
Citronella oil	30.90	21.28	42.02	68.55
Clove oil	30.71	22.06	40.85	72.29
Eucalyptus oil	32.33	26.90	38.18	62.96
Lemon oil	30.32	25.98	37.26	73.05
Mentha oil	30.57	25.43	36.05	66.71
Orange oil	29.91	20.83	40.13	68.57

recorded by Amer and Mehlhorn [23] with lemon oil; LC_{50} value of 50.2 ppm was noted against *Cx. quinquefasciatus* at 24 h exposure. Orange oil in the present study recorded the LC_{50} values of 54.97, 63.25 and 45.93 ppm against *Ae. aegypti*, *Cx. quiquefasciatus* and *An. stephensi* respectively. This result corroborates with the findings of Michaelakis *et al.* [24] who reported the LC_{50} value of 51.5 ppm against *Cx. pipiens*. Essential oil from *Zingiber officinalis* exhibited LC_{50} value of 50.78 ppm after 24 h exposure against *Cx. quinquefasciatus* [12]. Citronella oil in the present study showed LC_{50} value of 47.21 ppm against *Ae. aegypti* at 24 h exposure period. Nataya Sutthanont *et al.* [25] observed that *Zingiber zerumbet* showed LC_{50} value of 48.88 ppm at 24 hr exposure period against *Ae. aegypti*. Clove oil in the present study showed LC_{50} value of 39.80 ppm against *Anopheles stephensi*. Essential oil derived from *Syzygium aromaticum* showed the LC_{50} value of 169 ppm against *Ae. aegypti* [25]. Cinnamon oil in the present study exhibited the LC_{50} value of 67.16 ppm and 55.06 ppm against *C. quinquefasciatus* and *An. stephensi*, respectively at 24 h exposure. Zhu *et al.* [26] reported that LC_{50} value of 84 and 66 ppm against *A. albopictus* and *C. pipiens pipiens*, respectively. Eucalyptus oil in the present study showed LC_{90} value of 247.18 and 294.00 ppm against *An. stephensi* and *C. quinquefasciatus*, respectively at 24 h exposure. At 24 h exposure, eucalyptus oil showed LC_{90} value of 274.00 and 264 ppm against *A. albopictus* and *C. pipiens pipiens*, respectively [26].

In our study *Cymbopogon nardus* (Citronella oil) exhibited LC50 value of 47.21 and 47.62 ppm against *Ae. aegypti* and *An. stephensi*, respectively, which was lower than the LC_{50} value of 69 ppm reported by Sukumar *et al.* [27] for *Cymbopogon citratus* and 63 ppm for *Lippia sidoides* by Carvalho *et al.* [28] against *Ae. aegypti*. The LC_{50} values of lemon and Eucalyptus oil in our study were 43.79 and 64.64 ppm, respectively against *Cx. quinquefasciatus*. Traboulsi *et al.* [29] reported that LC_{50} values for oils from *Citrus sinensis* and *Eucalyptus* spp were 60.0 and 120.0 ppm, respectively against *Culex pipiens* larvae. Senthilkumar *et al.* [11] reported LC_{50} values of 71.79 ppm against *Cx. quinquefasciatus* for the essential oil extracted from *Blumea mollis*. Mentha oil in our study recorded the LC_{50} value of 42.25 ppm and LC_{90} value of 132.41 ppm against the larvae of *Cx. quinquefasciatus* at 24 h exposure. Koliopoulos *et al.* [30] reported that the LC_{50} values of 4 *Mentha* species ranged from 47.88 to 74.28 ppm and LC_{90} values ranged from 64.34 to 107.45 ppm against *Cx. pipiens* biotype *molestus* after 48 h exposure.

In the present investigation orange oil showed the most potent knockdown activity of 27.44, 26.22 and 29.91 min against *An. stephensi*, *Cx. quinquefasciatus* and *Ae. aegypti*, respectively. Tarelli *et al.* [31] reported that the knockdown time (KT_{50}) values obtained for orange oil was 10.1 minutes against the housefly, *Musca domestica*. KT_{50} values of 14, 20 and 18 min against *An. stephensi*, *Cx. quinquefasciatus and Ae. aegypti*, respectively [32] were recorded for an essential oil isolated from *Lantana camara*. Eucalyptus oil in the present study showed the KT_{50} values of 33.38, 31.34 and 32.33 min against *An. stephensi*, *Cx. quiquefasciatus* and *Ae. aegypti*, respectively. This is in agreement with Tolaza *et al.* [33] who recorded KT_{50} value of 31.39 min against head lice for Eaculyptus oil. Citronella oil in the present study showed KT_{50} values of 33.76 and 32.70 min against *An. stephensi* and *Cx. quinquefasciatus*. This value was much lower than 45.02 min which was observed in the same oil against *Ae. aegypti* by Zaridah *et al.* [34].

In short, through the larvicidal and knockdown effects, our study clearly demonstrated that mentha oil, clove oil and orange oil had high potency to control three species of vector mosquitoes. These essential oils can be used to develop herbal formulations with larvicidal and knockdown effects against the vector mosquitoes.

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