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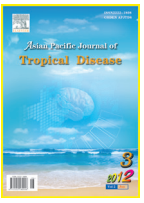

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Efficacy of essential oils of aromatic plants as larvicide for the management of filarial vector *Culex quinquefasciatus* Say (Diptera: Culicidae) with special reference to *Foeniculum vulgare*

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ABSTRACT

Objective: To evaluate the larvicidal activity of essential oils derived from ten aromatic plants with special reference to *Foeniculum vulgare* (*F. vulgare*) against early fourth instar larvae of *Culex quinquefasciatus* (*Cx. quinquefasciatus*) Say (Diptera: Culicidae). **Methods:** Essential oils were extracted from plant materials through hydro distillation and efficacy was determined through bioassay method. Two of the effective oils were evaluated further for the determination of their LC_{50} and LT_{50} values based on Probit analysis.Essential oils of one plant was analyzed through TLC and HPLC. **Results:** Most of the essential oils caused 100% mortality within 120 min at 250 ppm. Oil of *Tagetes patula* (*T. patula*) and *F. vulgare* gave more than 90% mortality within 40 min. LC_{50} values calculated after 60 min of treatment were (84.80 ± 7.65) and (24.69 ± 1.24) ppm for *T. patula* and *F. vulgare* respectively. At the same exposure period positive control temephos yielded (22.13±2.39) ppm LC_{50} value. At 50 ppm *T. patula*, *F. vulgare* and temephos exhibited LT_{50} values (113.71 ± 10.29), (11.02 ± 3.31) and (38.15 ± 5.90) mins respectively. Presence of high quantity of trans anethole in the essential oils of *F. vulgare* was confirmed by TLC and HPLC results. **Conclusions:** Present study indicates that essential oils of spices/aromatic medicinal plants particularly *F. vulgare* and *T. patula* carry huge potential as a mosquito larvicide. This potential could be exploited for the development of safer and effective botanical mosquito larvicidal tool for the management of *Cx. quinquefasciatus*.

1. Introduction

Several mosquito species belonging to genera Anopheles, Culex and Aedes are vectors for the pathogens of various diseases like malaria, filariasis, Japanese encephalitis, dengue and dengue haemorrhagic fever, yellow fever, *etc*. *Culex quinquefasciatus* (*Cx. quinquefaciatus*) is a vector of lymphatic filariasis, while this disease is widely distributed in tropical area with around 120 million people infected worldwide and 44 million people having common chronic manifestation[1].

Tropical areas are more prone to parasitic diseases and the risk has increased due to climate change and intensifying globalization[2]. Also, owing to poor drainage system, especially during rainy seasons, the presence of many fish ponds, irrigation ditches and the rice fields provide abundant mosquito breeding places. Mosquito vector-borne diseases contribute to the major disease burden in India[3].

Fight against adult mosquitoes is temporary, unsatisfactory, inadequate and environmental polluting. Larval treatment is much more effective for managing this notorious insect because in this stage larvae are localized and restricted to a small space due to the low mobility[4]. Moreover, vector control is facing a threat due to the emergence of resistance to synthetic insecticides. In this context, essential oils have received much attention as potentially useful bioactive compounds against insects[5-7]. The application of easily degradable plant compounds is considered one of the safest methods in controling insect pests and vectors[8,9].

Plant extracts are currently studied more and more because of their role in plant protection as well as in urban entomology[10]. Plant extracts are safer for non target organisms including man, therefore, plant based formulations would be more feasible from environmental perspective than synthetic mosquitocides[11]. Herbal

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products with proven potential as insecticide or repellent can play an important role in the interruption of the transmission of mosquito-borne diseases at the individual as well as at the community level. Aromatic plants and their essential oils are very important sources of many compounds that are used in different respects[12].

Great deal of research have been done by number of authors around the globe and significant progress has been made in the field of management of notorious mosquito species through the utilization of botanicals (essential oils/polar and non-polar extracts) derived from aromatic medicinal plant/spices[13-17].

In this perspective, essential oils have received considerable attention as potentially useful bioactive compounds against insects[18], exhibiting low mammalian toxicity and degrading rapidly in the environment. Studies of essential oils obtained from the plants, *Cymbopogan citratus*(*C. citratus*), *Tagetus minuta* (*T. minuta*), *Dalbergia sisoo*(*D. sisoo*), *Lippia sidoides*(*L. sidoides*)[19], *Hyptis martiusii* (*H. martiusii*)[20], *Tagetes erecta*(*T. erecta*)[21], *Mentha piperita* (*M. piperita*)[22], *Cedrus libani*(*C. libani*)[23], *Zanthoxylum armatum*(*Z. armatum*)[24] and many other plants[6,18,25-29] have demonstrated promising larvicidal activities against mosquito vectors.

Historically, thin-layer chromatography (TLC) and gas chromatography (GC) have been used for the analysis of lipids. The high temperatures used in the GC causes degradation of some molecules, whereas many fat molecules are not volatile enough to go through the GC. On the other hand, detection of fats on TLC is somewhat cumbersome. Due to the limitations of these techniques and the advances in high-performance liquid chromatography (HPLC) detection technology, HPLC is gaining popularity for lipid analysis.

In this study we have demonstrated larvicidal properties of the essential oils of ten aromatic plants against *Culex quinquefasciatus* (*Cx. quinquefasciatus*) and have determined LC_{50} and LT_{50} values for two potential oils. Also, one of the oil was analyzed through HPLC for determination of its active constituents.

2. Materials and methods

2.1. Extraction of essential oil

Ten plant varieties of aromatic medicinal plants were collected from the local market of Bhopal, India. Different plant parts were utilized for extraction of essential oils. Maximum oil yield was in *S. aromaticum* followed by *C. zeylanicum* while *P. nigrum* yielded only 0.2% (Table 1). Extraction was done through hydro distillation using a modified Clevenger-type apparatus for 4 h. The extracted essential oils were dried over anhydrous sodium sulphate and stored at 4℃ in glass vials.

Table 1
Plant species and their parts used for extraction of essential oil.

Plant species	Common name	Plant part	Essential oil content (%)
P. nigrum	Clove	Floral buds	0.2
T. patula	Cinnamon	Stem bark	0.5
F. vulgare	Cumin	Seeds	2.4
C. zeylanicum	Bay	Leaves	3.9
C. longa	Turmeric	Rhizome	2.8
S. aromaticum	Peppermint	Leaves	7.5
M. spicata	Black pepper	Seeds	0.6
A. sativum	Marigold	Whole plant	0.9
C. tamata	Fennel	Seeds	1.5
C. cyminum	Garlic	Buds	1.1

2.2. Sources of mosquito larvae

Eggs of *Cx. quinquefasciatus* were obtained from the Fungal Biotechnology and Invertebrate Pathology Laboratory, Department of Biological Sciences, Rani Durgawati University, Jabalpur, India and reared by the standard methods of Sandhu *et al*[30]. Larvae were kept in open trays exposed to light for couple of hours during daytime. The water in trays was routinely changed and larvae were fed with 0.01% (w/v) of larval diet (1:1, sterilized yeast powder and dog biscuit).

2.3. Preliminary screening through bioassays

In the preliminary screening early fourth instar healthy larvae were separated and fed with larval diet prepared from dog biscuit and yeast powder. The larvicidal activity was assessed by the procedure of WHO with some modification. Ten larvae were placed in each of clean bioassay glass jars (10 x 6 x 6 cm) containing 200 mL of chlorine free tap water. Each bioassay cup contained 50 μL of essential oils (250 ppm) and 25 μL of emulsifier (Polysorbate 80). Wide mouth tissue culture glass bottles (12 x 6 x 6 cm) were used for bioassay studies. The experiment was performed in triplicates and bioassay bottles were duly labeled. Bottles were held at (26 ± 2) ℃ and dead larvae were counted after 10, 20, 40, 60 and 120 mins of exposure to essential oils. Larvae were considered dead if they were immobile and unable to reach the water surface[31].

2.4. Bioassays for determination of LC_{50} and LT_{50}

Essential oils that yielded more than 90% mortality after 40 min of treatment in preliminary screening were further evaluated at different concentrations ranging from 250 to 6 ppm at different time intervals from 10 to 120 min for the determination of LC_{50} and LT_{50} values. Garlic essential oils was avoided due to its unacceptable odor. Organo phosphorate compound (Temephos) was used as positive control.

2.5. TLC analysis of F. vulgare essential oils

F. vulgare of essential oils was subjected to silica gel thin layer chromatography along with the standard trans anethole (4-Propenylanisole) 98% purchase from Merck Chemicals, International. Petroleum ether: ethyl acetate (8:2) was used as developing solvent. Plate was exposed to iodine vapors in

a chamber for 30 min for visualization of spots.

2.6. HPLC analysis of F. vulgare essential oils

HPLC analysis of *F. vulgare* essential oils was done in reverse phase isocratic mode by using Shimadu's prominent HPLC system using a reverse phase C18 column [Phenomenex Luna, 5μ C18 (2) 100A (250×4.60 mm)], eluent: Methanol/acetonitrile (70:30), UV detector: 254 nm, flow rate 0.5 mL/min, column temperature 40℃. 500 mg essential oils was dissolved in 5 mL of mobile phase solvent and 20 μL was injected.

2.7. Statistical analysis

Log probit analysis of the experimental data was done by using the computer softwares [StatPlus 2007 Professional software (AnalystSoft Robust Business solutions) and MS Excel 2007] to find the LC_{50}, LT_{50}, fiducial limits, regression equations (Y=mortality; x=concentrations), regression coefficient and chi-square values.

3. Results

Except for *C. longa* and *M. spicata* all the essential oils yielded 100% larval mortality at 250 ppm concentration (Tabel 2). High mortality (>90%) was obtained in *T. patula*, *F. vulgare*, *M. spicata* and *A. sativum* during 40 min. Order of mortality observed after 20 min was: *F. vulgare* > *T. patula* > *M. spicata* > *C. cyminum* > *A. sativum* > *P. nigrum* > *C. longa* > *C. zeylanicum* > *S. aromaticum* > *C. tamala* (Tabel 2).

Tabel 3 indicates that larval mortality depends on concentration of active ingredient and duration of exposure. In terms of concentration temephos was most effective followed by essential oils of *F. vulgare* and *T. patula*.

The LC_{50} values and their upper and lower fiducial limits, regression equations and Chi-square (χ^2) values of the essential oils of *T. patula* and *F. vulgare* along with temephos for 60 mins of exposure of *Cx. quinquefasciatus* are given in Table 4. LC_{50} value for *T. patula* is found to be (84.80±7.65) ppm which indicates that both *F. vulgare* and temephos are twice as effective as *T. patula*. The results of regression analysis indicated that the mortality rate (Y) is positively correlated with the concentration (x) having a regression coefficient (R) close to one in each case (Table 4). Similarly, results in Table 5 demonstrate that at 50 ppm volatile oils of *F. vulgare* killed 50% larval population in (11.02 ± 3.31) min followed by temephos (38.15 ± 5.90) and *T.*

Table 2
Percent larval mortality (%) (mean ± SEM) obtained with essential oils (250 ppm) at different time periods.

Plant species	10 min	20 min	40 min	60 min	120 min
P. nigrum	0	56.67±1.70	90.00±1.25	93.33±1.88	100
T. patula	0	76.67±3.30	93.33±2.05	100	–
F. vulgare	70.00±2.49	96.67±1.25	100	–	–
C. zeylanicum	0	33.33±1.70	80.00±0.94	100	–
C. longa	13.33±0.82	40.00±1.25	60.00±0.47	63.33±2.16	66.67±3.09
S. aromaticum	0	26.67±1.25	90.00±1.41	93.33±2.05	100
M. spicata	0	73.33±1.25	86.67±2.05	90.00±2.05	93.33±2.16
A. sativum	20.00±1.25	66.67±1.41	100	–	–
C. tamala	0	23.33±1.70	86.67±2.62	96.67±1.70	100
C. cyminum	63.33±2.16	70.00±0.94	76.67±2.16	100	–

Table 3
Effect of dose and time period on larvicidal activity of Temephos and essential oil of *T. patula* and *F. vulgare*.

Essential oils	Conc.(ppm)	10 min	20 min	40 min	60 min	80 min	100 min	120 min
T. patula	250	0	76.67	93.33	100	–	–	–
	100	0	30.00	43.33	46.67	76.67	96.67	100
	50	0	13.33	16.67	23.33	40.00	46.67	53.33
	25	0	0	0	0	0	0	0
	12	0	0	0	0	0	0	0
	6	0	0	0	0	0	0	0
F.vulgare	250	70.00	96.67	100	–	–	–	–
	100	60.00	86.67	100	–	–	–	–
	50	53.33	63.33	80.00	83.33	90.00	100	–
	25	0	40.00	46.67	50.00	56.67	56.67	60.00
	12	0	0	10.00	16.67	20.00	20.00	26.67
	6	0	0	0	0	0	0	0
Temephos	250	0	53.33	70.00	93.33	100	–	–
	100	0	46.67	63.33	76.67	100	–	–
	50	0	23.33	40	63.33	100	–	–
	25	0	10	33.33	50.00	96.67	100	–
	12	0	0	26.67	46.67	86.67	100	–
	6	0	0	16.67	20.00	30.00	83.33	100

Table 4
Log probit analysis (Finney's method) of the larvicidal activity calculated for larval mortality after 60 mins.

Essential oils	LC_{50} Value (ppm)	Upper fiducial limit	Lower fiducial Limit	Standard Error LC_{50}	Regression equations	R^2	Chi-Square (χ^2)
T. patula	84.80	110.65	67.08	7.65	Y=-2.9870+0.4242x	0.98	1894.860
F. vulgare	24.69	27.24	22.37	1.24	Y=33.0211+0.3428x	0.54	1.979
Temephos	22.13	27.07	17.74	2.39	Y=40.7656+0.2379x	0.75	3.605

Table 5
Log probit analysis (Finney's method) of the larvicidal activity calculated for larval mortality at 50 ppm.

Essential oils	LT_{50} Value (min)	Upper fiducial limit	Lower fiducial Limit	Standard Error LT_{50}	Regression equations	R^2	Chi-Square (χ^2)
T. patula	113.71	139.12	97.62	10.29	A=-1.525+0.4684b	0.97	8.587
F. vulgare	11.02	17.77	3.88	3.31	A=55.9315+0.4150b	0.92	2.029
Temephos	38.15	52.04	23.58	5.90	A=2.1323+0.9575b	0.91	19.485

patula(113.71 ± 10.29).

The result of TLC is shown in Figure 1 indicates the presence of high content of trans anethole in fennel essential oils compared with the standard. Due to its relatively low polarity it has traveled high on the plate. Other components of the oil were polar and remained close to the origin point.

HPLC chromatogram of *F. vulgare* essential oils is shown in Figure 2 indicates the principal components in the oil which shows that trans-anethole (88.69 area %) with retention time 7.268 min. The strong larvicidal activity of this oil could be attributed to trans-anethole due to its higher percentage as revealed by HPLC peak area (Table 6). However other minor components like estragole may have little impact on larvicidal action.

Figure 1. TLC of *F. vulgare* essential oils along with standard trans-anethole

Table 6
HPLC peak table of *F. vulgare* essential oils.

Peak No.	Retention time	Peak area	Peak height	Area%	Height%
1	6.043	10718536	1248212	9.655	25.613
2	6.275	1457440	144859	1.313	2.972
3	6.665	379850	20089	0.342	0.412
4	7.268	98458178	3460274	88.690	71.003
Total		111014004	4873434	100.000	100.000

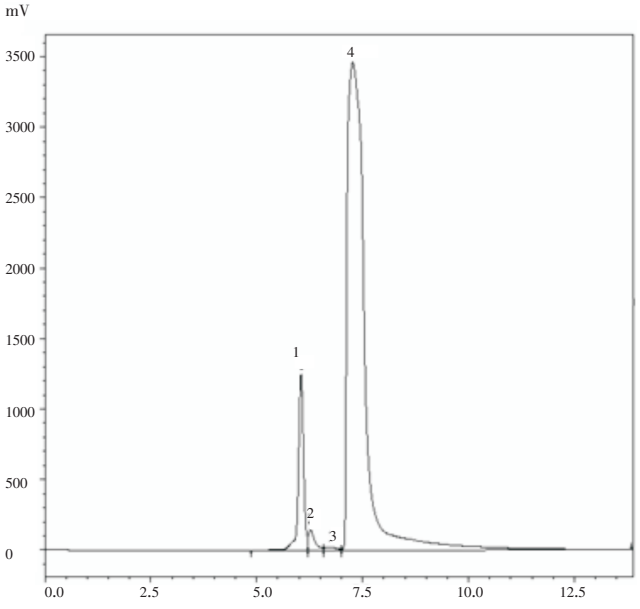

Figure 2. HPLC chromatogram depicting profile of *F. vulgare* essential oils

4. Discussion

Essential oils from plants may be an alternative source of mosquito larval control, since they have a rich source of bioactive compounds that are biodegradable into nontoxic products and potentially suitable for use in integrated management programs. To site a few examples, the essential oils from rhizomes of *C. longa* is composed mainly of turmerones such as β-turmerone, ar-turmerone and β-turmerone[31]. *C. zeylanicum* contain cinnamaldehyde, which is a kind of strong insecticidal principal compound, *A. sativum* has diallyl trisulfide and methyl allyl disulfide, *T. patula* essential oils have major constituents like limonene,

β-ocimene and β-caryophyllene. *F. vulgare* contains estragole, fenchone and anethole as potent larvicides.

During first 10 min after treatment larval mortality was observed in essential oils of *F. vulgare*, *C. longa*, *A. sativum* and *C. cyminum*. More than 60% mortality was observed with *F. vulgare* and *C. cyminum* after 10 min. Quick response was observed in *F. vulgare* essential oils as demonstrated earlier after 10 min. *T. patula* essential oils were effective only at higher concentrations, as no mortality was observed at 25 ppm even after 120 min of exposure. *F. vulgare* essential oils were effective upto 12 ppm at which it caused 20% mortality after 80 min but, temephos was most potent, and caused 100% mortality after 120 min of treatment even at lowest dose of 6 ppm.

In fact, many researchers have reported on the effectiveness of plant essential oils against mosquito larvae. Essential oils of *Juniperus macropoda* and *Pimpinella anisum* as larvicidal, adulticidal, ovicidal, oviposition-deterrent and repellent towards three mosquito species; *An. stephensi*, *Ae. aegypti* and *Cx. quinquefasciatus*[14]. Pitasawata *et al* [16] reported five aromatic plants, *Carum carvi* (caraway), *Apium graveolens* (celery), *F. vulgare* (fennel), *Zanthoxylum limonella* (mullilam) and *Curcuma zedoaria* (zedoary) having larvicidal potential against mosquito vectors *An. dirus* and *Ae. aegypti*. Knioa *et al* [17] tested oils obtained from various spices against *Ochlerotatus caspius* fourth instars and proved all of the tested oils to have strong larvicidal activity, with the most potent oil being thyme inflorescence extract, followed by parsley seed oil, aniseed oil, and then coriander fruit oil. Tiwary *et al*[24] reported larvicidal activity of the essential oil extracted from the seeds of *Zanthoxylum armatum* against three species of mosquito vectors, *Ae. aegypti*, *An. stephensi* and *Cx. quinquefasciatus*. Sutthanont *et al*[32] investigated the chemical compositions and larvicidal potential of *Citrus hystrix*, *Citrus reticulata*, *Zingiber zerumbet*, *Kaempferia galanga*, and *Syzygium aromaticum* against mosquito vectors. They suggested the use of these essential oils from edible herbs as a potentially alternative source for developing novel larvicides to be used in controlling vectors of mosquito-borne disease. Park *et al*[33,34] evaluated larvicidal activity of 11 Myrtaceae essential oils and their constituents against *Ae. aegypti* L. and concluded that Myrtaceae essential oils and their constituents could be developed as control agents against mosquito larvae. Cheng *et al*[28] suggested that the essential oil from *Eucalyptus camaldulensis* leaves and its effective constituents may be explored as a potential environmental-benign mosquito larvicide.

Based on the Probit analysis, *F. vulgare* essential revealed LC_{50} value (24.69 ± 1.24) ppm after 60 min of treatment which is very close to that of temephos (22.13 ± 2.39) ppm. Likewise lethal doses and lethal time periods have been determined by many authors. Dharmagadda *et al*[35] tested larvicidal activity of *T. patula* essential oil against the fourth instar larvae of *Ae. aegypti*, *An. stephensi*, and *Cx. quinquefaciatus*. Different concentrations of essential oil were studied and the results were compared with that of synthetic insecticide, malathion. Amer and Mehlhorn[12] evaluated oils of 41 plants for their effects against third-instar larvae of *Ae. aegypti*, *An. stephensi* and *Cx. quinquefasciatus*. The LC_{50} values of these oils ranged between 1 and 50.2 ppm for *Cx. quinquefasciatus*. Senthilkumar *et al*[27] extracted essential oil from the leaves of *Blumea mollis* and studied the chemical constituents and the larvicidal effects against *Cx. quinquefasciatus*. The essential oil had significant toxic effect against early fourth instar larvae of *Cx. quinquefasciatus* with LC_{50}=71.71 and LC_{90}=143.41 ppm. Govindarajan[29] investigated the larvicidal property of essential oils from various parts of four plant species. The highest larvicidal activity was observed in the essential oil from *Zingiber officinale* against *Cx. tritaeniorhynchus* and *An. subpictus* with the LC_{50} and LC_{90} values as 98.83, 57.98 ppm and 186.55, 104.23 ppm, respectively.

Tripathi *et al*[36] in WIPO patent No. WO/2003/079796 have stated a new herbal formulation, comprising essential oil of medicinal plant *F. vulgare* and other plants useful as insecticide against mosquito larvae. The formulation of their invention has toxic action against larval stages of malarial vector, *An. stephensi*. The property was attributed to the synergistic combination of essential oil of plant genus *F. vulgare* and other essential oils derived from medicinal plants.

HPLC is a sensitive and reliable method for analyzing components of essential oils. In this study essential oils of *F. vulgare* showed total four peaks with trans anethole as major peak (88.69% area). Similarly, Benincasa *et al*[37] analyzed essential oils by HPLC with standard columns in reversed and normal phase such as citrus. Volatile and non-volatile fractions were investigated, and in the non-volatile fraction some coumarins were identified. Rauber *et al*[38] developed a HPLC method for determination of citral in volatile oil of *Cymbopogon citratus*. Their method showed an excellent performance (linearity, precision, accuracy and specificity) for assay citral in volatile oil.

The current studies therefore suggest the use of the essential oil from the seeds of *F. vulgare* as a mosquito larvicide. The oil is easily available and the cost constraint may be overcome by the low LC_{50} value. The essential oils of this plant could be utilized by public for controlling mosquito larvae in small water bodies recognized as breeding sites such as pit and holes, coolers, waste containers *etc*. This will be quite beneficial from the pollution management view point. Future scope of this investigation will be to develop a suitable formulation with appropriate synergistic agents and field evaluation of the product for determination of toxicology effect and bioefficacy.

Conflict of interest statement

We declare that we have no conflict of interest.

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