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Evaluation of anti-mosquito properties of essential oils

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Essential oils from *Cedrus deodara*, *Eucalyptus citriodora*, *Cymbopogon flexuous*, *C. winterianus*, *Pinus roxburghii*, *Syzygium aromaticum* and *Tagetes minuta* were evaluated for bioactivity against the adults of *Culex quinquefasciatus* and *Aedes aegypti*. Serial dilutions of the oils were made in deodorized kerosene to obtain a range of concentrations (0.5-10%) and the adults were exposed to the vapour of the different oils for 1h in WHO kits for sensitivity testing. *C. winterianus* and *S. aromaticum* oils were equi effective and found most effective with LC_{50} and LC_{95} values respectively at 0.5 and 0.9 % for *C. quinquefasciatus* and 1.0 and 2.0 % for *A. aegypti*. Activity was found in the order: *S. aromaticum* > *C. flexuous* > *E. citriodora* > *C. winterianus* > *C. deodara* > *T. minuta*.

Keywords: *Culex quinquefasciatus*, *Aedes aegypti*, Essential oils, Mosquito repellents

Introduction

Mosquitoes are the major vectors for dengue fever, yellow fever, malaria, filariasis, Japanese encephalitis and other fevers[1]. Synthetic organic insecticides used to control mosquitoes have produced a feedback of environmental ill effect, non-targets organisms being affected and most mosquito species have becoming physiologically resistant to synthetic insecticides[2]. Several phytochemicals have detrimental effects on mosquitoes[3,4]. Essential oils and terpenoids are reported to show repellency to adult mosquitoes[5,6]. Strong repellent action of *Azadirachta indica, Cymbopogon martini* var *sofia, C. citratus, C. nardus* and *Oscimum* sp. have been reported against some species of mosquitoes[7]. These findings have reemphasized the possibility of developing an effective and safe insecticide from plant derived products.

India represents a vast repository of diverse flora of considerable medicinal importance. Many researchers have reported the bioactivity of *Eucalyptus* sp.[8-10], *Cedrus deodara*[7,8,11] *Cymbopogon* sp.[8,12-15] and *Tagetes minuta*[16] against mosquitoes. In all these studies, bioactivity of the plant/parts has been evaluated either as repellent or as larvicide. Some of the studies pertain to the evaluation of adult mortality activity is generally tested using laboratory bioassay[16]. However, scanty research work is available to determine the bioactivity of essential oils directly on the adult mortality. The present paper evaluates bioactivity of the oils from seven plants, *Cedrus deodara* (Roxb.) Loud (Pinaceae)*, Eucalyptus citriodora* (Myrtaceae)*, Cymbopogon flexuous* (DC.) Sapf (Graminae)*, Cymbopogon winterianus*(Linn.) Randle (Graminae)*, Pinus roxburghii* (Sarg.) (Pinaceae)*, Syzygium aromaticum* (Linn.) Merr & Perry (Myrtacae) and *Tagetes minuta* L. (Compositae) on the adults of two mosquito species, *Culex quinquefasciatus* and *Aedes aegypti.*

Materials and Methods

Extraction and Preparation of Test Solution

Plant materials were collected from naturally growing regions of North India. Clevenger's type apparatus[17] was used to obtain essential oils from freshly collected leaves of *C. winterianus*, *C. flexuous, E. citriodora and T. minuta*, crushed wood chips of *C. deodara* and crushed clove buds of clove oil. The oils were refrigerated and used as a test sample. The pine oil was obtained from resins.

GC Analysis of Chemical Constituents

Chemical constituents of oils were identified by Gas Chromatography using SE-30 column and the GC parameters as follows: Oven temperature, 80-200°C

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@ 10°C per min; Carrier gas, N_2, at 30 ml per min; and Detector, Flame Ionization Detector. Oil constituents were detected by comparing the time with those of standard compounds purchased from Sigma-Aldrich.

Rearing of Mosquitoes

Larvae of both the mosquito species were collected in a beaker (1000 ml) from the breeding pond (2'×10'×3') and maintained [Relative Humidity (RH), 70-80%; temp, 27±2°C]. Larvae were fed in the laboratory with powdered yeast and dog biscuit (3:1). Pupae of both the species, picked carefully and selectively with the help of a dropper and transferred into separate glass beakers containing fresh water, were placed inside mosquito cages (45×30×45 cm^3), kept inside a room (RH, 70-80%; temp, 27±2°C). The adults of mosquitoes emerging from pupa were caged and provided with sucrose solution (10%). In this way, large number of healthy mosquitoes of both the species was reared in the laboratory. Adults (3-4 d old) were taken for the study.

Bioactivity Test

Stock solution (10%) of all the essential oils was prepared in deodorized kerosene oil. Solution concentrations (0.5, 1, 2, 4, 8 %) were then prepared by serial dilution from the stock solution. 0.5 ml solution of each concentration of the different oils was uniformly spread on filter paper (12×15 cm) for lining the holding tubes of WHO test kits (125 mm long and 44 mm diam)[18]. Mosquitoes (10) were caught from the cage with the help of a mouth aspirator tube (12 mm internal diam), together with 60 cm of tubing and mouthpieces and released inside the test kits of each set of concentration. Each concentration together with an untreated control group was replicated 10 times for both the species. Mortality was recorded after 1 h exposure. Adults were considered dead when they displayed distorted body configuration and showed no movement when transferred to the treatment free environment. The control mortality was accounted by using Abbott's formula[19]. All the data were statistically analyzed using Excel Microsoft Programme and SPSS (Window, 2000).

Results and Discussion

The yield and major chemical constituents of essential oils were studied (Table 1). The toxicity of all the oils varied (Figs 1 & 2). All the oils tested were toxic to the adults of *C. quinquefasciatus* (Tables 2 & 3; Figs 1 & 2). *S. aromaticum* and *C. flexuous* oil showed strongest activity and were equi toxic to the adults of *C. quinquefasciatus* with LC_{50} and LC_{95} values at 0.53 and 0.93 percent respectively at the end of an exposure of 60 min whereas against the adults of *A. aegypti*, the LC_{50} and LC_{95} values were 2.65, 1.22 and 5.32, 1.92 percent respectively. This might be due to the presence of eugenol (63%) in *S. aromaticum* oil and citral (61%) in *C. flexuous* oil as major constituent. Eucamalol[9] was isolated as

Table1—Major active constituents of essential oils

Plant	Yield of essential oil %, v/w	Major constituents	Yield of major constituent %
C. deodara	6.9	Himachalene	59
(Woodchips)		Cis-Atlantone and α-Atlantone	19
E. citriodora	1.78	Citronellal	46.2
(Leaf)		Citronellol	10.22
C. flexuous	0.36	Citral a and citral b	61.6
(Leaf)			
C. winterianus	1.2	Citronellal	34.0
(Leaf)		Geraniol	16.9
		Citronellol	11.0
P. roxburghii	18.9	α-Pinene	18.1
(Resin)		Longifolene	13.8
		Carene	51.8
S. aromaticum	15.1	Eugenol	63
(Bud)			
T. minuta	0.45	Cis β- Ocimine	18
(Leaf)		Dihydrotagetone	56

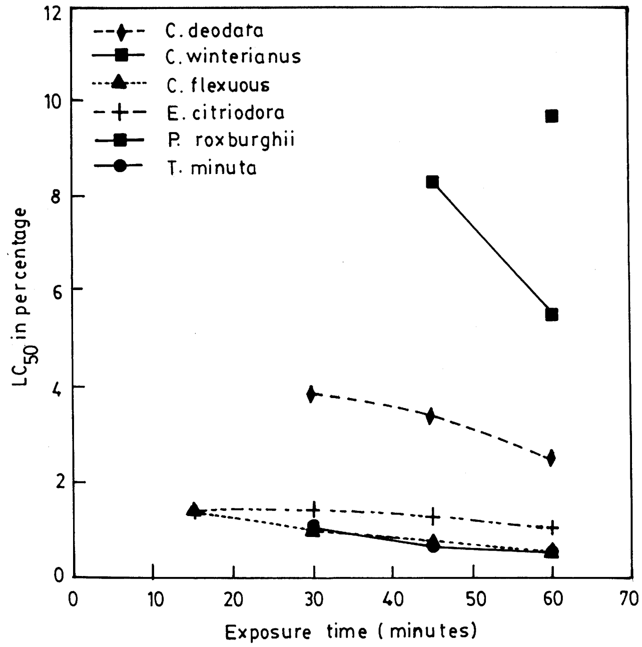

Fig. 1—Comparative illustration of test oils against *Culex quinquefasciatus*

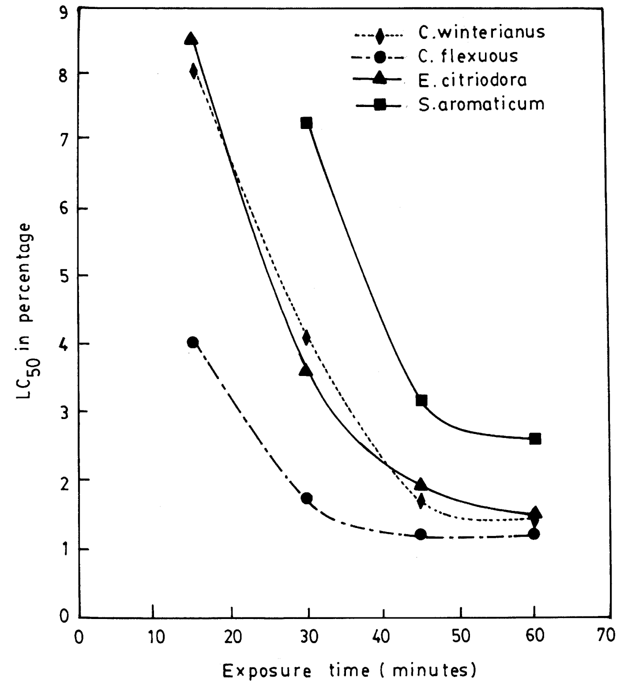

Fig. 2—Comparative illustration of activity of the test oils against *Aedes aegypti*

Table 2—Bioactivity of the oil against *Culex quinquefasciatus*

Oil's origin	Exposure time min	LC_{50} (95% Fudicial limit)	LC_{95} (95% Fudicial limit)	Slope ±SE	Intercept ±SE	df	X^2
C. deodara	15*	—	—	—	—	—	—
	30	3.9 (3-5.1)	7.5 (5.6-15.2)	5.8±1.5	-3.4±0.9	4	1.7
	45	3.4 (2.5-4.5)	7.3 (5.3-15.5)	5.0±1.2	-2.6±0.7	4	1.8
	60	2.5 (1.8-3.3)	5.9 (4.2-13.4)	4.3±1.0	-1.7±0.5	4	2.6
C. winterianus	15*	—	—	—	—	—	—
	30*	—	—	—	—	—	—
	45	8.3 (4.2-78.7)	*	1.2±0.4	-1.1±0.3	4	2.2
	60	5.5 (2.9-23.1)	*	1.2±0.4	-0.9±0.3	4	2.7
C. flexuous	15	1.4 (1.1-1.9)	1.9 (1.5-3.5)	12.6±4.1	-1.9±0.9	4	0.6
	30	1.0 (0.7-1.4)	1.6 (1.3-15.7)	8.3±3.5	-0.0±0.4	4	0.1
	45	0.8 (0.6-1.1)	1.3 (1.0-3.1)	8.0±2.7	0.8±0.5	4	1.2
	60	0.5 (0.3-0.8)	0.9 (.7-28.7)	6.8±3.0	1.8±0.8	4	0.3
E. citriodora	15	1.4 (1.1-1.9)	1.9 (1.5-3.5)	12.6±4.1	-1.9±0.9	4	0.6
	30	1.4 (1.1-1.9)	1.9 (1.5-3.5)	12.6±4.1	-1.9±0.9	4	0.6
	45	1.3 (1.1-2.5)	1.7 (1.4-9.3)	126±5.0	-1.4±0.3	4	0.1
	60	1.0 (0.8-1.4)	2.1 (1.5-5.4)	5.5±1.5	-0.1±20.3	4	0.6
P. roxburghii	15*	—	—	—	—	—	—
	30*	—	—	—	—	—	—
	45*	—	—	—	—	—	—
	60	9.7 (5.9-39.6)	*	1.9±0.6	-1.9±0.5	4	5.5
S. aromaticum	15*	—	—	—	—	—	—
	30	1.0 (0.7-1.5)	3.3 (2.1-10.1)	3.3±0.8	-0.1±0.2	4	0.8
	45	0.7 (0.4-0.9)	1.6 (1.1-8.1)	5.0±1.7	-1.0±0.4	4	0.1
	60	0.5 (0.3-0.8)	0.9 (0.7-28.7)	6.8±3.0	1.8±0.8	4	0.3

*Due to low mortality LC_{50} and LC_{95} dose could not be determined

Table 3—Bioactivity of the oil against *Aedes aegypti*

Oil's origin	Exposure time min	LC_{50} (95% Fudicial limit)	LC_{95} (95% Fudicial limit)	Slope $\pm$SE	Intercept $\pm$SE	df	X^2
C. nardus	15	8.1 (4.8-27.9)	*	1.9$\pm$0.5	-1.5$\pm$0.4	4	1.2
	30	4.1 (2.6-7.4)	*	1.9$\pm$0.5	-1.2$\pm$0.3	4	1.4
	45	1.7(1.2-2.4)	6.0 (3.9-15.7)	3.0$\pm$0.7	-0.7$\pm$0.3	4	3.0
	60	1.4 (1.0-2.0)	4.2 (2.8-10.7)	3.5$\pm$0.8	-0.6$\pm$0.3	4	1.5
C. flexuous	15	4.0 (2.8-5.7)	*	3.1$\pm$0.7	-1.9$\pm$0.5	4	4.1
	30	1.7 (1.3-2.3)	3.3 (2.4-8.2)	5.7$\pm$1.7	-1.4$\pm$0.5	4	0.3
	45	1.2 (1.0-1.7)	1.9 (1.5-6.0)	8.4$\pm$2.9	-0.7$\pm$0.4	4	0.5
	60	1.2 (1.0-1.7)	1.9 (1.5-5.9)	8.4$\pm$2.9	-0.7$\pm$0.4	4	0.5
E citriodora	15	8.5 (6.3-12.7)	*	6.1$\pm$2.6	-5.7$\pm$2.4	4	0.3
	30	3.7 (2.8-4.8)	7.0 (5.2-14.7)	5.9$\pm$1.5	-3.3$\pm$0.9	4	0.9
	45	2.0(1.5-2.7)	3.9 (2.8-9.7)	5.6$\pm$1.5	-1.6$\pm$0.5	4	1.5
	60	1.5 (1.2-2.0)	2.6 (2.0-6.0)	7.2$\pm$2.2	-1.3$\pm$0.5	4	0.0
S. aromaticum	15*	—	—	—	—	—	—
	30	7.3 (5.3-9.2)	*	6.4$\pm$2.2	-5.5$\pm$2.0	4	4.5
	45	3.2 (2.4-4.2)	6.7 (4.9-14.5)	5.2$\pm$1.3	-2.6$\pm$0.7	4	0.9
	60	2.7 (2.0-3.5)	5.3 (3.9-12.4)	5.4$\pm$1.5	-2.3$\pm$0.7	4	0.4

*Due to low mortality LC_{50} and LC_{95} dose could not be determined

mosquito repellent against *A. aegypti* from *E. camaldulensis*. Similarly, citronellal at 46 per cent in *E. citriodora* oil and at 34 percent in *C. winterianus* showed activity against *A. aegypti*. The bioactivity effect depends upon the concentration of terpene aldehydes in particular oil. The pre sent study showed the activity in the order *C. flexuous*> *C. winterianus* > *E. citriodora* against *A. aegypti and C. flexuous*> *E. citriodora*> *C. winterianus* against *C. quinquefasciatus* after 60 min exposure.

Adults of *A. aegypti* were insensitive towards the oils of *C. deodara, P. roxburghii* and *T. minuta* under treated concentration range and 1 h of exposure whereas against *C. quinquefasciatus*, LC_{50} for the first two oils was 2.48 and 9.69 percent respectively indicating low effectivity. *T. minuta* did not show any effect. Both the species, however, didnot show much difference in their sensitivity against the oil from *E. winterianus* with LC_{50} and LC_{95} values at 1.03, 2.07 percent and 1.52, 2.57 percent respectively for *C. quinquefasciatus* and *A. aegypti*. For *C. winterianus* oil, *A. aegypti* was more sensitive than *C. quinquefasciatus* with LC_{50} values at 5.53 and 1.43 per cent respectively.

Investigations are currently underway to isolate active compounds from different essential oils for mosquito control. Further studies may be required by using pure terpene aldehyde constituents isolated from different essential oils against adult mosquitoes, which might be used to replace synthetic products.

Conclusions

The present study demonstrates the adulticidal activities of different essential oils with varied degree against two important mosquito species (*C. quinquefasciatus* & *A. aegypti*). *C. winterianus* and *S. aromaticum* oils were equi effective and found most effective against both the mosquito species with LC_{50} and LC_{95} respectively values at 0.5 and 0.9 percent for *C. quinquefasciatus* and 1.0 and 2.0 percent for *A. aegypti*. In general, the activity was found in the order: *S. aromaticum*> *C. flexuous* > *E. citriodora* > *C. winterianus* > *C. deodara*> *T. minuta.* The present study would be quite helpful in developing plant based anti-mosquito agents.

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