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MOSQUITO-LARVICIDAL ACTIVITY OF CEYLON CITRONELLA {*CYMBOPOGON NARDUS* (L.) Rendle} OIL FRACTIONS

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Abstract: Fractional distillation of Ceylon citronella {*Cymbopogon nardus* (L.) Rendle} oil yielded 13 fractions. Monoterpene hydrocarbon fractions were highly lethal to late 3rd instar *Culex quinquefasciatus* larvae. The results suggest that myrcene in the monoterpene fractions is responsible for the larvicidal activity. Elemol and/or methyl iso-eugenol were identified as active principles responsible for the larvicidal activity in later fractions. The residue after fractional distillation of oil also possessed considerable larvicidal activity. Hydrocarbon fraction and the residue after fractional distillation of Ceylon citronella oil can be used as mosquito-larvicides.

Key words: Citronella oil, *Culex quinquefasciatus, Cymbopogon nardus*, elemol, essential oil, fractional distillation, larvicide, monoterpenes, methyl isoeugenol, myrcene.

INTRODUCTION

Most of the phytochemicals with insecticidal activity are biodegradable and less harmful to mammals than synthetic insecticides.[1] Therefore, there is a possibility of replacing synthetic insecticides with potent bio-insecticides of plant origin. The insecticidal[2] and insect repellent[2,3] activities of *Cymbopogon nardus* (L.) Rendle (known as "Ceylon citronella") oil against *Sitroga cerealella* (Oliver) have been reported. Mosquito-larvicidal activity of *Cymbopogon nardus* oil against six mosquito species was previously reported by Ranaweera.[4] The present study describes the larvicidal activity of *Cymbopogon nardus* oil and its fractions against *Culex quinquefasciatus* mosquitoes.

METHODS AND MATERIALS

Source of material: Citronella oil was obtained by the steam distillation of Ceylon citronella {*Cymbopogon nardus* (L.) Rendle} grass obtained from a plantation at Walasmulla, Sri Lanka. Plant specimens were compared with the standard specimens deposited at the National Herbarium, Peradeniya.

Fractionation of essential oil: Citronella oil (5kg) was fractionally distilled using Vacum Fractionation Unit (LT Labortechnic Normschliff) equipped with glass column packed with sulzer packing, installed at the Pilot Engineering Division of CISIR, Colombo.

Mosquito bioassays: Fifty percent lethal concentration (LC_{50}) values of plant extracts for 3rd instar *Culex quinquefasciatus* Say larvae were determined by the procedure followed by World Health Organization[5] with slight modifications. For bioassays, healthy late, 3rd instar *C. quinquefasciatus* larvae were distributed in batches of 20 in small beakers containing 25ml water. Test dispersions (25ml) were prepared in separate beakers by adding different amounts of the extract to give a series of ten final concentrations ranging from 2.0 to 20.0 mg/l, when contents with larvae in small beakers were added to the latter. Tween 80 at 0.1% was used as a surfactant to disperse citronella oil. Mortality counts were taken after 24 h. The negative control contained 0.1% Tween 80 in distilled water. Bioassays were carried out at 29^{0}C with five replicates. LC_{50} values were estimated from a probit/log-concentration graph.

Gas chromatographic analysis: Essential oil and its fractions were analysed by using a Varian 2700 Gas chromatograph; Column: Carbowax 20M 10% coated on Gaschrom Q (3m x 20mm); Injection block temp. 230^{0}C; Detector oven temp. 230^{0}C; Carrier gas (argon) 30ml/min; Samples (0.2μl), with programming from 100 to 230^{0}C at 2^{0}C/min; Recorder, Shimadsu Chromatopak 6A.

RESULTS

Identification of fractionated citronella oil: Fractional steam distillation of *Cymbopogon nardus* oil carried out under different temperature and pressure conditions yielded 13 fractions (Table 1). Gas chromatographic analysis of oil fractions revealed that the fractions F1-F5, contained varying amounts of monoterpene hydrocarbons: α-pinene, camphene, myrcene, limonene, *cis*-ocimine and γ-terpinene. Fractions F6-F13, contained varying amounts of citronellal, linalool, B-caryophyllene, L-borneol, geranyl formate, citronellol, nerol, geraniol, geranyl butyrate, nerolidol, methyl eugenol, elemol, methyl *iso*-eugenol, farnesol etc. The residue (F13) contained high amounts of elemol, methyl *iso*-eugenol and farnesol.

Mosquito-larvicidal activity of oil fractions: The results indicate that fractions, F2, F3, F4 and F13 were highly lethal to mosquito larvae (Table 1). The F2 fraction had the highest larvicidal activity with a LC_{50} value of 1.7mg/l.

As a result of the fractional distillation, myrcene content in the fraction (F2) was increased to 8.73% from 0.8% in unfractionated oil. Myrcene also displayed the highest larvicidal activity (LC_{50}, 0.3mg/l) among standard compounds tested (Table 3). Coefficients of correlation calculated between LC_{50} values obtained for each oil fraction and the essential oil components of fractions are presented in Table 2. The highest negative correlation (r= -0.89) was observed for myrcene (F1-F5 fractions). Larvicidal activity of fractions, F5-F8 was observed to be low and their LC_{50} values ranged from 10.0 to 12.6mg/l. However, the fractions, F9-F12 and the residue (F13) after fractional distillation displayed considerable larvicidal activity comparable to hydrocarbon fractions. When final fractions of distillation were considered high negative correlations were observed between elemol (r= -0.95), methyl *iso*-eugenol (r= -0.95) and LC_{50} values observed for F9-F13 fractions.

Table 1: Activity of citronella oil and its fractions against late 3rd instar *Culex quinquefasciatus* larvae.

Oil/Fraction	Conditions of distillation			LC_{50} value mg/1
	Temperature, ^0C		Vacuum, m.bar	
	Flask head	flask		
*Oil	-	-	-	6.3
Fractions:				
F1	60-65	112	100	5.3
F2	80-85	120	100	1.7
**F2(S)	80-85	120	100	1.4
F3	85-90	120	100	2.0
F4	90-95	125	100	2.8
F5	90-95	125	60	12.6
F6	105-110	130	60	12.6
F7	110-115	130	60	11.0
F8	115-120	135	40	10.0
F9	120-125	135	40	8.3
F10	125-130	140	35	7.2
F11	130-135	145	25	6.2
F12	135-140	150	25	4.4
F13	Residue	-	-	3.4

*Unfractionated oil; **F2 fraction was tested for activity after three months of storage at 29°C.

Table 2: Coefficients of correlation observed between the essential oil constituents of citronella oil fractions (F1-F5; F10-F13) and their LC_{50} values against late, 3rd instar *Culex quinquefasciatus* larvae.

Essential oil component	Coefficient of correlation
Fractions (F1-F5)	
α-Pinene	0.10
Camphene	-0.22
Myrcene	-0.89
Limonene	-0.26
cis-Ocimine	0.21
γ-Terpinene	-0.53
Fractions (F10-F13)	
Nerolidol	0.40
Methyl eugenol	0.95
Elemol	-0.95
Methyl *iso*-eugenol	-0.95
Farnesol	-0.13

Table 3: Effects of standard compounds against late, 3rd instar *Culex quinquefasciatus* larvae.

Standard compound		LC_{50} Value (mg/l)
α-Pinene	(98.5%	6.7
Myrcene	(96.7%)	0.3
γ-Terpinene	(98.2%)	0.8
Limonene	(100.0%)	4.6
Linalool	(97.6%)	>20.0
Citronellal	(80.6%)	>20.0
Terpineol	(100%)	>20.0
Nerol	(95.9%)	>20.0

DISCUSSION

High negative correlation (r= -0.89) observed between myrcene and LC_{50} values obtained for hydrocarbon fractions and also high larvicidal activity of the myrcene standard indicate that myrcene was the major active principle of hydrocarbon fractions responsibile for the larvicidal activity. As γ-terpinene also displayed considerable activity it can be concluded that both myrcene and γ-terpinene are responsible for the larvicidal activity of monoterpene hydrocarbon fractions. Results also showed that fractions, F5- F8 which contained mostly acetylisable compounds possessed very low activity when compared to other fractions. When the final fractions, F9-F13 were considered, high negative correlations observed between elemol (r= -0.95), methyl *iso*-eugenol (r= -0.95) and LC_{50} values for fractions indicates the role of elemol and/or methyl *iso*-eugenol as possible active principles responsible for the larvicidal activity of these fractions.

Total amount of total acetylisable compounds ("total geraniol") in citronella oil determines its market quality.[6] Two citronella species, *Cymbopogon nardus* (L.) Rendle ("Ceylon citronella") and *Cymbopogon winterianus* Jowitt ("Java") are distinguished morphologically, anatomically[7] and chemically.[8] One of the striking differences observed by Wijesekera[9] was the presence of monoterpene hydrocarbons amounting to more than 20% of the oil in *C. nardus* as against 3-4% in *C.winterianus*. The total geraniol content of *C. nardus* oil is low (55-65%) when compared to *C.winterianus* which contains around 85%.

Results showed that a three fold increase in the larvicidal activity of hydrocarbon fractions (F2-F3) resulted from fractionation of oil. Therefore, it is possible to fractionate the hydrocarbon fraction from Ceylon citronella oil and use it as a mosquito larvicide. The separation of monoterpene hydrocarbons from Ceylon citronella oil will also improve its perfume quality by increasing its total geraniol (acetylisables) content. In addition to the hydrocarbon fraction, it is also possible to use the residue obtained from the fractional distillation of oil as a mosquito larvicide. However, in order to use the monoterpene hydrocarbon fraction and the residue obtained from the fractionation of "Ceylon" citronella oil as mosquito-larvicides, their bioactivity and stability under field conditions should be investigated.

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