Original Article

Adulticidal Activity of Five Essential Oils against *Culex pipiens quinquefasciatus*

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The aim of this study is to observe the adulticidal activity of five essential oils against the mosquito *Culex pipiens quinquefasciatus*. Fumigating adulticidal activity was investigated by airtight fumigation in conical flasks. The result showed that the toxic effect of the five essential oils varied with the period of fumigation. Rutaceae oil was the most toxic of the five. Carvacryl oil had the shortest adulticidal time (6.087 min). The chemical components of rutaceae oil were analyzed by GC/MS. The major components were α-citral (33.50%) and citral (35.77%). Citral showed marked adulticidal activity in a short-term fumigation. All five essential oils had considerable adulticidal effects on *Cx. pipiens quinquefasciatus*. © Pesticide Science Society of Japan

Keywords: essential oil, adulticidal activity, *Culex pipiens quinquefasciatus*.

INTRODUCTION

The control of mosquitoes is an important public health concern around the world. Mosquitoes not only are a nuisance but also transmit severe diseases like malaria, filariasis, Japanese encephalitis, dengue fever, and yellow fever.[1] Chemical control is an effective strategy used extensively in daily life. There are many kinds of compounds toxic to mosquitoes, including organochlorine, orgnaophosphorus, carbamates, pyrethroid and so on.[2] They are both effective and residual. However, the environmental threat these chemicals pose and the resistance of mosquitoes to insecticides have increased during the last five decades.[3] There is an urgent need to develop new materials for controlling mosquitoes with environment safety, rapid biodegradation and low cost. Plants are one potentially important source of candidates.

Several compounds obtained from plants possess potential insecticidal or repellent activity. Several herbal products had been used as natural insecticides even before the discovery of chemicals,[4] such as nicotine from tobacco (*Nicotiana tabacum*) leaves, anabasine and lupinine, the alkaloids extracted from Russian weed (*Anabasis aphylla*),[5] rotenone from *Derris eliptica*, and pyrethrums from flowers of *Chrysanthemum cinererifolium*.[6] Numerous plant products have been reported either as insecticides for killing larvae or adult mosquitoes, or as repellents for mosquito biting.[7] Unfortunately, few of them have been made into marketable products.

Essential oils are products of secondary metabolism in plants. Their components and quality vary with geographical distribution, harvesting time, growing conditions and method of extraction. Several experiments on the insecticidal activities of essential oils against various mosquitoes have been conducted. It was found that the hydrogenate fraction of a *Tagetes minuta* floral extract had a strong effect on the larvae and adults of *Aedes aegypti* and *Anopheles stephensi*.[8] Grace reported that the oil of *Pluchea dioscoridis* had marked larvicidal activity against *Cx. pipiens*, and moderate activity against *An. melampodina*.[9] A petroleum ether extract of *Thymus capitatus* was found to be toxic to the eggs, larvae and adults of *Cx. pipiens*. Among different fractions extracted from *T. capitatus*, the volatile oil, thymol and the unsaponifiable portion exhibited strong larvicidal activity, while the unsaponifiable portion and volatile oil had the greatest adulticidal effect, and thymol reduced egg hatchability.[10] Toxicological studies on three-ethanol extract preparations of *Solanum nigrum* leaves showed larvicidal activity against *Ae. caspius* and *Cx. pipiens*,[11] and sunlight, pH value and turbidity during the extraction process did not affect the activity. In this study, five essential oils with possible insecticidal activity were examined for adulticidal effect on *Cx. pipiens quinquefasciatus*.

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MATERIALS AND METHODS

1. Essential Oils

Five essential oils were used in this study. Asteraceae oil was extracted from whole plants of *Ajania tenuifolia* collected from the Haibei Alpine Meadow Ecosystem, Qinhai Province, China. Rutaceae oil was extracted from leaves of *Citrus sinensis* collected from Deqing County, Guangdong Province, China. Both oils were extracted by steam distillation. Metha piperta oil, carvacryl oil, and citronella oil were the products of Shanghai Charoma Perfumery & Bio-Chem Co., Ltd. China. Their source plants were *Mentha piperita*, *Mentha spicata* and *Cymbopogon citrates*, respectively. Citral was provided by Shanghai Medicine Co., China. All testing solutions were dissolved in acetone.

2. Test Mosquitoes

The mosquitoes were a laboratory colony of *Cx. pipiens quinquefasciatus*, provided by the Institute of Parasitological Diseases, Zhejiang Academy of Medical Sciences, China. The mosquitoes were kept at 26±1°C and 65±5% (RH), under a 12 : 12 hr (light : dark) photoperiod. Females 2/3-days-old were used in this study.

3. Bioassays

Fumigating adulticidal activity was tested by airtight fumigation in conical flasks as described by Jiang *et al.*[12] The mosquitoes were placed in 250 mL conical flasks, the number of which was 10–15. A cork sealed with pledget was tightly placed on the conical flask and filter paper (1 cm×3 cm) was pegged to the cork. A 10 μL volume of the essential oil solution was immediately dropped onto the filter paper with a pipette tube. The control mosquitoes were treated under the same conditions with pure acetone. The conical flask was sealed tightly and left for 24 hr at the rearing room.

4. LC_{50} Test

The median lethal concentration (LC_{50}) was the concentration killing half of the adult mosquitoes. The mosquitoes were considered dead when they overturned. Mortality was estimated at 0.5, 1, 2, 4, 6 and 24 hr. Five different concentrations of the essential oil were tested to produce a range of mortality from 5% to 90%. The testing concentrations of rutaceae oil were 0.025, 0.05, 0.1, 0.2 and 0.4%. Those of the others were 0.2, 0.4, 0.8, 1.6 and 3.2%. Each batch of adult mosquitoes was treated with five concentrations for three replicates.

5. LT_{50} Test

The median lethal time (LT_{50}) was the time taken to kill half of the adult mosquitoes. The killing time was also determined by airtight fumigation in conical flasks. The concentration of the five essential oils was the LC_{95} at 0.5 hr. The number of deaths was checked every minute till 30 min, and after 24 hr fumigating.

6. Oil Component Analysis

The components of rutaceae oil were analyzed by Voyage 2000 GC/MS (Finnigan, USA). The operating conditions for GC/MS were as follows. Half a microliter of rutaceae oil was injected into a DB-WAX, fused to a silica capillary column (30 m×0.25 mm and 0.25 μm thick). Helium was used as the carrier gas at 1 mL/min, with a splitting ratio at 200 : 1. The temperature program was as follows: 50°C for 2 min, then rising to 300°C at 10°C/min, with a final hold time of 10 min. The injection temperature was 250°C, ionization source 200°C, and EI 70 eV. Identification of the components was confirmed by comparing spectra with standards of the NIST Library of Voyage 2000 GC-MS.

7. Data Analysis

Probit analysis was used to determine the LC_{50}, LT_{50}, 95% FL (Fiducial Limit) and slopes according to methods outlined by Finney.[13)]

RESULTS

1. Toxicity

All five essential oils had considerable adulticidal effects on *Cx. pipiens quinquefasciatus* under laboratory conditions (Table 1). There were no dead mosquitoes in the control group. Rutaceae oil showed the most effective adulticidal activity. The ranking of the oils based on LC_{50} values differed with period and oil type. The order was: asteraceae oil > metha piperta oil > citronella oil > carvacryl oil > rutaceae oil in the short term (0.5 hr); asteraceae oil > citronella oil > carvacryl oil > metha piperta oil > rutaceae oil in the medium term (4 hr); and asteraceae oil > carvacryl oil > metha piperta oil > citronella oil > rutaceae oil in the long term (24 hr).

2. Killing Speed

The order based on LT_{50} values was: carvacryl oil < asteraceae oil < metha piperta oil < rutaceae oil < citronella oil (Table 2), when the LC_{95} values were used to treat the adult mosquitoes. Arvacryl oil had the fastest adulticidal effect (6.09 min); all the mosquitoes died overnight except when metha piperta oil was used (97% mortality).

3. Components

Figure 1 shows a typical GC/MS chromatogram of rutaceae oil. A totally of 51 compounds were identified (Table 3). The major components were α-citral (33.50%) and citral (35.77%). The contents of epoxy-linalooloxide (4.12%), **D**-limonene (4.10%), neric acid (3.24%) and cis-geraniol (2.36%) were all above 2%.

4. Effect of Citral

Citral showed strong fumigating toxicity against *Cx. pipiens quinquefasciatus* in the short-term (0.5–2 hr). The LC_{50} val-

Table 1. Fumigating toxicity of five essential oils and citral against *Culex pipiens quinquefasciatus*

Essential oil	0.5 hr LC$_{50}$ (%) (95%FL)	0.5 hr Slope[a]	1 hr LC$_{50}$ (%) (95%FL)	1 hr Slope[a]	2 hr LC$_{50}$ (%) (95%FL)	2 hr Slope[a]	4 hr LC$_{50}$ (%) (95%FL)	4 hr Slope[a]	6 hr LC$_{50}$ (%) (95%FL)	6 hr Slope[a]	24 hr LC$_{50}$ (%) (95%FL)	24 hr Slope[a]
A	0.0133 (0.0038–0.0466)	0.8	0.0112 (0.0042–0.0299)	1.2	0.0143 (0.0068–0.0303)	1.5	0.0554 (0.0378–0.0814)	1.5	0.0936 (0.0644–0.1360)	1.4	0.0513 (0.0334–0.0788)	1.3
B	0.4831 (0.3755–0.6215)	2.7	0.2609 (0.1807–0.3767)	2.1	0.1675 (0.0961–0.2921)	1.9	0.2862 (0.1982–0.4133)	1.9	0.3726 (0.2742–0.5062)	2.1	0.6356 (0.5145–0.7851)	3.0
C	0.3633 (0.2597–0.5083)	1.8	0.2220 (0.1486–0.3318)	2.1	0.1728 (0.1042–0.2867)	1.9	0.2879 (0.1901–0.4361)	1.6	0.6532 (0.4668–0.9140)	1.6	0.8508 (0.6683–1.0831)	2.3
D	0.9477 (0.6766–1.3273)	1.8	0.2034 (0.1228–0.3368)	1.8	0.2755 (0.1824–0.4162)	1.8	0.427 (0.3118–0.5849)	2.0	0.8162 (0.6440–1.0344)	2.5	1.7106 (1.1539–2.5357)	2.2
E	0.3971 (0.2663–0.5921)	1.4	0.0523 (0.0074–0.3714)	0.9	0.1692 (0.0973–0.2943)	1.7	0.4174 (0.3227–0.5398)	2.2	0.5715 (0.4685–0.6973)	3.1	0.5495 (0.4482–0.6736)	3.0
F	0.0012 (6.62E–06–0.2188)	0.4	0.0015 (5.3E–05–0.0410)	0.7	0.0007 (5.17E–06–0.0904)	0.5	0.0727 (0.0552–0.1013)	1.6	0.1412 (0.1122–0.1776)	2.3	0.3336 (0.2389–0.4659)	1.9

A, rutaceae oil; B, mentha piperita oil; C, carvacryl oil; D, asteraceae oil; E, citronella oil; F, citral. [a] The slope of the regression equation.

Table 2. The fumigating time of the five essential oils against *Culex pipiens quinquefasciatus*

Essential oil	Slope	LT$_{50}$ (min)	95% FL	LC$_{95}$ (%)	24 hr mortality (%)
A	2.2	18.15	15.43–21.34	1.19	100
B	2.8	13.28	11.76–15.00	2.00	97
C	1.3	6.09	4.66–7.95	3.02	100
D	1.3	8.23	6.30–10.74	8.07	100
E	1.9	21.14	17.89–24.98	6.08	100

The code used for the essential oils was the same as in Table 1.

ues were 0.0012% (0.5 hr), 0.0015% (1 hr) and 0.0007% (2 hr), which were much higher than those of rutaceae oil (Table 1). However, LC$_{50}$ values were 0.0554% (4 hr), 0.0936% (6 hr) and 0.0578% (24 hr) for rutaceae oil and 0.0727% (4 hr), 0.1412% (6 hr) and 0.3336% (24 hr) for citral. The LC$_{50}$ value was higher for citral than rutaceae oil at 4, 6 and 24 hr.

DISCUSSION

Essential oils are usually extracted with ether or distilled with water from different parts of plants. They are mixtures of simple compounds and volatile. The five oils used in this study had the same characteristics, and resources rich in these oils are abundant in China. All five had adulticidal activity against *Cx. pipiens quinquefasciatus* and could serve as potential insecticides in field tests.

The LC$_{50}$ is usually used to evaluate the toxicity of an insecticide. A strong adulticidal effect should mean the mosquitoes die in a shorter period of time. The evaluating marker is the LT$_{50}$. Rutaceae oil had the greatest fumigating toxicity of the five oils, but its killing speed was not the fastest. Carvacryl oil had the shortest killing time. A mix of carvacryl oil and rutaceae oil might have the greatest adulticidal effect. Essential oils are safe to humans and the environment, and

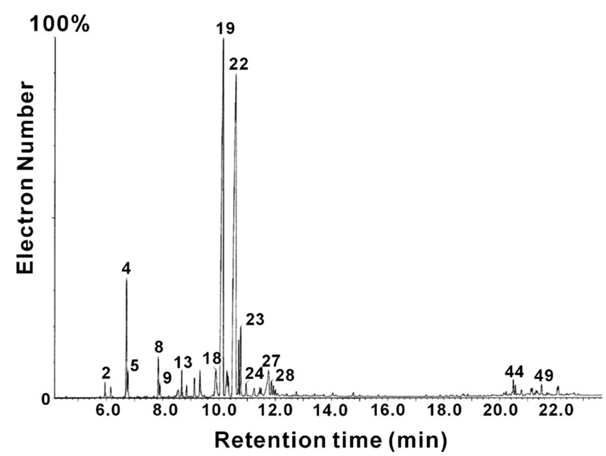

Fig. 1. GC/MS chromatogram of rutaceae oil.

Table 3. List of compounds of rutaceae oil identified by GC/MC in Fig. 1

Peak No.	Retention Time (min)	Name	*m*/*z*	Relative Content (%)
1	5.793	α-Phellandrene	136 (M^+), 93, 91, 79, 77	0.05
2	5.960	6-Methyl-5-hepten-2-one	126 (M^+), 108, 69, 55, 43	0.59
3	6.160	Tetrahydro-2,2-dimethyl-5-(1-methylethyl)-furan	143, 99, 81, 59, 43	0.36
4	6.710	D-Limonene	136 (M^+), 107, 93, 68, 67	4.10
5	6.760	Cineole	154 (M^+), 139, 108, 81, 71	0.89
6	7.352	(1α,2α,5α)-2-Methyl-5-(1-methylethyl)-bicyclo[3.1.0]hexan-2-ol	154 (M^+), 111, 93, 71, 43	0.04
7	7.427	Tetrahydro-α,α,5-trimethyl-5-vinyl-furfuryl alcohol	155, 111, 94, 93, 59	0.11
8	7.835	α-Linalool	136, 93, 71, 55, 43	1.33
9	7.894	*n*-Nonaldehyde	124, 98, 70, 57, 56	0.37
10	8.210	*trans*-*p*-Mentha-2,8-dienol	152 (M^+), 137, 109, 94, 79	0.08
11	8.527	5-(2-Hydroxyethylidene)-2(5*H*)-furanone	126 (M^+), 98, 83, 82, 54	0.20
12	8.552	3,3,5-Trimethyl-1,4-hexadiene	109, 108, 81, 69, 41	0.28
13	8.685	(*R*)-(+)-Citronellal	154 (M^+), 121, 95, 69, 41	0.96
14	8.852	(*S*)-cis-Verbenol	152 (M^+), 109, 94, 81, 67	0.40
15	9.136	1,3,4-Trimethyl-3-cyclohexenyl-1-carboxaldehyde	137, 109, 94, 81, 67	0.75
16	9.327	*p*-Menth-1-en-8-ol	136, 121, 93, 81, 59	1.33
17	9.777	3-Methyl-3-(4-methyl-3-pentenyl)-oxiranecarboxaldehyde	110, 109, 69, 67, 41	0.14
18	9.894	*cis*-Geraniol	154 (M^+), 121, 93, 69, 41	2.36
19	10.144	α-Citral	152 (M^+), 109, 94, 69, 41	33.50
20	10.277	*trans*-Geraniol	154 (M^+), 123, 69, 68, 41	1.46
21	10.327	2-Isopropyl-5-methyl-3-cyclohexen-1-one	152 (M^+), 137, 110, 95, 82	0.75
22	10.594	Citral	152 (M^+), 109, 94, 69, 41	35.77
23	10.769	Epoxy-linalooloxide	127, 109, 81, 59, 43	4.12
24	10.962	1,3,3-Trimethyl-2-oxabicyclo[2.2.2]octan-6-ol	170, 126, 108, 83, 43	0.49
25	11.445	2,7-Dimethyl-2,7-octanediol	110, 95, 82, 59, 41	0.33
26	11.495	1-Methyl-4-(1-methylethenyl)-1,2-cyclohexanediol	152, 137, 108, 71, 43	0.34
27	11.753	Neric acid	168 (M^+), 123, 100, 69, 41	3.24
28	11.862	*S*-(+)-5-(1-Hydroxy-1-methylethyl)-2-methyl-2-cyclohexen-1-one	153, 110, 109, 95, 59	0.47
29	11.937	(*Z*)-3,7-Dimethyl-2-octen-1-ol, isobutyrate	109, 97, 85, 81, 59	0.36
30	12.012	Acetic acid, geraniol ester	136, 121, 93, 69, 43	0.20
31	12.079	2-Methyl-2-(3-methyl-2-oxobutyl)-1-cyclohexanone	153, 135, 111, 71, 43	0.12
32	12.412	4α-Methyl-*trans*-2-decalinone	166 (M^+), 151, 111, 67, 55	0.10
33	12.679	Caryophyllene	204 (M^+), 161, 133, 93, 79	0.08
34	12.754	4-(1,3,3-Trimethyl-7-oxabicyclo[4.1.0]hept-2-yl)-2-pentanone	169, 151, 126, 83, 43	0.17
35	13.404	2,2,3,3-Tetramethyly ciclopropanoic acid, 1-ethylpentyl ester	142, 127, 83, 56, 43	0.10
36	14.046	2,3-Pentadienoic acid, ethyl ester	126 (M^+), 98, 82, 81, 53	0.26
37	14.146	5,5-Diethyl-6-methyl-spiro[2.3]hexan-4-one	166 (M^+), 137, 98, 69, 41	0.13
38	14.771	Caryophyllene oxide	177, 161, 109, 93, 79	0.23
39	15.896	3,4-Dihydro-5,5-dimethyl-4-ethoxycarbonyloxazole	113, 98, 85, 57, 43	0.11
40	17.363	1-(2,2,5α-trimethylperhydro-1-benzoxiren-1-yl)-2-buten-1-one	193, 151, 123, 69, 41	0.09
41	18.855	2,6-Dimethyl-2,6,11-undecatrien-8-ol	153, 123, 95, 69, 41	0.08
42	20.138	2,4,5,5,8α-Pentamethyl-4α,5,6,7,8,8α-hexahydro-2*H*-chromene	208 (M^+), 150, 123, 109, 69	0.17

Table 3. (Continued).

Peak No.	Retention Time (min)	Name	*m*/*z*	Relative Content (%)
43	20.222	ε-Damascone	192 (M^+), 137, 123, 109, 69	0.20
44	20.480	*cis*-Geranic acid methyl ester	182 (M^+), 151, 123, 83, 69	0.57
45	20.555	5,9-Dimethyl-4,8-decadienal	139, 123, 115, 82, 69	0.35
46	20.780	4-(2,2,6-Trimethyl-bicyclo[4.1.0]hept-1-yl)-butan-2-one	208 (M^+), 150, 109, 81, 69	0.27
47	21.172	(*Z*)-3,7,11-Trimethyl-2,10-dodecadien-1-ol	224 (M^+), 123, 109, 95, 69	0.28
48	21.314	1,4,7,7-Tetramethyl-bicyclo[2.2.1]heptan-2-one	166 (M^+), 122, 109, 82, 41	0.16
49	21.505	2-Isopropenyl-5-methylhex-4-enal	152 (M^+), 123, 109, 69, 41	0.50
50	22.072	2-(1-Hydroxybut-2-enylidene)cyclohexanone	166 (M^+), 123, 69, 55, 41	0.29
51	22.106	2,6-Dimethyl-2,6-dodecadiene	194 (M^+), 151, 123, 69, 41	0.34

many of them have a pleasant smell. Therefore, they have good prospects as new insecticides.

Fifty-one components were identified in rutaceae oil using the GC/MS technique; the major compounds were citral and α-citral (69.27%). Both rutaceae oil and citral exhibited noticeable adulticidal activity in this study. Citral was found to be more effective in the short-term; rutaceae oil showed adulticidal activity long-term. Essential oils are mixtures, and different components may act together. Different compounds act on different terms.

Citral is primarily used in foods, beverages, candies, detergents, perfumes and toiletries. It was chosen for its smell in this study and was proven safe.[14] We found a new role for citral in this study. The peel oils extracted from bitter orange (*Citrus aurantium*), orange (*C. sinensis*) and lemon (*C. limon*) were found to have potential insecticidal effects on larvae and adults of *Cx. pipiens quinquenfasciatus*. The volatile extract of *C. sinensis* was also found to possess insecticidal activity.[15] Judging from the toxicity results, the insecticidal activity may be due to citral.

The target of many insecticides is the nervous system, and in particular, acetylcholinesterase (AChE).[16] Methyl bromide and chloropicrin react with nucleophilic sites such as OH, SH or NH_2 groups in vital enzymatic systems.[17] However, little is known about the mode of adulticidal activity of essential oils. The only way volatile insecticides can enter the pest's body is through the respiratory system.[18] In our fumigating tests, inhalation (through spiracles) was the only way the mosquitoes could have received the volatile chemicals in the oils. Judging from the symptoms, the mode of action may be similar to that of a neurotoxin. The primary results showed that AChE was the main target enzyme for carvacryl oil and citronella oil, and a non-specific esterase was the main target for rutaceae oil, mentha piperita oil, carvacryl oil and citronella oil (unpublished data). But the mechanism involved needs further study.

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