

## Effect of culex pipiens against marjoram and bitter orange and its chemical constituents

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### ABSTRACT

In this study; the two oils were analyzed by GC/MS. About 20 and 32 volatile compounds were identified from the volatile oils of *O. majorana* and *C. aurantium* respectively. Terpinen-4-ol and terpinene were the major isolated constituents of *O. majorana*, while limonene and 2-methyl-5-(1-methyl ethenyl)-2-cyclohexene-1-one were the major compounds of *C. aurantium* oil. Larvicidal activity of marjoram (*Origanum majorana*), (family: *Verbenaceae*) and bitter orange (*Citrus aurantium*), (family: *Rutaceae*) volatile oils were tested against the early fourth instar larvae of *Culex pipiens*. The larval mortality was observed after 24 hours of exposure. Both of the two oils showed high larvicidal effects; however, the highest larval mortality was found in the treatment with marjoram volatile oil. The LC<sub>50</sub> values are 188.5 ppm and 251.4 ppm for marjoram and bitter orange respectively. © 2015 Trade Science Inc. - INDIA

### KEYWORDS

Marjoram;  
Bitter orange;  
GC/MS;  
Larvicidal evaluation,  
Culex pipiens.

### INTRODUCTION

Mosquito-transmitted diseases remain the major cause of the loss of human life worldwide with more 700 million people suffering from diseases annually<sup>[12]</sup>. Mosquito-borne diseases have an economic impact, including loss in commercial and labor outputs, particularly in countries with tropical and subtropical climates; especially in Africa and Asia. Plants and their essential oils may be the alternative source for larvicidal agents. In this direction, various plant essential oils have been evaluated<sup>[9]</sup>. The composition of oils from various *origanum* species has been investigated. <sup>1</sup> It was postulated that the oil exist in two forms. One with terpinen-4-ol and sabinene hydrate as major components, while the other with thymol and/or carvacrol as predominant com-

pounds. Also Vera *et al*<sup>[14]</sup>; found that the volatile oil of marjoram is rich in terpinen-4-ol, cis-sabinene hydrate, p-cymene and  $\gamma$ -terpinene. However, the bitter orange volatile oil is found to be rich in linalyl acetate and linalool<sup>[6]</sup>.

Main objectives of this study were to evaluate the efficacy of volatile oils derived from marjoram and bitter orange against of *C. pipiens* as well as the analyses of chemical composition of the applied volatile oils.

### MATERIALS AND METHODS

#### Isolation of the volatile oils

Air-dried aerial parts were cut in small pieces and subjected to steam distillation for three hours using the

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method described by Marcus *et al.*<sup>[8]</sup>; and Weaver *et al.*<sup>[15]</sup>. The volatile oils were dried over anhydrous sodium sulfate and stored under N<sub>2</sub> atmosphere in amber vials at 4 °C until they were analyzed.

### GC-MS analysis conditions

For qualification, the essential oil was analyzed on Gas Chromatography Mass Spectrometry HP 6890 Series A (Agilent) by using A Thermo Scientific (TR-5MS), (5% Phenyl Polysil Phenylene Siloxane) capillary column (30 m x 0.25 mm i.d.; 0.25 µm film thickness). Helium (He), having a flow rate of 1.00 ml/min, was used as carrier gas. The GC oven temperature was kept at 50 °C for 5 minutes and programmed to 250 °C. The injector temperature was 250 °C. The amount of injection was 1 µL.

### Identification of components

Retention indices for all compounds were determined according to the Van Den Dool method<sup>[5]</sup>. While identification of the components was based on comparison of their mass spectra with those of internal (computer) library, Wiley7n.1 and PMW\_Tox3.1 libraries and some reference compounds.

### Mosquito rearing

The larvicidal properties of the tested materials were evaluated under laboratory conditions against larvae of the mosquito species *Culex pipiens* (Diptera: Culicidae). Mosquito larvae were collected from a colony that has been maintained in Faculty of Agriculture, Zagazig University. Adults were kept in wooden framed cages (33x 33 x 33 cm) with a 32x32 mesh at 25 ± 2 °C, 80 ± 2% relative humidity and photoperiod of 14: 10 (L: D) hours. Cotton wicks saturated with 10% sucrose solution were used as food source of the mosquitoes. Females laid eggs in round, plastic containers (10 cm diameter x 5 cm depth) filled with 150 ml of tap water. Egg rafts were removed daily and placed in cylindrical enamel pans (with diameter of 35 cm and 10 cm deep), in order to hatch. Larvae were reared under the above mention conditions of temperature and light and were fed daily with baby fish food until pupation. Pupae were then collected and introduced into the adult rearing cages.

### Larvicidal bioassays

The method recommended by WHO<sup>[16]</sup>, for testing mosquitoes larvicides was followed. One gram of each volatile oil was mixed with 10 mg of emulsifier, Tween 80 and diluted to 100 ml with dechlorinated tap water to give 1% suspension. Further dilutions of 1000, 500, 250, 100 and 50 ppm were prepared in water from the volatile oil of marjoram and 800, 600, 400, 200 and 100 ppm were prepared from bitter orange oil. Twenty five larvae were placed in glass beaker with 250 ml of each concentration. Four replicates were made per concentration, and a control treatment with tap water and emulsifier was included in each bioassay. Beakers with larvae were placed at 25 ± 2 °C, 80 ± 2% relative humidity and photoperiod of 14: 10 (L:D) hours.

### Data analysis

Larvicidal effect was recorded 24 hours after treatment. Data obtained from each dose (Total mortality) were subjected to probit analysis in which probit-transformed mortality were regressed against Log<sub>10</sub>-transformed dose, LC<sub>50</sub>, LC<sub>90</sub> values and slopes were generated.

TABLE 1 : Volatile oil constituents of *Origanum majorana*

Name of compound	Retention time (RT)	Compound percent
Butyl ethanoate	2.9	0.06
α-Terpinene	3.14	0.71
Sabinene	5.3	10.30
Linalyl propionate	6.8	0.25
1-Terpineol	8.4	11.68
B-phellandrene	8.6	1.68
γ-Terpinene	9.7	18.92
Trans-Caryophyllene	10.02	0.04
(+)-Spathulenol	10.7	1.40
Cis-Sabinene hydrate	11.38	18
Isopulegol	11.65	0.01
Nonyl Phenol	12.1	2.3
3-Phenyl (3- <sup>2</sup> H <sub>1</sub> ) Propanal	12.2	0.04
Terpinene-4-ol	14.6	30.6
3-Carene	15.012	3.21
5-Caranol	15.4	0.36
Hexadecanoic acid, methyl ester	15.6	0.046
Isophytol	16.5	2.61
Cineole	17.6	2.02
Linalool acetate	22.9	1.86

TABLE 2 : Volatile oil constituents of *Citrus aurantium*

Name of compound	Retention time (RT)	compound percent
1,5,8-p-Menthatriene	9.79	0.2
Limonene	9.93	34.08
Linalool oxide	12.6	1.15
Trans-sabine hydrate	13.2	0.59
1-Cyclopentyl ethanone	13.43	0.15
Cis-Limonene oxide	13.54	5.58
Trans- Limonene oxide	13.69	6.94
Cis-p-2,8-Menthadien-1-ol	14.14	1.77
2,2-Dimethyl-5-methylene- Norbornane	14.76	0.61
Trans-isolimonene	14.9	0.28
E-Citral	15.008	0.83
1-p-Tolyl ethanone	15.18	0.55
2,4,7-Decatriene-1-ol	15.25	1.96
O-Mentha-1(7),8-dien-3-ol	15.34	1.71
Trans- Ocimene	15.42	3.36
Trans (+) carveol	16.05	7.03
2-methyl-5-(1-methyl ethenyl)-2-Cyclohexen-1-one	16.45	14.17
Camphene	17.24	0.54
Trans-Carvone oxide	17.48	0.83
Perilla aldehyde	17.65	0.91
2-Hydroxy-5-methyl acetophenone	17.74	1.03
Cis-Carvone oxide	17.87	1.87
3-tert Butyl-2-methyl cyclohexen-1-one	18.19	1.03
7,7-dimethyl-2-methylene-bi cyclo[2,2,1] Heptane	18.33	0.96
1-methyl-4-(1-methyl ethenyl)-2-Cyclohexen-1-one	18.45	1.12
2-methyl-5-(1-methyl ethenyl)- Cyclohexanol	18.63	1.69
1-methyl-2-vinyl cyclohexanol	18.96	1.9
1-phenyl propane-1,3-diol	19.12	2.82
3-picoline	21.22	0.54
2-decalone	22.26	1.53
Trans (+) carveol	22.37	1.12
2,3-dimethyl-3-(1-methyl ethenyl)- Cyclopentanol or 2,3-dimethyl-3-(prop-1-en-2-yl)- Cyclopentanol	23.59	0.57

## RESULTS AND DISCUSSION

### Phytochemical analysis

Total of twenty and thirty two components were identified from the *O. majorana* and *C. aurantium* volatile oils respectively. The analysis of *O. majorana* volatile oil showed it to be rich in terpinen-4-ol (30.60%),  $\gamma$ -terpinene (18.92%), cis-sabinene hydrate (18.00%), 1-terpineol (11.68%) and sabinene (10.30%), (TABLE 1).

According to TABLE (2) the analysis of *C. aurantium* showed it to be rich in limonene (34.08%), 2-methyl-5-(1-methyl ethenyl)-2-cyclohexene-1-one (14.17%), trans(+) carveol (7.03%), trans-limonene oxide (6.94%) and cis-limonene oxide (5.58%), were the major compounds of oil.

### Larvicidal bioassay

The insecticidal activity of the *O. majorana* and *C. aurantium* volatile oils were evaluated against fourth

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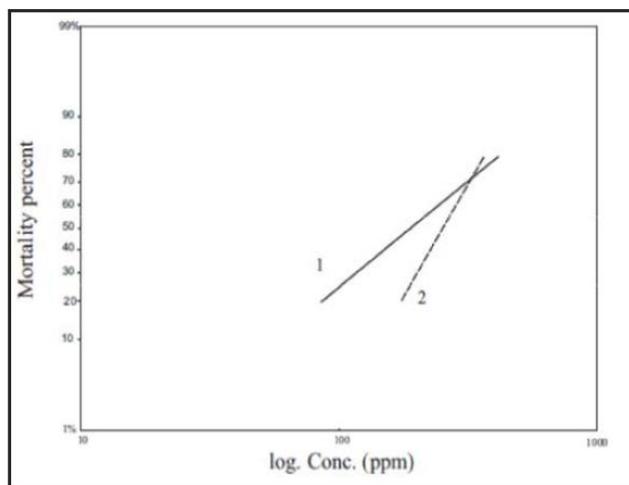
instar larvae of *Culex pipiens*. The toxicity of the two volatile oils showed that the volatile oil of marjoram (*O. majorana*) is more effective than the bitter orange (*C. aurantium*). The  $LC_{50}$  values are 188.5 ppm and 251.4 ppm for marjoram and bitter orange respectively (TABLE 3 and Figure 1).

Aromatic plants are known to produce essential oils which are complex, multi-component system com-

tained from various plants has been evaluated in several studies for example; Chavan *et al.*<sup>[2]</sup>, noted the larvicidal nature of the essential oil *Ocimum basilicum*, which induced 100% mortality against *C. quinquefaciatus* at concentration of 0.12%. Tawatsin *et al.*<sup>[13]</sup>, evaluated and reported repellent effects of essential oils extracted from 18 Thai plants against *Aedes aegypti* *Ae. Albopictus*, *Anopheles dirus* and

**TABLE 3 : The toxicity and slope function of the *Origanum majorana* and *Citrus aurantium* volatile oils against *Culex pipiens* larvae**

Tested plants	Conc. ppm	Mortality %	$LC_{50}$ (fiducial limits) ppm	$LC_{90}$ (fiducial limits) ppm	Slope function
<i>Origanum majorana</i>	50	14	188.5 (96.8-340.1)	628.1 (540.3-2138.6)	2.4
	100	25			
	250	40			
	500	94			
	1000	99			
<i>Citrus aurantium</i>	100	4	251.4 (231.9-271.3)	433.3 (403.7-496.7)	5.2
	200	24			
	400	87			
	600	99			
	800	99			



**Figure 1: Log. Conc.-Mortality regression lines of the plant volatile oils against *Culex pipiens* larvae, 1) *Origanum majorana* and 2) *Citrus aurantium***

posed mainly of terpenes with strong interactions among them.

It is difficult to point out which chemicals are responsible for the larvicidal effect against *Culex pipiens* in this study. The larvicidal activity may be due to the synergetic effects of a combination of phytochemicals in each essential oil<sup>[4]</sup>. The efficiency of volatile oils ob-

*C. quinquefaciatus*. Also, Koliopoulos *et al.*<sup>[7]</sup>, found that the most effective oils against *C. pipiens* larvae were *Mentha suaveolens* (major constituent piperitenone oxide, 62.4%), *M. spicata* (piperitenone oxide, 35.7% and 1,8 cinole, 14.5%) and *M. longifolia* (piperitenone oxide, 33.4%; 1,8 cinole, 24.5% and trans-piperitone epoxide, 17.4%), while *Mellisa officinalis* essential oil was rich in pinenes, limonene, p-cymene and terpen-4-ol had medium larvicidal values. However, the two oils used in this study are belonging to terpene group in their structure act as juvenoids or IGRs. Our results are in accordance with Singh *et al.*<sup>[11]</sup>, Schearer<sup>[10]</sup> and Cruichashank *et al.*<sup>[3]</sup>, who mentioned that the terpenoid amides act as insect juvenile hormone.

## CONCLUSION

About 20 and 32 volatile compounds were identified from the volatile oils of *O. majorana* and *C. aurantium* respectively. Terpinen-4-ol and terpinene were the major isolated constituents of *O. majorana*, while limonene and 2-methyl-5-(1-methyl ethenyl)-2-

cyclohexene-1-one were the major compounds of *C.aurantium* oil. Also the basis of the mortality percentage, it was found that the volatile oils marjoram (*Origanum majorana*) and bitter orange (*Citrus aurantium*) showed a promising insecticidal activity against *Culex pipiens* larvae.

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