# Larvicidal Activity of Essential Oils from Three Plants Grown in South West Region of Saudi Arabia against *Culex pipiens*

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## ABSTRACT

The essential oils isolated from the aerial parts of *Pluchea dioscoridis* (L) (Astracese), *Clutea myricoides* (Euphorbiaceace) and *Lavandula pubescens* (Lamiaceae) were analysed by GC-MS and tested for their larvicidal activity against the 3rd - 4th instars larvae of *Culex pipiens*. The main constituents identified in the three oils were carvacrol, caryophyllene oxide, β-bisabollene and thymol (*P. dioscoridis*); 1, 3-dimethyl-1-cyclohexene, 2-furanecarboxaldehyde-5-methyl, Phenol,3,4,5-trimethyl, bicyclo [2.2.1] hyptene,2-(2-methyl-1-propenyl) and 2-methoxy-4-vinylphenol (*C. myricoides* ); β-eudesmol, Naphthalene,1,2,3,4,4a,5,6,8a-octahydro-4-a,8-dimethyl-2-(1-methylethyl), unidentified compounds, caryophyllene, spathulenol and furan, 2-[(2-ethoxy-3,4-dimethyl-2-(cyclohexene-1-ylidene)-methyl] (*L. pubescens*), respectively. The oil of *P. dioscoridis* was the most active against *C. pipiens* larvae ($LC_{50}$= 8.03 ppm), followed by the oil of *C. myricoides* ($LC_{50}$= 165.22 ppm) and *L. pubescens* ($LC_{50}$= 280.07 ppm), respectively.

The oil of *P. dioscoridis* deserves further investigation to isolate its components which need an individual evaluation for their biological activity.

**Key Words:** Larvicidal activity, Mosquito control, *Culex pipiens*, *Pluchea dioscoridis*, *Clutea myricoides, Lavandula pubescens*.

## INTRODUCTION

Mosquitoes are dangerous medical insect-pest worldwide. In addition to skin allergies and annoying effects their bites cause to man, they transmit a number of serious diseases, such as malaria, yellow fever, dengue fever, West Nile fever and Rift valley fever (Service, 1993; Nasci and Miller, 1996; Tsai *et al*., 1998; Hubalek and Halouzka, 1999). Synthetic insecticides are the main method for controlling mosquitoes. However, many problems associated with using synthetic insecticides have been occurred, for example, insect resistance and environment contamination (Ascher *et al*., 1995; Amalraj *et al*., 2000; Gunasekaran *et al*., 2004; Senthil, 2006). Therefore, searching for safe and effective natural alternatives is necessary. Plants are promising source for environmentally-friend alternatives to chemical pesticides (Jacobson, 1982; Secoy and

Smith, 1983). Many attempts have been made to find plant-derived compounds or extracts to be used against mosquitoes (Omolo *et a*l., 2005; Prajapati *et al*., 2005; Senthil, 2007; Knio *et al*., 2008).

The aim of the present work is to explore and evaluate the potential of the essential oils isolated from three wild plants growing in south western region of Saudi Arabia [*Pluchea dioscoridis* (L) (Astraceae), *Lavandula pubescens* (Lamiaceae) and *Clutea myricoides* (Euphorbiaceae)] as natural larvicides against *Culex pipiens* larvae. Few studies have been performed on the chemical composition or biological activity of the essential oils of these plant species (Hafez *et al*., 1989; Mahmoud, 1997; Al-Taweel *et al*., 2001; Grace, 2002).

## MATERIALS AND METHODS

### Plants.

Samples of whole fresh, *Lavandula pubescens, Clutia myricoides and Pluchea dioscoridis* plants were collected from Asir region, Saudi Arabia. Plants were identified by the Botany Department, College of Science, King Saud University, Riyadh, Saudi Arabia.

### Isolation of the volatile oils.

Aerial parts of the tested plants were chopped with distilled water, and they were directly steam distillated; the distillates were extracted with diethyl ether. The ether extracts were dried over anhydrous sodium sulfate. The solvent was evaporated under vacuum at 40 $^{\circ}$C using a rotary evaporator. The oily products were kept in glass vials with Teflon screw caps at – 20 $^{\circ}$C.

### Instrumental Analysis

Agilant Technologies 6890 N Net work gas chromatograph system with Agilant 5973 mass selective detector was used for MS identification of the components of the three isolated oils. The gas chromatogrphy was equipped with Agilant 7683 injector. A HP1- MS, 30 m x 0.25 mm i.d and film thickness 0.25 µm capillary column was used in combination with the following oven temperature programme: initial temperature 40 $^{0}$C, held for 2 min, 2 $^{0}$C / min ramp to 230 $^{\circ}$C held for 10 min. The carrier gas (helium) flow rate was in constant flow mode at 1.1ml / min. Splitless injection of one µl volume of the sample, dissolved in methanol, was carried out at 250 $^{\circ}$C. The mass spectrometer was operated in electron ionization mode with a transfer line temperature of 280 $^{\circ}$C, ion source 230 $^{\circ}$C and selected ion-monitoring mode.

### Mosquito colony

The susceptible strain of *C. pipiens* used in this study was obtained from The High Institute of Public Health, Alexandria Univ., Alexandria, Egypt, and was maintained in our laboratory for more than five years. Hatched larvae were fed on finely powdered mouse feed until they reached the $3^{rd}$- $4^{th}$ larval instar.

### Larvicidal activity

Twenty mosquito larvae were placed in a 50 ml glass beaker containing 50 ml of the desired concentration in distilled water. Tested concentrations, dissolved in ethanol (containing 1% Tween 80) were added to give the required final concentrations and stirred quietly with a glass rod. Each concentration was replicated 3 times. Three controls that received only the solvent were maintained during the test. Dead larvae were counted 48 h after treatment and larvae that did not move when touched with a thin needle were considered to be dead. Data were corrected according to Abbott formula (1925) and probit analysis of data was done according to Finney (1971).

## RESULTS AND DISCUSSION

The main chemical constituents of the essential oils that isolated from the aerial parts of *P. dioscroidis*, *C. myricoides* and *L. pubescens* are presented in Table1. The major constituents of *P. dioscoridis* oil were carvacrol (49.55 %), followed by caryophyllene oxide (7.81%), β-bisalbollene (7.71%), Benzene-1-methoxy-4-methyl-2-(1-methylethyl) (7.18%), thymol (6.63%), and the rest of the components shown in Table 1. Bioassoys showed that *P. dioscoridis* oil was the most toxic against third-fourth instar larvae of *C. pipiens* ($LC_{50}$ = 8. 03 ppm) followed by *C. myricoides* oil ($LC_{50}$ = 165.22 ppm) and *L. pubescens* oil ($LC_{50}$ = 280. 06) as presented in Table 2. The high content of corvacrol, thymol, caryophyllene, β-bioabollene, and caryophyllene oxide in the oil of *P. myricoidis* could be responsible for its high toxicity to *C. pipiens* larvae.

These compounds were the main constituents in the essential oil of *Satureja spinosa*, with $LC_{50}$ of 56.1 ppm, against *C. pipiens* larvae (Michaelakis *et al*., 2007). Mansour *et al*. (2000) found that the oil of *Thymus capitatus* was toxic to *C. pipiens* larvae and the oil contained high percentages of thymol, carvacrol and caryophyllene. Carvacrol and thymol were the main components in *Origanum syriacum* oil and there $LC_{50}$ values against *C. pipiens* larvae were 37.6 and 36 ppm, respectively (Traboulsi *et al*., 2002).

The only available report on the biological activity of *P. dioscoridis* against *C. pipiens* larvae was published by Grace (2002); the $LC_{50}$ of the tested oil was 71.86 ppm. The main constituent of the oil isolated from *P. discoridis* grown in Egypt was farneosol (16.5%). The variation in the chemical composition and in insecticidal activity between the oil of *P. dioscoridis* grown in Saudi Arabia and that grown in Egypt is common. Variation of chemical composition and biological activity of many plant species depends on geographic location, time of collection, growing conditions, age and developmental stage of plant (Wells, 1993; Singh *et al*., 2001).

As shown in Table 1, the main chemical constituents of *C. myricoides* oil are 1, 3-dimethyl-1-cyclohexene (20.45%), bicyclo [2.2.1] heptene, 2-(2-methyl-1-propenyl) (17.8%), followed by different furanyl and phenolic compounds that compose (53.12%). The oil showed moderate activity (Table 2) against *C. pipiens* larvae ($LC_{50}$ = 165.22 ppm).

For our knowledge, this could be the first report on the chemical analysis and biological activity of the volatile oil extracted from this plant species.

The major components in the oil of *L. pubescens* were β-eudesmol (37.32%), followed by caryophyllene, spathulenol and a number of sesquiterpene and oxygenated sesquiterpene compounds (Table1). Oils containing eudesmol, caryophyllene and spathulenol were shown to have mosquitocidal activity (Mansour *et al*., 2000, Albuquerque *et al*., 2004, Hadjiakhoondi *et al*., 2003).

In conclusion, the oils of *C. myricoides* and *L. pubescens* were moderately toxic to *C. pipiens* larvae. However, the oil of *P. dioscoridis* was highly toxic and further investigations are needed to isolate and evaluate its components individually for their biological activity.

Table1. Main chemical constituents of essential oils extracted from Saudi plants and tested for their larvicidal activity against *Culex pipiens.*

| Constituent | Rt | Constituent (%) | | |
|---|---|---|---|---|
| | | *P. dioscoridis* | *C. myicoides* | *L. pubescens* |
| (+) -4-cerene | 21.41 | 2.44 | | |
| Benzene-1-methoxy-4-methyl-2-(1-methylethyl) | 33.01 | 7.18 | | |
| Thymol | 36.19 | 6.63 | | |
| Carvacrol | 38.77 | 49.55 | | |
| Caryophyllene | 44.54 | 3.2 | | |
| β-bisabollene | 50.65 | 7.71 | | |
| Caryophyllene oxide | 55.00 | 7.87 | | |
| Furon, 2-5-dimethyl | 80.66 | 4.32 | | |
| Furon, 2-5-dimethyl | 7.49 | | 5.19 | |
| Furanmethanol | 8.47 | | 2.6 | |
| Ethanone, 1-(2-Furanyl) | 10.34 | | 8.29 | |
| 2-Furanecorboxaldehyde,5-methyl | 13.29 | | 9.67 | |
| Furancarboxylic acid, methyl ester | 14.41 | | 2.54 | |
| Phenol, 2-methoxy | 22.03 | | 5.91 | |
| Phenol, 2-ethyl | 28.99 | | 2.73 | |
| ,2-(2-methyl-1-propenyl) Bicyclo[2.2.1] heptene | 30.64 | | 17.8 | |
| 4-pyridazinamine | 30.87 | | 5.9 | |
| 1.3-Dimethyl-1-cyclohexene | 34.04 | | 20.45 | |
| Phenol,3,4,5-trimethyl | 37.48 | | 9.56 | |
| 2-Methoxy-4-vinylphenol | 38.02 | | 9.36 | |
| 2-Hexnal (E) | 7.75 | | | 2.66 |
| Benzeneacetaldehyde | 18.39 | | | 2.83 |
| NI | 41.67 | | | 13.95 |
| NI | 42.31 | | | 9.31 |
| Naphthalene,1,2,3,4,4a,5,6,8a-octahydro-4-a,8-dimethyl-2-(1-methylethyl) | 43.18 | | | 4.25 |
| Caryophyllene | 44.18 | | | 5.39 |
| Eudesma-4(14), 11-diene | 48.39 | | | 3.63 |
| β-eudesmol | 59.18 | | | 37.32 |
| Spathulenol | 64.98 | | | 4.14 |
| 2(3H)-Benzoofuranone,6-ethenyllhexahydro-6-methyl-3-methylene-7(1-methylethyl) | 68.78 | | | 4.93 |
| Furan,2-[(2-ethoxy-3,4-dimethyl-2-(cyclohexene-1-Ylidene)-methyl] | 75.42 | | | 8.04 |

Rt= Retention time NI = Not identified

Table 2. Probit analysis of larvicidal activity of the three tested oils against *Culex*

| Plant | Family | $LC_{50}$ (ppm) ( 95% CL) | $LC_{95}$ (ppm) ( 95% CL) | Slope test |
|---|---|---|---|---|
| *P. dioscoridis* | Astraceae | 8.03 (6.56-9.57) | 46.8 (35.75 - 67.53) | 2.15 |
| *C. myicoides* | Euphorbiaceae | 165.22 (132.3- 203.29) | 735.86 (472.4 - 1951) | 2.54 |
| *L. pubescens* | Lamiaceae | 280.07 (241.9- 331.55) | 429 (428.38 - 802.45) | 8.86 |

*pipiens* 3rd – 4th instars larvae after 48 h of exposure.

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## الملخص العربي

# النشاط الإبادي للزيوت الطيارة من ثلاث نباتات تنمو جنوب غرب السعودية ضد يرقات البعوض

**علي سرار**

**قسم وقاية النبات - كلية الزراعة - جامعة الملك سعود.**

تم التعرف على المكونات الأساسية للزيوت الطيارة المعزولة من الأجزاء الهوائية لنباتات *L. pubescens, C. myricoides, P. dioscoridis* بواسطة كروماتوجراف الغاز- مطياف الكتلة. وكانت أهم المكونات الأساسية التي تم التعرف عليها لنبات *P. dioscoridis* هي: كارفاكرول، كاريوفيللين أكسيد، ب- بيسأبوللين، ثيمول. أما مكونات *C. myricoides* هي3,1 ثنائي الميثيل-1- هكسين حلقي، 2-فيوران كاربوكسالدهيد-5- ميثيل، فينول5,4,3ثلاثي ميثيل، [1,2,2] هبتين ثنائي الحلقة2-(2-ميثيل-1-بروينيل)، 2-ميثوكسي-4-فينيل فينول. وبالنسبة لمكونات نبات

*L. pubescens* كانت بيتا إيوديسمول، نفثالين 1، 2، 3، 4، 4أ، 5، 6، 8أ أوكتا هيدرو-4أ-8 ثنائي ميثيل( 1- ميثيل إثايل). كاريوفيللين، سباثولينول، فيوران 2- [2-إيثوكسي-3، 4 ثنائي ميثيل-2- هكسين حلقي-1- يليدين)- ميثيل] . وقد أظهر زيت نبات *P. dioscoridis* أعلى كفاءة إبادية ضد يرقات البعوض(العمر3-4) حيث كان التركيز القاتل لنصف الافراد المعاملة 8.03 جزء في المليون يليه زيت نبات *C. myricoides* (165.33 جزء في المليون) ثم أخيرا نبات *L. pubescens* (280.07 جزء في المليون). وعليه فإن زيت نبات *P. dioscoridis* يحتاج إلى فصل مكوناته كل على حده وإختبار نشاطها البيولوجي منفردة لتحديد المركب المسئول عن هذا النشاط الإبادي ضد يرقات البعوض.