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# Larvicidal Activity of Essential Oil of *Lactuca sativa* against *Culex pipiens* (Diptera: Culicidae)

**Amal M. F. Al-Barty**
Biological Science Department, Faculty of Science,
Taif University, Taif, El-Hawyeia 888, Saudi Arabia.
Email: aalbarty@yahoo.com

***Abstract* – The main aim of the study is to evaluate the larvicidal activity of essential oil of*Lactuca sativa*against Culex pipiens (Diptera: Culicidae).The analysis and the identification of the various constituents of essential oil were carried out by gas chromatography. This oil mainly consisted of α-pinene (5.11% and 0.62%), p-Cymene (2.07% and 1.92%), thymol (11. 55% and 10.73%), durenol (52.00% and 49.97%), α-terpinene (1.34% and 1.34%), thymol acetate (0.99% and 0.67%), caryophyllene (2.11% and 1.98%), linalool (3.09% and 2.98%), camphene (4.11% and 3.65%), limonene (1.28% and 1.11%) as the major compounds. The lethal concentrations (LC50 and LC90) measured for the essential oil *Lactuca sativa*, were respectively of the order of 258.71 mg/L and 580.49 mg/L. Biological test was performed according to a standard methodology inspired by the World Health Organization protocol. The results could be useful in search for newer, safer, and more effective naturallarvicidal agents.**

***Keywords* – *Culex pipiens*, *Lactuca sativa*, Larvicidal, Essential Oil, Natural Agents.**

## I. INTRODUCTION

The species of the genus Culex are incumbent vectors for several pathogens such as West Nile virus, affecting humans and/or animals Culex pipiens (*Cx. pipiens*) has been strongly suspected as the vector responsible for transmission[1-3].

These mosquito species usually breed in stagnant water with high levels of organic matter, such as artificial containers[4,5], and blocked drainages or the ditches in urban and suburban areas[6].

Almost all the medicinal plants available in the world have great potential sources for discovery as well as production of new drugs benefit to mankind. Presently, there are lot of approaches available to search for new biologically active ingredients in the medicinal plants for the preparation of safe drugs. Scientifically many works have been expended to evaluate and discover newantioxidant, antimicrobial and antifungal ingredients from different kinds of natural sources like soil, microorganisms, animals and plants. Different types of folk medicine or herbal medicine are among the most important resources. Systematic screening of these available traditional herbs may result in the discovery of novel effective bioactive compounds for the formulation of drugs[7].

These specific essential oils are made up of many different types of terpenoids and volatile organic compounds and have been shown to possess antimicrobial, antifungal and anti-bacteridal properties [8].

The essential oils and organic plant crude extracts are of particular interest because of their safety and their wide acceptance by consumers and their uses for potential multi-purpose functional uses[9].

Lettuce is a vegetable plant belonging to Asteraceae family. It is often grown everywhere as a leaf vegetable. This leaf vegetable was first cultivated by the Egyptians. After first cultivation during 16$^{th}$to 18$^{th}$century Europeans first saw and found many varieties and species of lettuce. But nowadays the consumption of lettuce has spread tremendously throughout the world due to their medicinal importance. Lettuce is easily cultivated in relative low temperatures countries. However, currently it is cultivated in tropical and subtropical countries with special nursing [5-8].

The aim of this present study is to examine the chemical composition of the essential oils and antioxidant activity of the crude extracts from the fresh and dry leaves of lettuce and studying thelarvicidal activity of this oil against *Culex pipiens* species.

## II. MATERIALS AND METHODS

### *2.1. Plant harvest and extraction of essential oil*

The fresh green leaves and stems of lettuce were collected. The plant samples were collected in the morning at 10:00 am and identified by morphological features and data base present nowadays. The samples were instantly packed in the plastic bags and stored in freeze until the isolation of essential oil. The plant samples were dried under shade. About 50 g of dried leaves were ground using a grinder (Jaipan, Super Deluxe) for 20 seconds.

### *2.2. Isolation of the essential oil*

The air-dried and fresh plant material (100 g) was subjected to hydrodistillation individually for 3h using a Clevenger type apparatus. The isolated essential oils were re-extracted using organic solvent dichloromethane. Finally, the essential oil from fresh and dry leaves samples were dried over anhydrous sodium sulphate and preserved in a sealed vial at 4 °C until further analysis.

### *2.3. GC-MS analysis*

The GC -MS analysis of the essential oils isolated from the fresh and dry leaves of locally grown lettuce was performed using a Perkin Elmer Clarus 600 GC-MS system (equipped with an 30 µm 0.25 i.d., film thickness 0 .25 µm) coupled with a Perkin Elmer Clarus 600C MS. The fused silica capillary column (model Rtx®-5MS) was used for separation of essential oils. For the detection of gas chromatography-mass spectroscopic data an electron ionization system with ionization energy of 70 eV was

used. Inert gas, helium was used as a carrier gas at a constant flow rate of 1 m L/min. Mass transfer line and injector temperatures were set at 220 °C and 290 °C, respectively. The oven temperature was programmed from 60 °C (hold 2 min ) to 270 °C at 4 ° C/min, then held isothermal for 20 min and finally raised to 290 °C at 10 °C/min. One micro litre oil sample was injected into the column with split mode condition. The split ratio was 200:1. The calculative percentage of the chemical constituents in the crude essential oil was expressed as a percentage by peak area.

### *2.4. Identification of essential oil*

The chemical compounds of essential oils were identified Based on the retention time on silica capillary column and the matching of mass spectra with the standard library such as NIST 2005 v.2.0 and Wiley Access Pak v.7, 2003. Whenever possible, the mass spectra was matched by co-injection with the authentic compounds[10].

### *2.5. Characteristic breeding site*

The collection of larvae of *Cx. pipiens* was performed in a breeding site located in one of the urban area. This site is characterized by a very high density of larval belonging to Culicidae. The warm water from this site promotes the proliferation of larvae of Cx.pipiens.

### *2.6. Collection of larvae of Cx. pipiens*

Larvae were collected using rectangular plastic tray that inclined 45 ° to the water surface. L arvae harvested were maintained breeding in rectangular trays with an average temperature of (25.6) °C in the Entomology Unit.

### *2.7. Identification of larvae*

The identification of morphological characters of larvae has been performed using the identification key of Culicidae and the identification software dealing with mosquitoes of Mediterranean Africa[11].

### *2.8. Larval susceptibility testing*

A stock solution (10%) of essential oil in dichlormethan and a dilution series: 100 , 200, 300, 400, 500 and 600 mg/L were prepared. Preliminary experiments enabled us to select a range of concentrations for test. About 1 mL of each solution prepared was placed in beakers containing 99 mL of distilled water in contact with 20 larvae of stage 3 and 4. The same number of larvae was placed in a beaker containing 99 mL indicator of distilled water plus 1 mL ethanol (control). Three replicates were carried out for each dilution and for the control. After 24 h of contact, living and dead larvae were counted.

The results of susceptibility testing were expressed in percentage of mortality versus concentrations of essential oils used. If the percentage of mortality in control is greater than 5%, the percentage of mortality in larvae exposed to the essential oil shall be corrected by using Abbott's formula[12]. % Mortality corrected=( % Mortality observed-% Mortality Control)/(100-% Mortality Control)X100.

### *2.8. Statistical analysis*

Statistical analysis of data was based on SAS's program. The data were subject to analysis of variance (ANOVA). Means were compared by Duncan's multiple range test (Duncan, 1955) at $P < 0.05$. Percentages of the mortalities were corrected according to Abbott's formula [12] as follows:

$$\% \text{ Corrected mortality} = (T - C)/(100 - C)$$

Where: T : % mortality in treatment,
C: % mortality in check (control).

## III. RESULTS

### *3.1. Chemical composition of essential oil*

The total ion chromatogram spectra (TIC) of the isolated essential oils from the fresh and dry leaves of lettuce by gas chromatography-mass spectrometry are shown in Table(1) and Figure (1and 2).

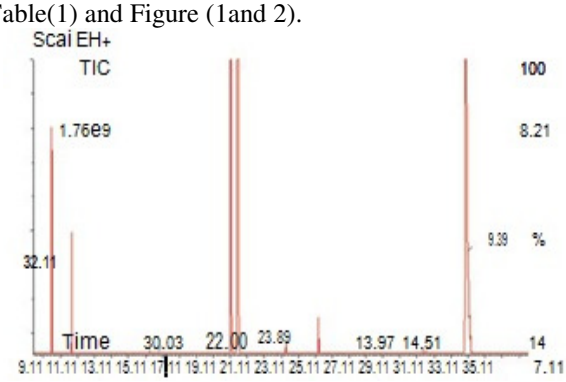


Fig.1. A typical chromatogram of the constituents of essential oil

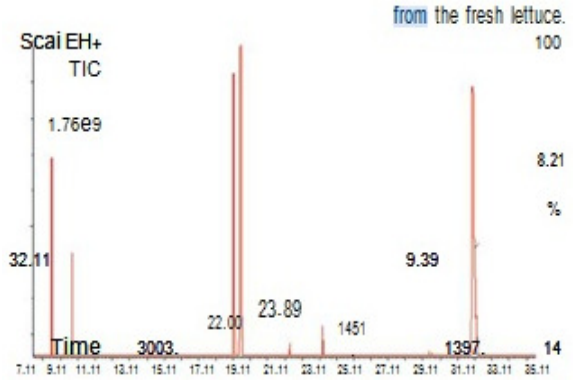


Fig.2. A typical chromatogram of the constituents of essential oil

The TIC chromatogram showed that most of the peak heights are higher in essential oil from fresh lettuce than the essential oil from the dry lettuce. T he analysis of the chemical constituents in both essential oils by using GC-MS had led to the identification of 20 different classes or groups and different concentration of volatile terpenoids and organic compounds; representing 83.07% and 79.88% of the total essential oils isolated from fresh and dry leaves samples of lettuce, respectively. The identified chemical compounds in the isolated essential oil by GC-MS are listed in Table 1 according to their elution order on the fused silica capillary column (model Rtx®-5MS). Both the essential oil contain a mixture of compounds consisting of mainly oxygenated mono and sesquiterpene hydrocarbons and their derivatives. The major volatile organic compounds detected in the fresh and dry leaves oils, respectively were α-pinene (5.11% and 0.62%), p-Cymene

(2.07% and 1.92%), thymol (11. 55% and 10.73%), durenol (52.00% and 49.97%), α-terpinene (1.34% and 1.34%), thymol acetate (0.99% and 0.67%), caryophyllene (2.11% and 1.98%), linalool (3.09% and 2.98%), camphene (4.11% and 3.65%), limonene (1.28% and 1.11%) as the major compounds.

Table 1: Chemical formula of essential oils of *Lactuca sativa*.

| Dry Leaves (%) | Fresh Leaves (%) | Retention Formula | Molecular | Compound name |
|---|---|---|---|---|
| 0.62 | 5.11 | 5.600 | 136 | α- Piene |
| 0.12 | 0.12 | 6.960 | 128 | 3-Octanone |
| 0.22 | 0.22 | 7.110 | 136 | B------- |
| 0.31 | 0.31 | 7.950 | 136 | α |
| 1.92 | 2.07 | 8.190 | 134 | P-Cymene |
| 1.35 | 1.35 | 9.390 | 136 | α |
| 2.98 | 3.09 | 10.862 | 154 | Linalool |
| 0.46 | 0.46 | 13.968 | 154 | 4-Terpinol |
| 0.46 | 0.46 | 14.513 | 154 | α |
| 0.30 | 0.30 | 16.699 | 164 | O-Methylthymol |
| 10.73 | 11.55 | 18.750 | 150 | Thymol |
| 49.98 | 52.00 | 19.195 | 150 | Durenol |
| 0.67 | 0.99 | 22.001 | 192 | Thymolaetate |
| 1.98 | 2.11 | 23.892 | 204 | Caryophyllene |
| 0.29 | 0.29 | 24.673 | 204 | L-Alloaromadendrene |
| 0.17 | 0.17 | 26.904 | 204 | Viridiflorene |
| 0.54 | 0.54 | 30.035 | 220 | α |
| 0.62 | 0.62 | 30.240 | 220 | 3-Octanone |
| 1.11 | 1.28 | 32.700 | 137 | Limonene |
| 3.65 | 4.11 | 32.710 | 136.2 | Camphene |
| 79.88 | 83.07 | | | Total |

In this present study, there are some minor compounds that were also isolated and identified from fresh and dry lettuce such as β-pinene, α-terpinolene, linalool, 4-terpineol, α-terpineol, o-methylthymol, L-alloaromadendrene, viridiflorene (Table1). Almost all the chemical constituents identified in both the essential oil isolated from the fresh and dry lettuce exhibited very high potent biological activity.

### *3.2. Larvicidal activity of essential oil of Lactuca sativaagainst Cx. pipiens*

#### *3.2.1. Variation in mortality rate*

After exposure to different concentrations of essential oil of *Lactuca sativa* for 24 h, the mortality rate of larvae of Cx.pipiens at stage 3 and 4 varied from 17.3% to 100% (Figure 3).

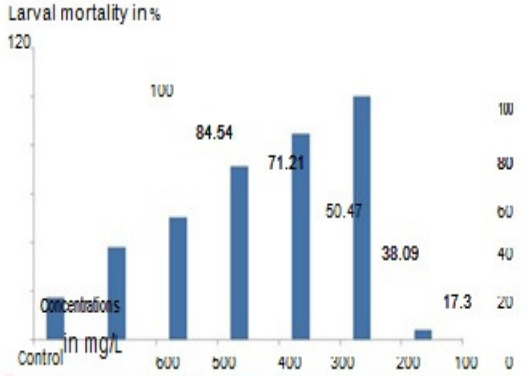


Fig.3. Mortality(%)of larvae of*Cx. pipiens*varied depending on theconcentration of essential oil (mg/L) of *Lactuca sativa*after 24 h exposure.

The lowest concentration necessary to achieve mortality of larvae of *Cx. pipiens* was evaluated at 600 mg/L (Figure 3).

#### *3.2.2. Lethal concentrations LC50 and LC90*

After 24 h, the essential oil from the leaves of *Lactuca sativa* exhibited significant larvicidal activity; the LC50 and LC90 of the essential oil of *Lactuca sativa*is 258.71 mg/L (lower limit-upper limit: 126.99-527.06 mg/L) and 580.49 mg/L (lower limit-upper limit: 354. 51-950 .53 mg/L) respectively. Chi-square values (equation of the regression line Y=3.65193+8.81146X and calculated $\chi^2$=16.3978) of the essential oil show significant larvicidal activity.

## IV. DISCUSSION

The essential oils from the fresh and dry lettuce by hydrodisstillation were yellowish in colour and the main chemical constituents in the essential oils were oxygenated monoterpenes, sesquiterpenes, hydrocarbons volatile organic compounds and their derivatives. The majority of the researchers have reported that the major chemical constituents in the essential oils from the medicinal plant origin are monoterpenes and sesquiterpenes hydrocarbons and their oxygenated derivatives. These monoterpenes and sesquiterpenes hydrocarbons and their oxygenated derivatives have low molecular weight organic volatiles and enormous potential to strongly exhibit microbial pathogens [13,14]. But in most of cases the antimicrobial ingredients in the essential oils are terpene derivatives,

which are almost phenolic in character. It would seem reasonable that their antimicrobial and antifungal mode of action might be related to that of other chemical compounds.

The medicinal effects of plant materials typically result from the combinations of secondary metabolic products or compounds present in the plant origin. These secondary metabolic products or compounds are not essential for cell structure and maintenance of life but often involved in plant protection against biotic or abiotic stresses. Natural products, as essential oils, as pure compounds or as standardized extracts, provide unlimited opportunities for the preparation of new drug discoveries because of the unmatched availability of chemical diversity inside the sources[15,16].

The essential oil yield of *Lactuca sativa* (0.8%) obtained in this study, is relatively low compared to some plants that are exploited industrially as the source of essential oils[17]. Plant essential oils, in general, have been recognized as an important natural resource of insecticides[18].

Taking into account the absence of studies on the essential oils of *Lactuca sativa* against specifically the species *Cx. pipiens*, we tried to compare the action of a plant of the species of *Lactuca sativa* against Culex. Thus, the LC50 and LC90 obtained from the plant Origanumvulgare Euro-Asian species against the mosquito Culex sp. were respectively 256 and > 500 mg/L[19]. These results are close to those found in our study.
This study has shown the larvicidal action of the essential oil of *Lactuca sativa* against *Cx. pipiens*. This essential oil can be an effective alternative in the fight against mosquito vectors of disease.

This paper reports the isolation and GC-MS analysis of the essential oils from the fresh and dry leaves of lettuce.

Thus, lettuce could become an alternative to synthetic bactericides for using in agro industries and also to screen and develop such novel types of selective and natural bactericides in the treatment of many microbial phytopathogens causing severe destruction to crops, vegetables and ornamental plants. Therefore, the essential oils isolated from this species are potential active candidates to be used as antimicrobial and antifungal agents in new drugs preparation for therapy of infectious diseases. Further advance research on toxicological and clinical studies are required to prove the safety of the oil asa medicine[20].

## V. Conclusion

This study is aimed to evaluate the larvicidal activity of essential oil of*Lactuca sativa* against *Culex pipiens* (Diptera: Culicidae). The results proved the efficiency ad activity of essential oil of *Lactucasativa* against larval stages of *Culexpipiens* and this is safer and natural way against larvicidal stage of *Culex pipiens.*

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