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Research Article

# Chemical Composition and Larvicidal Activity of *Mentha pulegium* Essential Oil from Central Morocco Against Larvae of Mosquito *Culex pipiens* (Diptera: Culicidae)

Fouad El-Akhal[1], YassineEzzoubi[2], Taghzouti Khalid[3], Abdelhakim El Ouali Lalami[1,4]

[1]*Regional Diagnostic Laboratory of Epidemiological and Environmental Hearth, Regional Health Directorate, ELGhassani Hospital, 30000 Fez, Morocco.*
[2]*Laboratory of Applied Organic Chemistry, Faculty of Sciences and Technology, Sidi Mohamed Ben Abdellah University, B.P. 2202 – Route d'Imouzzer, Fez, Morocco.*
[3]*Laboratory of Animal Physiology, Department of Biology, Faculty of Science, University Mohammed V, Rabat, Morocco.*
[4]*Institute of Nursing Professions and Health Techniques, EL Ghassani Hospital, 30000 Fez, Morocco.*

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**ABSTRACT**
Background: In Morocco, the species *Culex pipiens* (Diptera: Culicidae) has developed resistance to the synthetic insecticide. Therefore, there is an urgent need to find alternatives to the insecticides, as natural herbal biocides. Objective: The essential oil of *Mentha pulegium* grown the North center of in Morocco has been analyzed and their larvicidal activity on *Culex pipiens* was determined. Methods: The analysis and the identification of the various constituents of essential oilobtained by hydro-distillation of the aerial part were carried out by Gas Chromatography coupled with Mass Spectrometry (GC/MS). Biological tests were realized according to a methodology inspired from standard World Health Organization protocol. The larvicidal activity of medicinal plant extracts were tested against early fourth-instar larvae of *Culex pipiens*. The repellent efficacy was determined against of mosquito species (*Culex pipiens*) at six concentration's (5, 10, 20, 40, 80 and 160 ppm) under the laboratory condition. The mortality was observed 24h after treatment; data were subjected to probit analysis to determine the Lethal Concentrations ($LC_{50}$ and $LC_{90}$) to kill 50 and 90 percent of the treated larvae of tested species. Results: The major constituents of *Mentha pulegium* essential oil were identified as pulegone (53.23%), α-pinene (12.1%), piperitone (9.62%), menthone (9.26) and piperitenone (6.6%). The $LC_{50}$ and $LC_{90}$ were estimated at 25.45 ppm and 98.66 ppm respectively. Conclusion: This investigation indicates that the *Mentha pulegium* essential oil could serve as a potential larvicidal and could be used as an ideal ecofriendly approach for the control of the *Culex pipiens*.

**Keywords:** *Mentha pulegium*, Essential oil, Chemical composition, *Culex pipiens*, Larvicidal activity, Fez, Morocco.

## INTRODUCTION

Vector-borne diseases remain a public health problem. Mosquitoes constitute an important group of arthropods for public health[1]. They transmit a wide range of human diseases such as Filariasis, Malaria, Dengue, Yellow fever and Japanese encephalitis, causing millions of deaths worldwide each year[2,3].

The continuous application of synthetic insecticides causes development of resistance in vector species, contamination by of toxic substances through the food chain and adverse effects on environmental quality and non target organisms including human health[4].

It was found that Culex (Diptera: Culicidae) populations could develop resistance on account of the widespread use of insecticides[5-9]. Considering the above, there is an urgent need to develop new insecticides which are biodegradable and environmentally safe[10]. Natural products such as extracts and essential oils of plant could be suitable alternatives to use of synthetic insecticides in the fight against the vectors of diseases in particular versus the *Culex pipiens* species[11-13].

Several plants were chosen for their traditional use as insect repellents[14]. Among these plants, *Mentha pulegium* (*M. pulegium*) belonging to the genus of Mentha (Lamiaceae), is one of the most popular essential oils crops[15]. Essential oil obtained from plants of the genus Mentha is a potent medicinal herb with many activities in food, flavours, cosmetics and for pharmaceutical purposes[16]. Besides medicinal applications, insecticidal properties of several Mentha spp essential oils are reported against ants, mosquitoes, wasps, hornets and cockroaches[17].

This is in order to make our contribution to this part of the research we have harvested, extracted and analyzed the chemical composition of essential oil of *M. pulegium* as well as determined their larvicidal activity on *Culex pipiens* (*C. pipiens*), suspected to be the vector of West Nile in Morocco in 1996[18,19], in 2003[20]. The insecticidal

**Author for Correspondence: eloualilalami@yahoo.fr*

Figure 1: Photo of plant *Mentha pulegium.*

activity of *M. pulegium* plant against *C. pipiens* has never been studied before in the North center of Morocco.

## MATERIALS AND METHODS

*Plant material and authentication.*

A sample of plant *M. pulegium* (leaves, stems and roots) (Figure 1) was collected in February and March 2014 from natural habitat in province of Taounate (North east of Morocco).

This plant has been identified and a specimen was deposited in the herbarium of The National Institute of Medicinal and Aromatic Plants of Taounate city falling within of the Sidi Mohamed Ben Abdellah University, Fez, Morocco. Samples of 200g of the fresh aerial parts of *M. pulegium* were subjected to hydrodistillation for 2 hours using a Clevenger apparatus; the obtained essential oil was stored at 4°C so that can be used in the upcoming experiments. Essential oil yield was calculated as the percentage of essential oil volume obtained by dry weight of plant used.

*Chemical characterization of essential oil.*

The chromatographic analysis of essential oil was conducted in ''Centre Universitaire Régionale d'Interface'' (CURI) in Fez city. The Gas Chromatography (GC) analysis were performed using a Hewlett Packard Gas Chromatographer (HP 6890) with electronic pressure control, equipped with a HP-5MS capillary column (30 m x 0.25 mm, film thickness 0.25 µm), a FID detector set at 300°C and using a H2/Air mixture, and a *split-splitless*injector set at 220 °C. The injection mode was split (split ratio: 1/50, flow rate: 1.4 ml/min) and the injected volume was about 1 µl. Nitrogen was used as carrier gas with a flow rate of 1.7 ml/min. The column temperature was programmed from 40 to 300 °C at a heating rate of 4°C/min, during 5 min. The apparatus was controlled by a "Chemstation" computer system.

The Gas Chromatography/Mass Spectrometry (GC/MS) analysis were performed by a Hewlett-Packard Gas Chromatographer (HP 6890) coupled with a mass spectrometer (HP 5973). Fragmentation was performed by electron impact at 70 eV. The carrier gas is helium whose flow is fixed at 1.5 ml/min. The injection mode was split (split ratio: 1/70). The apparatus was controlled by a "Chemstation" computer system.

The identification of the components is based on the comparison of their mass spectra (GC/MS), respective with spectra of the library (NIST 98), of the bibliography[20], Kovats index for each compound on OPTIMA-5 column was calculated in reference to n-alkanes.

*Characteristic of breeding site*.

The collection of larvae of *C. pipiens* was performed in a breeding site located in the urban area of the city of Fez, named "Hafat My Driss" (395 m altitude; 34°04'00.76'' latitude and 4°59'28.96'' longitude). This site corresponded to very high density of *C. pipiens* larvae.

*Collecting C. pipiens larvae.*

Larvae were collected using a rectangular plastic in May 2014. This collection of larvae were maintained in breeding at an average temperature of at 23 ± 2°C, 70 ± 10% relative humidity in the Entomology Unit at the Regional Diagnostic Laboratory Epidemiological and Environmental Hygiene falling within Regional Health Directorate of Fez.

*Morphological identification of larvae*.

Larvae were determined using a Moroccan Culicidae identification key[22] and the Mediterranean Africa mosquito's identification software[23].

*Protocol for larvicidal efficacy of plant extracts on C. pipiens.*

Biological tests were inspired from standard World Health Organization protocol[24]. A stock solution (1000 ppm) and dilution series for *M. pelugium* extract, was prepared. Preliminary experiments were used to select a range of concentrations to be tested. About 1 ml of each solution prepared was placed in beakers containing 99 ml of distilled water in contact with 20 larvae of stadium 3 (L3) and 4 (L4)[24], and the same number of larvae was placed in a beaker containing 99 ml indicator of distilled water plus 1 ml of ethanol. Three replicates were carried for each dilution and for the control.

After 24 h contact, living and dead larvae were counted. The results of susceptibility testing sites were expressed in percentage of mortality versus concentrations of essential oils used. If the % mortality in controls is greater than 5%, the % mortality in larvae exposed to the essential oil is corrected by using Abbott's formula[25].

% Mortality Corrected = [(% Mortality Observed - % MortalityControl) / (100 - % Mortality Control)] × 100.

If the control mortality exceeds 20%, the test is invalid and must be repeated.

*Data processing*.

Probit analysis of concentration-mortality data (Windl version 2.0) software developed by CIRAD-CA/MABIS was conducted to estimate the $LC_{50}$ values[26] and associated 95% confidence limits for each treatment[27]. The analysis of the averages and standard deviation was also performed by using the test of analysis of variance ANOVA. Mean and standard deviation (± SD) were determined from at least three independent experiments.

## RESULTS

Table 1: Percent yield and physical characters of *M. pulegium* from center of Morocco.

| Botanical name | Part used | Physical characteristics Color | Odor | Density (g/ml) | Yield(%) |
|---|---|---|---|---|---|
| *M. pulegium* | Areal part | Pale Yellow | Persistent, fragrant Aromatic mint-like | 0.934 | 1.65±0.15 |

Table 2: Chemical composition of *M .pulegium* essential oil analyzed by GC-MS.

| Number | RT | Compound | % Area |
|---|---|---|---|
| 1 | 773 | 4-isopropyl-1,3-cyclohexanedione | 0.6 |
| 2 | 929 | α-pinene | 12.1 |
| 3 | 913 | d-limonene | 1.3 |
| 4 | 920 | caryophyllene | 0.7 |
| 5 | 929 | α-humulene | 0.2 |
| 6 | 940 | 3-octanol | 1.4 |
| 7 | 963 | cyclohexene, 4-methyl-1-(1-methylethenyl) | 0.5 |
| 8 | 1021 | 1.8-cineole | 0.2 |
| 9 | 1163 | menthone | 9.26 |
| 10 | 1078 | 2-cyclohexen-2-one | 0.7 |
| 11 | 1189 | α-terpineol | 0.9 |
| 12 | 1241 | pulegone | 53.23 |
| 13 | 1247 | piperitone | 9.62 |
| 14 | 1323 | menthylacetate | 0.1 |
| 15 | 1347 | piperitenone | 6.6 |
| 16 | 1410 | β-caryophyllene | 0.8 |
| Total | | 98.21 % | |

*Yield of essential oil*

The yield of essential oil of *M. pulegium* was founded of the order of 1.65%±0.15. This essential oil yield was calculated on the basis of the dry matter.

*Chemical composition of essential oil*

The composition of essential oil was analyzed by GC and GC/MS showed that the major components of *M. pulegium* are pulegone (53.23%), α-pinene (12.1%), piperitone (9.62%), menthone (9.26) and piperitenone (6.6%) (Table 2).

*Variation of mortality*

After exposure to different concentrations of essential oil of *M. pulegium* for 24 h, the
mortality rate of larvae of *C. pipiens* at stage 3 and 4 varied from 10% to 100% (Figure 2).

The lowest concentration necessary to achieve 100% mortality of larvae of *C. pipiens* was evaluated at 160ppm (Figure 2).

*The $LC_{50}$ and $LC_{90}$ Lethal Concentrations*

According to the table below (Table 3), $LC_{50}$ and $LC_{90}$ show the larvicidal effect of the essential oils tested. The essential oil of *M. pulegium* has the lowest $LC_{50}$ of 25.45 ppm and an $LC_{90}$ = 98.66 ppm (Equation of the regression line: $Y = -3.06179 + 2.17811 * X$; calculated $Chi^2$: 9.835). According to the shown $LC_{50}$and $LC_{90}$ values (Table 3), there are no significant differences between the tested essential oils ($CI_{95}$ overlap).

**DISCUSSION**

Average yield of *M. pulegium* essential oil was 1.65%±0.15. Which is in agreement with that reported by (Derwich et al., 2010)[28] which has found a yield of order of 1.54%, from the species *M. pulegium* grown at Skoura near Boulmane, 90 km in the south east of Fez City, central of Morocco. Similar study realized by (Foganholi, et al. 2015)[29], demonstrated that the yield of *M. pulegium* essential oil was between 0.17 % and 0.23 %. Oliveira et al., (2011)[30]also reported that the yields of *M. pulegium* essential oils grown in Brazil varied between 0.2 % and 0.09 % in the spring and winter, respectively. The differences in the yield of Mentha essential oils with respect to geographical regions were reported by Aziz and Craker (2009)[31].

In our research, the major constituents of *M. pulegium* essential oil found are: pulegone, α-pinene, piperitone, menthone and piperitenone. In the study realized by (Derwichet al., 2010)[28], twenty eight compounds were identified in leaves oil of *M. pulegium* collected from Skoura near Boulemane City (central of Morocco) and the major component was piperitone (35.56%) and other predominant constituents were: piperitenone (21.18%), α-terpineol (10.89%), pulegone (6.452%), pipéritone oxide (4.02%), menthol (3.28%), menthone (3.09%), neomenthol (2.80%), menthofuran (2.15%), isomenthone (1.56%), carvone (1.13%), geranylacétate (1.06%), germacrène D (1.03%) and limonène (1.02%). Daniel et al., (2002)[32], studied the composition of *M. Pulegium* oil collected from the Uruguay, they reported that the composition is characterized by a high content of pulegone (73.4%) and isomenthone (12.9%). While, the *M. pulegium* essential oil from Egypt was characterized by pulegone (43.5%) and piperitone (12.2%)[33]; and that from Tunisia was composed of: pulegone (41.8%) and isomenthone (11.3%)[34]. However, the pulegone was found as the main constituent in samples collected in the spring (61.43 %) and in the winter (28.40 %) by (Oliveira et al., 2011)[30], followed by trans-caryophyllene (18.68 % and 10.20 %), respectively. Derwich et al., (2010)[35] also reported that the piperitone (35.56 %) and piperitenone (21.18 %) as the major constituents of the essential oil of *M. pulegium* from the region of Boulmane (Skoura) of Morocco.

In our study, the insecticidal activity of the oil of *M. pulegium* can be considered very important. Indeed, more than 90% of *C. pipiens* larvae were killed using 98.66 ppm of the essential oil ($LC_{50}$: 25.45 ppm). Pavela et al. (2014)[36], conducted a study on the activities of essential oils of Mentha species on *Culex quinquefasciatus* (Diptera: Culicidae) and also he found a significantly different larvicidal efficacy. In the literature, it has been shown that Mentha species have insecticidal activity against various insects such as *Aedes aegypti* ($LC_{50}$=

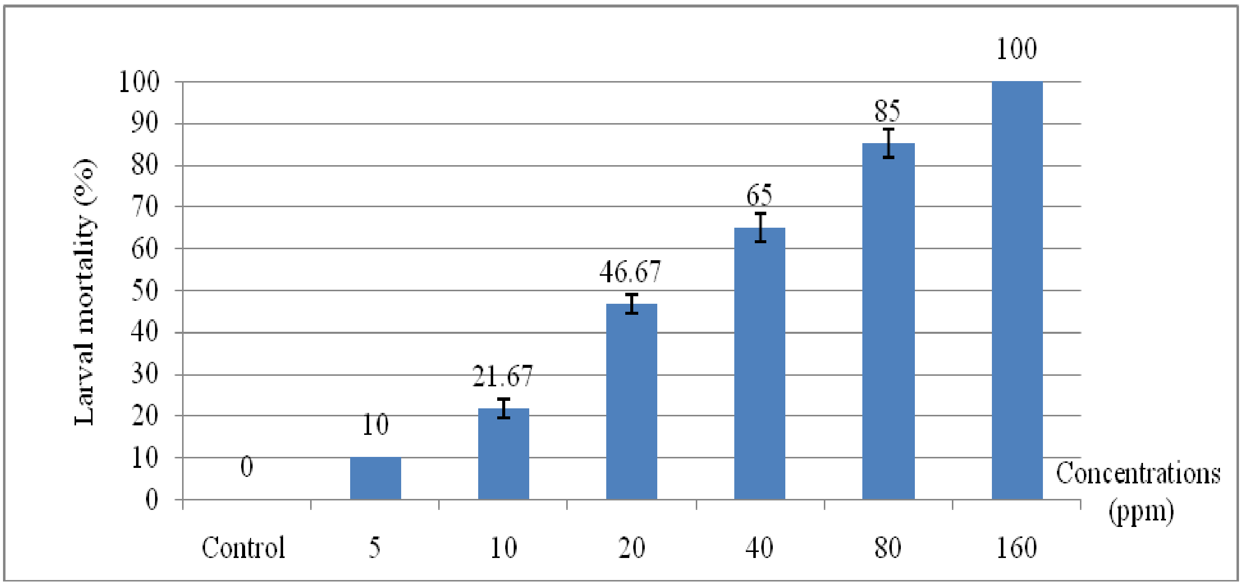


Figure 2: Mortality (%) of larvae of *C. pipiens* depending on the plant species *M. pulegium* essential oil concentration after 24 h of exposure.

Table 3: Concentrations $LC_{50}$ and $LC_{90}$ lethal larvae of *C. pipiens* after 24 h of exposure.

| Plant species | $LC_{50}$(ppm) (Ll-Ul)* | $LC_{50}$(ppm) (Ll-Ul)* |
|---|---|---|
| *M. pulegium* | 25.45 (13.86- 44.34) | 98.66 (58.50- 130.44) |

* Ll-Ul: Lower limit-Upper limit.

114.33 ppm) and *Culex quinquefasciatus* ($LC_{50}$= 112.18 ppm)[37].
Also, the literature reports that the Mentha oil species, from India, recorded the $LC_{50}$ value of 42.25 ppm and $LC_{90}$ value of 132.41 ppm against the larvae of *Culex quinquefasciatus* at 24 h exposure[38]. Koliopoulos et al., (2010)[39] reported that the $LC_{50}$ values of Mentha species, from Central Greece, ranged from 47.88 to 74.28 ppm and $LC_{90}$ values ranged from 64.34 to 107.45 ppm against *Culex pipiens* after 48h exposure. The important larvicidal activity observed among the essential oil of *M. pulegium* might be explained by the action or the effect of the major components. Inhere results are the first in Morocco; this might allow developing an effective low cost and eco-friendly larvicides.
As a perspective of this study and as part of our researchs on bio-insecticide conducted in the laboratory[40,41], we plan to conduct studies on the larvicidal activity of *M. pulegium* essential oil against other species of the Culicidae family, including the species *Anopheles labranchaie*, responsible for indigenous malaria in Morocco.

## CONCLUSION

In the present work, realized for the first time in Morocco, we were interested to examinate the chemical composition and to evaluate the effect of *Mentha pulegium* essential oil on the toxicity of mosquito larvae (*Culex pipiens*). This study demonstrated that the GC/MS analysis of *Mentha puegium* showed that the major components were: pulegone (53.23%), α-pinene (12.1%), piperitone (9.62%), menthone (9.26) and piperitenone (6.6%). The essential oil of *M. pulegium* showed a larvicidal activity against *Culex pipiens* larvae in stages 3 and 4, with respective values of $LC_{50}$ and $LC_{90}$ about 25.45 ppm and 98.66 ppm. Therefore, this essential oil could be a promising alternative against mosquitoes.

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## CONFLICT OF INTERESTS

The authors declare that they have no conflict of interests.

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