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Chemical composition and larvicidal activity of essential oil of *Origanum majorana* (Lamiaceae) cultivated in Morocco against *Culex pipiens* (Diptera: Culicidae)

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ABSTRACT

Objective: To evaluate the larvicidal activity of essential oil of *Origanum majorana* (Lamiaceae) cultivated in Morocco against *Culex pipiens* (Diptera: Culicidae).

Methods: The analysis and the identification of the various constituents of essential oil were carried out by gas chromatography coupled with mass spectrometry. Biological test was performed according to a standard methodology inspired by the World Health Organization protocol with slight modification.

Results: This oil mainly consisted of monoterpene and sesquiterpenes. The majority compounds are 4–terpinene (28.96%), γ–terpinene (18.57%), α–terpinene (12.72%) and sabinene (8.02%). The lethal concentrations (LC_{50} and LC_{90}) measured for the essential oil *Origanum majorana*, were respectively of the order of 258.71 mg/L and 580.49 mg/L.

Conclusions: The results could be useful in search for newer, safer, and more effective natural larvicidal agents.

1. Introduction

The species of the genus *Culex* are incumbent vector for several pathogens such as West Nile virus, affecting humans and/or animals[1–3]. The outbreaks of West Nile virus infection have been reported in Morocco in 1996, 2003 and 2010[4,5]. *Culex pipiens* (*Cx. pipiens*) has been strongly suspected as the vector responsible for transmission[4–7].

These mosquito species usually breed in stagnant water with high levels of organic matter, such as artificial containers[8,9], and blocked drainages or the ditches in urban and suburban areas[10]. In the region of Fez, the species of *Culex* genus are found all the year[11]. Their presences may pose a threat to the population of Fez region, which is considered as crossroads and place of residence of several travelers and nationals of sub saharienns countries affected by vector–borne diseases[12].

To fight against these vectors of disease, insecticides are the most widely used products, but they have several disadvantages since they can be the source of various environmental problems[13–15].

Indeed, most chemical insecticides utilized caused a major problem in development of resistance by certain mosquitoes[16–19]. In addition, researchers and scientists are currently trying to find effective and accessible alternative from natural products, which are of renewed interest and growing popularity[20].

In Morocco, studies on the insecticidal activity of vegetable extracts against the mosquito larvae are very limited[21,22], at least to our knowledge the essential oil of *Origanum majorana* (*O. majorana*) has not been the subject of previous studies. Furthermore, the comparison of natural products with synthetic chemicals helps to better exploit these natural bio–insecticides.

The literature reports that the genus *Origanum* (Lamiaceae) is characterised by a large number of biological activities, including antioxidant, antiinflammatory and

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anticholinesterase effects, as well as activities against ageing and neurodegenerative disease[23]. Recently, the species of this plant have attracted more attention of consumers due to the antimicrobial, antifungal, insecticidal and antioxidative effects of this herb on human health[24].

Regarding *O. majorana* which is an herb and perennial native to southern Europe and the Mediterranean, it is used in food for flavoring sausages, meats, salads and soups. Traditionally, it is used as a folk remedy against asthma, the headache, and rheumatism[25].

In this research, authors aimed to study the chemical composition and larvicidal activity of essential oil of *O. majorana* cultivated in central Morocco against *Cx. pipiens* for the first time.

2. Materials and methods

2.1. Plant harvest and extraction of essential oil

Plant material of the *O. majorana* plant was harvested in March 2010 at the city of Taounate. A total of 200 g of the plant material of *O. majorana* was subjected to a water distillation for 3 h, using a modified Clevenger type apparatus. The essential oil collected by decantation at the end of the distillation has been dried over anhydrous sodium sulfate to remove traces of residual water. The essence thus obtained was opaque in small vials and stored at 4 °C before use.

2.2. Chemical study and identification composed

Chemical analysis of the essential oil was performed with the aid of a gas chromatograph coupled to mass spectrometry (GC-MS).

Then gas phase chromatographic analyses were carried out with the aid of a Trace GC Ultra apparatus equipped with one injector in Split Play, a VB-5 column (30 m×0.25 mm, film thickness 0.25 μm). The operating conditions are as follows: carrier gas: helium; solvent: ethyl acetate; injection temperature: 220 °C; injection volume: 1 μL; flow rate: 1.4 mL/min; temperature program: from 40 °C to 180 °C to 4 °C/min, with a level of 20 min to 300 °C.

The coupling with the mass spectrometer Polaris QMS was done with a temperature of 300 °C interface. The operating conditions are as follows: type electron impact ionization (70 eV); injector temperature was 200 °C. The database used was NIST M Search.

The identification of the constituents was assigned on the basis of comparison of their retention indices and mass spectra with those given in the literature[26,27].

2.3. Characteristic breeding site

The collection of larvae of *Cx. pipiens* was performed in a breeding site located in the urban area of the city of Fez, appointed Grand Canal (402 m altitude, 30°03′37′′ N and 5°08′35′′ E). This gite is characterized by a very high density of larval belonging to Culicidae. The warm water from a thermal spring named Ain Lah promotes the proliferation of larvae of *Cx. pipiens*.

2.4. Collection of larvae of *Cx. pipiens*

Larvae were collected using rectangular plastic tray that inclined 45° to the water surface. Larvae harvested were maintained breeding in rectangular trays with an average temperature of (22.6±2.0) °C in the Entomology Unit at the Regional Diagnostic Laboratory Epidemiological and Environmental Hygiene falling within Regional Health Directorate of Fez.

2.5. Identification of larvae

The identification of morphological characters of larvae has been performed using the identification key of Moroccan Culicidae and the identification software dealing with mosquitoes of Mediterranean Africa[28].

2.6. Larval susceptibility testing

A stock solution (10%) of essential oil in ethanol 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[29]. % Mortality corrected=(% Mortality observed-% Mortality Control)/(100-% Mortality Control)×100.

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

2.7. Processing of data

For the entry and processing of data, the log-probit analysis (Windl version 2.0) software developed by CIRAD-CA/MABIS was used[30].

3. Results

3.1. Yield of essential oil

The yield of essential oil of *O. majorana* is 0.8%. This essential oil yield was calculated on the basis of the dry matter.

3.2. Chemical composition of essential oil

The results of the analysis of essential oil by GC-MS showed that the major components of *O. majorana* are 4-terpinene (28.96%), γ-terpinene (18.57%) and α-terpinene (12.72%) (Table 1). The monoterpene hydrocarbons constitute the major fraction (51.7%), followed by oxygenated monoterpenes with 44.38%. The sesquiterpene hydrocarbons represented only 3.67% of all the constituents identified (99.75%).

Table 1

Chemical composition of essential oil of *O. majorana* analyzed by GC-MS.

Chemical composition	Retention Index	Percentage (%)
α-thujene	928	0.65
α-pinene	939	0.76
Sabinene	975	8.02
β-pinene	-	1.79
α-phellandrene	-	0.50
α-terpinene	1 018	12.72
p-cymene	1 026	0.80
β-phellandrene	-	3.83
γ-terpinene	1 062	18.57
Terpinolene	1 085	4.06
Cis hydrate sabinene	-	0.47
Trans hydrate Sabinene	-	0.44
α-terpineol	-	1.42
Acetate de linalyle	1 094	5.63
Cis hydrate sabinene acetate	1 078	0.95
Monoterpenes	1 189	3.32
Hydrocarbon monoterpenes oxygenated	1 257	1.09
Monoterpenes	-	0.26
Trans dihydrocarvone	-	0.17
4-terpinene	-	28.96
p-menth-2-en-1-ol	-	1.67
β-caryophyllene	1 419	2.10
Valencene	-	1.48
α-humulene	1 454	0.09
Monoterpenes	-	96.08
Monoterpenes hydrocarbon	-	51.70
Monoterpenes oxygenated	-	44.38
Sesquiterpenes	-	3.67
Sesquiterpene hydrocarbon	-	3.67

3.3. Larvicidal activity of essential oil of O. majorana against Cx. pipiens

3.3.1. Variation in mortality rate

After exposure to different concentrations of essential oil of *O. majorana* for 24 h, the mortality rate of larvae of *Cx. pipiens* at stage 3 and 4 varied from 17.3% to 100% (Figure 1).

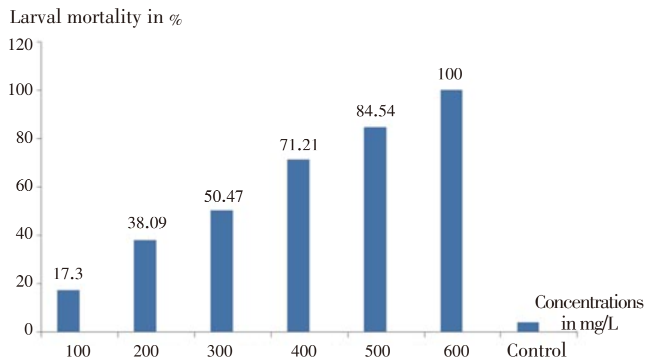

Figure 1. Mortality (%) of larvae of *Cx. pipiens* varied depending on the concentration of essential oil (mg/L) of *O. majorana* after 24 h exposure.

The lowest concentration necessary to achieve 100% mortality of larvae of *Cx. pipiens* was evaluated at 600 mg/L (Figure 1).

3.3.2. Lethal concentrations LC_{50} and LC_{90}

After 24 h, the essential oil from the leaves of *O. majorana* exhibited significant larvicidal activity; the LC_{50} and LC_{90} of the essential oil of *O. majorana* 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 χ^2=16.3978) of the essential oil show significant larvicidal activity.

4. Discussion

The essential oil yield of *O. majorana* (0.8%) obtained in this study, is relatively low compared to some plants that are exploited industrially as the source of essential oils[20]. The yield of the plants *O. majorana* cultivated in a nursery located in Soliman in the North-East of Tunisia was found between 0.04% to 0.09%[31], but that obtained from a species of Indian country is around 1.7%[32]. The yield of *Origanum vulgare*, which is an Eurasian species and belong to the same family (Lamiaceae) with *O. majorana* was found in the order of 7.4%[33].

Plant essential oils, in general, have been recognized as an important natural resource of insecticides[34]. The leaves of the *Origanum* herb are rich in essential oil which confers its characteristic and fragrance. Several studies have shown that the essential oil of *Origanum* is composed of majority constituents, giving it the biological activities. The extraction product can vary in quality, quantity and composition according to climate, soil composition, geographical location, seasonal variation, plant organ, age and vegetative cycle stage, and harvesting time[35,36].

In this work, major components of essential oil of *O. majorana* are: 4-terpinene with an pourcentage of 28.96%, γ-terpinene with 18.57% and α-terpinene with 12.72%. The composition of the essential oil of that same species cultivated in Tunisia has levels of 64.01% to 71.4% in oxygenated monoterpenes, 21.73% to 29.92% in hydrocarbon monoterpenes and 1.47% to 4.05% in sesquiterpene hydrocarbons[31]. According to work by Banchio *et al*.[37], the major components of *O. majorana* have been terpinen-4-ol (55.09%), cis-sabinene hydrate (8.37%), α-terpineol (9.09%) and trans-sabinene hydrate (13.20%).

The very important larvicidal activity observed in the essential oil of *O. majorana* could be explained by the chemical composition of this oil and the action or effect of compound majority. Indeed, it was recently reported by some authors that *Origunum* species have an insecticidal activity against insects[24,33,38].

Thus, essential oils of the *Origanum vulgare*, an Eurasian plants species, have been also found to have a greater larvicidal activity against mosquito *Culex* sp. The percentage mortality of species *Origanum vulgare* has been found in the order of (88.6±7.2)%[33].

Similar studies performed by Traboulsi *et al*. have shown the insecticidal activity of four plants including genus *Origanum* against larvae of *Cx. pipiens*. LC_{50} obtained were between 16 and 89 mg/L[39]. Phenolic compounds such as carvacrol (61%) and thymol (21.8%) were quantitatively the most important in the essence of *Origanum syriacum*. The evaluation of larvicidal activity of these compounds, in the same conditions, demonstrated that thymol (LC_{50}=36 mg/L) and carvacrol (LC_{50}=37.6 mg/L) were responsible for this activity[39].

Taking into account the absence of studies on the essential oils of *O. majorana* against specifically the species *Cx. pipiens*, we tried to compare the action of a plant of the species of *Origanum* against *Culex*. Thus, the LC_{50} and LC_{90} obtained from the plant *Origanum vulgare* Euro-Asian species against the mosquito *Culex* sp. were respectively 256 and >500 mg/L[33]. These results are close to those found in our study.

This study has shown the larvicidal action of the essential oil of *O. majorana* against *Cx. pipiens*. This essential oil can be an effective alternative in the fight against mosquito vectors of disease.

In our study, realized for the first time in Morocco, we evaluated the chemical composition of essential oil of *O. majorana* by GC-MS, which allowed us to identify 24 compounds. The major compounds are: 4-terpinene (28.96%), γ-terpinene (18.57%), α-terpinene (12.72%) and sabinene (8.02%). This oil showed larvicidal property against larvae of *Cx. pipiens*. The oil also showed an interesting larvicidal property against larvae of *Cx. pipiens* with LC_{50} value of 258.71 mg/L LC_{90} value of 580.49 mg/L.

We plan to continue this study to clarify the nature of the compounds responsible for the activity by fractionation carried out in parallel with biological tests and study evaluating the larvicidal activity against other mosquito larvae and other plants including harvested aqueous extracts.

Conflict of interest statement

We declare that we have no conflict of interest.

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