Chemical composition and insecticidal activity of *Anacyclus pyrethrum* essential oil from the Bensliman area against *Culex pipiens*

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Abstract: *Anacyclus pyrethrum* is a herbaceous plant that belongs to the Asteraceae family. The focus of the present study is to extract the essential oil from this plant, to determine its chemical composition and to evaluate its insecticidal activity against the larvae of the mosquito *Culex pipiens*. The essential oil from *A. pyrethrum* collected from the region of Bensliman in Morocco was extracted by hydrodistillation and analyzed by gas chromatography coupled to mass spectrometry were studied. Essential oil yield obtained by hydrodistillation was 0.09%. The major aroma constituents were Spathulenol (20.47%), Germacrene D (16.48%), Caryophyllene oxide (13.20%), 4(14)-Salvialene-1-one (8.27%) and Caryophyllene 4(14),8(15)-dien-5α-ol (7.30%). The larvicidal test carried out according to a methodology based on the standard protocol of the World Health Organization was studied on 4th instar larvae of *C. pipiens* and showed that *A. pyrethrum* essential oil possesses remarkable insecticidal properties. After 24 hours of exposition, larvicidal assays revealed a 100% mortality of *C. pipiens* larvae. The dose of 40 μL/mL was toxic enough to cause 100% larval mortality of *C. pipiens*. The lethal concentrations LC50 and LC90 calculated for the essential oil studied were of the order of 14.79 μL/mL and 19.95 μL/mL, respectively. To control mosquitoes, this essential oil extracted from *A. pyrethrum* might be used as a natural insecticide and therefore could be an alternative to synthetic insecticides already present on the market.

Keywords: *Anacyclus pyrethrum*; essential oil; hydrodistillation; GC-MS; insecticidal activity; *Culex pipiens*.

1. Introduction

*Anacyclus pyrethrum* is a perennial plant; this herb reaches 30 to 50 cm in height. This plant is an endemic species of Algeria and Morocco. It is also harvested for export, especially to the Middle East, India, Algeria and Morocco are the traditional suppliers 1. Its root is used in traditional medicine. *A. pyrethrum* is a plant which has interesting therapeutic effects. This plant is commonly featured in medical journals mainly due to the sesquiterpene lactones it contains. Its therapeutic use is mainly due to its anti-inflammatory and antimicrobial activity. It is also used in the treatment of liver diseases, in the treatment of rheumatism, sciatica, colds, neuralgia and paralysis 2. In Algeria (Tlemcen region) this plant can be used for the treatment of diabetes. The root of *A. pyrethrum* purifies the blood, used to control anemia thanks to its richness in vitamin B12 and Iron. In Morocco, the roots of *A. pyrethrum* are used in traditional medicine to treat various pathologies; in fact, this plant is widely known for its medicinal properties and especially its efficacy against skin infections, especially fungal infections. Also, infusion of the roots is recommended against toothache and in case of problems related to salivary secretion 3.

Chemical analysis of *A. pyrethrum* shows that they contain an alkaloid N-isobutylamide, called “pellitorine”, and mainly accumulate alkamides of which pellitorine is the major active constituent, they also contain anacycline, enetriyne alcohol, hydrocaroline, inulin and volatile oils which have antibacterial, larvicidal and insecticidal properties. Alkamides are a large and growing group of natural bioactive compounds found in *A. pyrethrum*. These natural products have a wide range of biological activities: antimicrobial, antiviral, diuretic, antioxidant and analgesic. In addition, alkamides are involved in the potentiation of antibiotics 3.

Hydrodistillation is the routine method recommended by pharmacopoeias to extract essential oils from plant

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materials. The main advantage of this method is due to its simplicity of implementation, its reduced cost and the absence of organic solvent. The extracted essential oils are analyzed by different techniques; the most used is high-performance liquid chromatography (HPLC) and thin layer chromatography (TLC). However, due to the high volatility of the compounds, the most appropriate technique is gas chromatography (GC); in addition, the most accurate information in qualitative analysis is obtained by gas chromatography coupled with mass spectrometry (GC-MS).

Mosquitoes are among the vectors of many vector-borne diseases. The species C. pipiens is the most common mosquito in the world that is responsible for the transmission of parasitic diseases and its impact on human health is very significant. C. pipiens was responsible for the transmission of several epidemics that affected Morocco in 2003 and 2010. The mosquito population control generally involves the use of synthetic chemical products; however, the use of these products caused adverse effects to humans and the environment.

For this reason, synthetic chemicals have been replaced by insecticides based on natural products extracted from plants. For this, we contributed to our part, to finding other natural substances that are alternative and harmless to the environment for mosquitoes control.

In this context, our study aims to extract A. pyrethrum’s essential oil, to identify its chemical composition in order to highlight the specific compounds of this oil; also, we sought to assess the larvicidal potential of this oil as a botanical insecticide for the control of mosquitoes’ larvae mainly the larvae of C. pipiens which is a public health problem worldwide.

2. Experimental section

2.1. Materials
The plant was cultivated in the region of Benslimane in Morocco: (Latitude: 33°42’6” N; Longitude: 7°11’8” W; Altitude: 233 m). The climate is humid with an annual average temperature of 23.7°C. The vegetal product was harvested during the flowering stage in May 2019. After collection, the plant was air-dried at room temperature (25°C) for 20 days.

2.2. Extraction procedure
Dried leaves, stems and roots, were hydro-distilled on a Clevenger-type apparatus for three hours. Preliminary tests revealed that three hours of the distillation of the plant studied is the optimum period which allows obtaining a good yield. The plant material (300g) was placed in a 5 L round-bottom flask with 3 L distilled water. After this, the essential oil was recovered by a syringe, then; this oil was dried over anhydrous sodium sulfate, filtered, and stored in a sealed vial at 4°C for subsequent analyses, in order to avoid any alteration of the essence before chromatographic analysis.

2.3. GC-MS analysis
The extracted essential oil was analyzed by gas chromatography coupled with mass spectrometry (GC-MS). The profile of volatile compounds was characterized by gas chromatography (GC) (Agilent 7890A Series) coupled to mass spectrometry (MS) equipped with a multimode injector and a 123-BD11 column with dimensions 15 m × 320 μm × 0.1 μm and the injected volume was 4μl.

The soluble extract was injected into the column in 1/4 split mode using helium as carrier gas at 3 mL.min⁻¹. The ion source and quadrupole temperatures were 230 and 150°C, respectively. The oven temperature program was initially programmed at 30°C for 5 min and was programmed to increase at a rate of 4°C / min until it reached 360°C. The identification of the components was done using NIST 2014 MS Library.

2.4. Larvae
The larvae of the mosquito C. pipiens subjected to the insecticide test were collected from a permanent larval shelter located at Mohammedia in Morocco. This study area is located north-east of Oued N’fifikh (latitude 33°43’12”N and longitude 72°20’24”W). This zone is characterized by its humidity. This humidity is a source of proliferation of mosquito larvae which are harmful to the population, and it is characterized by a very high density of the species C. pipiens.

The collection of larvae was carried out by using a rectangular plastic tray, which was introduced into the water by tilting at 45° to the water surface. After the harvest, the larvae were identified with a binocular microscope. The identification was carried out based on their morphological characters. The first instar larvae of C. pipiens harvested placed in rectangular trays and kept in rearing in these trays filled with water and were maintained continuously at a temperature between 25 and 27°C in (70% ± 2%) relative humidity. The larvae were fed on fish food and water was renewed every two days. After 6 to 8 days, we obtained 4th instar larvae which were used in larvalicidal tests.

2.5. Larvicidal assay
The insecticidal tests were carried out according to the standard protocol of the World Health Organization (1985). A solution containing acetone with different doses of A. pyrethrum essential oil (5, 10, 15, 20 and 40 μL / mL of acetone) was prepared. Preliminary experiments made it possible to select these doses. 1 ml of each prepared solution is placed in a beaker containing 99 ml of distilled water in contact with 20 C. pipiens larvae of 4th instar; the same number of larvae was placed in a control beaker containing 99 ml of distilled water and 1 ml of acetone.
The beakers were placed in a laboratory room under the temperature conditions (27°C ± 2°C) and humidity of (70% ± 2%). Five repetitions were performed for each dose as well as for the control. After one day of contact, we counted the dead larvae. Corrected mortality for treated larvae is expressed according to Abbott’s formula 19.

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M\% = \frac{M_o - M_c}{100 - M_c} \times 100
\]

M: Corrected mortality
M_o: Mortality observed in larvae
M_c: Mortality observed in control

2.6. Statistical analysis
In order to assess the efficacy of the toxicity of A. pyrethrum’s essential oil, we calculated LC50 and LC90, defined as the lethal concentrations causing respectively 50% and 90% mortality in the population of larvae of C. pipiens treated. These values were determined by probit analysis, according to Finney.

3. Results and discussions
3.1. Essential oil yield
The essential oil extracted from A. pyrethrum is characterized by its orange color. For calculations of essential oil yield, three replicates were performed for plant material. This yield was 0.09%, which is slightly higher than that found by Elazzouzi 20, which was around 0.07% for the A. pyrethrum essential oil from the Timahdite region. Moreover, this yield is higher than that found in Algeria 1, which was 0.019%.

However, only some studies report on the performance of A. pyrethrum’s essential oil. This oil yield is relatively low (<1%) for all the studies carried out on this plant. Many factors can influence the yield of an essential oil, such as environmental conditions, extraction technique, drying, the harvesting period of the plant; this period constitutes a parameter that influences both the chemical yield and quality of the essential oil, as well as the harvesting environment and the age of the plant material 21-24.

Similarly, after harvesting, the biosynthetic activity is reduced, which would lead to a drop in the production of essential oil in the plant material 7. This plant has a considerable advantage thanks to the essential larvicidal power of its essential oil which was proved against mosquito larvae; it can, therefore, be said that the essential oil of A. pyrethrum is a candidate for the development of new botanical insecticides applied against the mosquito. As a result, it has become required to highlight research for the optimization and improvement of the yield of the essential oil extracted.

The increase in essential oil yield can be attributed to several important factors, including the production of essential oil, stimulated or modified using environmental triggers, including nutritional changes. Several studies have reported the effects of agricultural practices on secondary metabolites in plants. The mineral amendments contribute to the increase in plant mass and therefore, to the increase in the yield of essential oil without affecting the composition of this oil. The use of nitrogen fertilizers has produced the best effects concerning the yield and production of essential oil in Origanum vulgare L 25.

Similarly, an increase in the fertilizer rate in the cultivation of chamomile (Chamaemelum nobile L) results in an increase in essential oil content but does not change its composition. Also, the essential oil yield can be increased by applying elicitors which have a role in activating genes to stimulate the production of secondary metabolites in plants 26.

3.2. Chemical composition
Analyses of the essential oil revealed the presence of 32 compounds. These compounds represent about 92.67% of the total chemical composition. The results obtained are shown in Table 1.

Table 1. Chemical composition of the essential oil of A. pyrethrum of Benslimane

| KI | Compound | Molecular mass | Molecular formula | Percentage (%) |
|----|----------|----------------|-------------------|----------------|
| 770 | Hexanal  | 100            | C₆H₁₂O            | 0.03           |
| 930 | α-Pinene | 136            | C₁₀H₁₆            | 0.63           |
| 944 | Camphene | 136            | C₁₀H₁₆            | 2.30           |
| 971 | β-Pinene | 136            | C₁₀H₁₆            | 0.56           |
| 978 | Myrcene  | 136            | C₁₀H₁₆            | 0.72           |
| 1011| p-Cymene | 134            | C₁₀H₁₄            | 0.50           |
| 1021| Limonene | 136            | C₁₀H₁₆            | 0.05           |
| 1080| Linalool | 154            | C₁₀H₁₈O           | 0.1            |
| 1083| Nonanol  | 144            | C₉H₂₀O            | 0.40           |
| 1175| Estragol | 148            | C₁₀H₁₂O           | 0.15           |
Oxygenated sesquiterpenes were found to be predominant in the chemical composition identified for *A. pyrethrum* essential oil studied (58.96%), followed by the hydrocarbon sesquiterpenes (24.19%). Also, the study carried out by Selle 1 in Algeria on the *A. pyrethrum* showed that the oxygenated sesquiterpenes also represent the most abundant group in the chemical composition of the essential oil with a percentage rises from 37.1% to 58.6% respectively before and after flowering. Similarly, in Morocco the study carried out by Elazzouzi 20 showed that the oxygenated sesquiterpenes were the most abundant group in the composition of the essential oil of *A. pyrethrum* harvested from the Timahdite region with a percentage rises from 89.17% in April to 90.58% in June.

|   |   |   |    |   |
|---|---|---|----|---|
| 1178 | α-Terpineol | 154 | C₁₀H₁₈O | 0.02 |
| 1263 | E-Anethole | 148 | C₁₀H₁₂O | 0.29 |
| 1267 | Thymol | 150 | C₁₀H₁₆O | 0.01 |
| 1268 | Bornyl Acetate | 196 | C₁₅H₂₉O₂ | 0.07 |
| 1277 | Carvacrol | 150 | C₁₀H₁₆O | 1.35 |
| 1342 | Nerylacetate | 196 | C₁₅H₂₉O₂ | 0.06 |
| 1428 | Geranylacetone | 194 | C₁₅H₂₉O₂ | 0.08 |
| 1448 | (E)-β-Fernesene | 204 | C₁₅H₃₀ | 0.62 |
| 1457 | β-Humulene | 204 | C₁₅H₃₀ | 0.91 |
| 1471 | γ-Murolene | 204 | C₁₅H₃₀ | 4.20 |
| 1480 | Germacrene D | 204 | C₁₅H₃₀ | 16.48 |
| 1500 | b-Bisabolene | 204 | C₁₅H₃₀ | 1.98 |
| 1509 | Cubebol | 222 | C₁₅H₂₆O | 4.01 |
| 1554 | cis-3-Hexenylbenzoate | 204 | C₁₅H₁₆O₂ | 0.04 |
| 1557 | Spathulenol | 220 | C₁₅H₂₆O | 20.47 |
| 1576 | Caryophyllene oxide | 220 | C₁₅H₂₆O | 13.20 |
| 1578 | 4(14)-Salviale-1-one | 220 | C₁₅H₂₆O | 8.27 |
| 1626 | Caryophyllene-4(14),8(15)-dien-5α-ol | 220 | C₁₅H₂₆O | 7.30 |
| 1641 | Vulgarone-beta | 218 | C₁₅H₂₆O | 1.22 |
| 1645 | α-Cadinol | 222 | C₁₅H₂₆O | 2.28 |
| 1668 | α-Bisabolol | 222 | C₁₅H₂₆O | 2.21 |
| 1835 | Phytone | 269 | C₁₅H₂₆O | 2.16 |
| Total |   |   |   | 92.67 |

KI: Kovats Index

According to the results of GC-MS analysis of *A. pyrethrum* essential oil harvested from the Bensliman area, the most abundant compounds were: Spathulenol (20.47%), Germacrene D (16.48%), Caryophyllene oxide (13.20%), 4(14) - Salvialone-1-one (8.27%) and Caryophyllene- 4(14),8(15) -dien-5α -ol (7.30%).

However, other compounds exist in relatively small percentages: γ-Murolene (4.20%), cubebol (4.01%), Camphene (2.30%), α-cadinol (2.28%), α-Bisabolol (2.21%), Phytone (2.16%), b-Bisabolone (1.98%) and Carvacrol (1.35%).

Similarly, in Morocco, spathulenol was the most abundant compound with a percentage of (16.90%), as showed by Zair 27 and (13.31%) as reported by Elazzouzi 20 for *A. pyrethrum* collected from the
Meknes region with a relatively small percentage than our essential oil, which is more abundant in spathulenol (20.47%).

The same species in Ifrane (Morocco); Remok et al. 28, contain two significant compounds which are spathulenol (16.9%) and Germacrane-4 (15%).

The study carried out by Selles 1 on the essential oil of A. pyrethrum in Algeria revealed the presence of 87 compounds of which Germacrane D (13.4%) is the most abundant. Our oil also contains this compound but with a relatively high percentage (16.48%).

According to the results obtained, slight variations are observed in the chemical composition of the A. pyrethrum essential oil collected from different regions. These differences can be explained by factors such as soil and weather conditions, also the extraction method and the drying of the material previous extraction 29-35. However, spathulenol and germacrane D are compounds known for their insecticidal activity; which makes the essential oil of A. pyrethrum an excellent natural insecticide. Despite the low yield of essential oil of A. pyrethrum, it was chosen for larvicidal tests; this is due to its chemical composition rich in compounds known for their insecticidal activity.

3.3. Insecticidal activity of Anacyclus pyrethrum essential oil on the larvae of Culex pipiens

Analysis of the results of the larvicidal assay (Figure 1) of the essential oil showed that total mortality (100%) of C. pipiens larvae was found at a dose of 40 μL/mL. For control, no mortality was recorded. This activity can be explained by the effect of the majority compounds and can also be due to the activity of individual minor constituents or to the synergistic effect between the several constituents. In a general way, the essential oil is a mixture of several compounds; thus, the active compounds contained in this oil which have for example insecticidal activity can have different mechanisms of action against insects. Indeed, synergistic effects between these active compounds have been observed in various studies 36. In a previous study on larvicidal activity against the larvae of Spodoptera littoralis, Pavela determined the efficacy of 30 aromatic compounds and their binary combinations which showed a significant synergistic effect on the mortality of S. littoralis. Generally, each compound contained in essential oils is unique in terms of structure and biological activity. Thus various individual substances can in their combinations with other substances provide a significant increase in the effectiveness of essential oil 37. Therefore; the synergistic relationships between the substances contained in the essential oil have an essential role in terms of insecticidal efficacy.

Indeed, this oil is characterized by a high content of spathulenol (20.47%), known for its insecticidal properties 1. A. pyrethrum essential oil contains other major compounds of which Germacrane D (16.48%) which has significant insecticidal properties. Also, caryophyllene and its derivatives are widely known for their insecticidal, repellent and attractive properties against insects 1. The presence of these main compounds, in particular, spathulenol (13.5%) and caryophyllene oxide (14.2%) was also noticed in the essential oil extracted from E. tereticornis in Benin and which showed significant insecticidal activity against A. gambiae which is a vector of malaria 38. As well, the essential oils extracted in Chile, obtained by hydrodistillation from the leaves of H. foliosus and B. ambrosoides were very rich in caryophyllene (3.97%) and germacrone D (8.81%) respectively. These oils proved also significant insecticidal properties against the housefly Musca domestica 39. Also, the essential oil extracted from G. blepharophylla proved very useful insecticidal activity against Aedes aegypti; therefore, GC-MS analyzes confirmed that essential oil of this plant was rich in caryophyllene oxide (70%) 40. The present study describes for the first time, insecticidal activity of this oil against C. pipiens mosquito species. The nature of the chemical composition of this oil as well as the interesting larvicidal results obtained against the larvae of C. pipiens confirm its use as a natural insecticide.

Thus, for the practical application of A. pyrethrum essential oil as a new botanical insecticide, studies on the stability of this essential oil are needed, especially the post-application temperature which can have a significant impact on the efficacy of botanical insecticides based on essential oils 41. In a previous study carried out by Pavela 42 on the larvicidal activity of Pimpinella animus’s essential oil against 3rd instar larvae of Culex quinquefasciatus, it was found that the temperature has a significant effect on the larvicidal efficacy of the essential oil and it was found that the highest mortality was achieved at high temperatures.

Another study was carried out by the same author concerning the effect of the post-application temperature on the insecticidal efficacy of the essential oil of Thymus vulgaris L, applied at different environmental temperatures (15; 20; 25 and 30 ± 1°C) on the larvae of the Egyptian cotton leafworm Spodoptera littoralis boisd (Lepidoptera: Noctuidae) and on the larvae of Culex quinquefasciatus say. When the essential oil was topically applied against the larvae of S. littoralis, the insecticidal efficacy increased with temperature. However, the opposite effect was observed when the oil was applied in the water against C. quinquefasciatus larvae. Water at the lower temperature achieved higher mortality of the C. quinquefasciatus larvae 43.
3.4. Lethal concentrations LC50 and LC90

Table 2 shows the values obtained for the lethal concentrations LC50 and LC90. The LC50 and LC90 values recorded for the essential oil of *A. pyrethrum* are around 14.79 μL/mL and 19.95 μL/mL, respectively.

**Table 2.** Toxicity parameters of *A. pyrethrum* essential oil against mosquito larvae of *C. pipiens*

| LC50 (μL/mL) [CI95] | LC90 (μL/mL) [CI95] |
|---------------------|---------------------|
| 14.79 [11.96-16.76] | 19.95 [15.05-29.10] |

CI95: 95% confidence interval

In the literature, we have not found any authors who have evaluated the larvicidal action of *A. pyrethrum* essential oil on *C. pipiens*. We have, therefore in this work, compared the toxicity of other oils on the species *C. pipiens*. For this, other essential oil of *Kelussia odoratissima* Mozaffarian against *C. pipiens* showed a significant effect with an LC50 value of 2.69 ppm (2.69 μL /L) and an LC90 of 7.90 ppm (7.90 μL /L) \(^ {42}\). According to El Ouali Lalami \(^ {11}\), the essential oil obtained from *Thymus vulgaris* cultivated in Morocco has been tested for the mortality of the larvae of mosquito’s *C. pipiens*. This oil was found useful at an LC50 of 102.027 ppm (102.027 μL /L) and an LC90 of 179.186 ppm (179.186 μL /L).

Also, the essential oils of *Citrus aurantium* and *Citrus sinensis* tested on *C. pipiens* mosquitoes were effective. The calculated LC50 and LC90 of *Citrus aurantium* and *Citrus sinensis* were around 139.48 ppm (139.48 μL /L) and 280 ppm (280 μL /L) and of 212.04 ppm (212.04 μL /L) and 516 ppm (516 μL /L), respectively \(^ {15}\).

Similarly, Tine-Djebbar evaluated the larvicidal activity of the essential oil of *Ocimum basilicum* on mosquitoes *C. pipiens*, and the values of lethal concentrations LC50 and LC90 were respectively 73.45 ppm (73.45 μL /L) and 101.20 ppm (101.20 μL /L) \(^ {43}\). Also, we compared the toxicity with other essential oils containing the same majority compounds as our oil. In a previous study on the biological activity of *salvia* essential oil against two species of mosquitoes, it was reported that caryophyllene oxide and spathulenol were the majority compounds. This oil proved significant larvicidal activity against larvae of *Anopheles quadrivittatus* and *Aedes aegypti* mosquitoes and showed significant toxicity with an LC50 of 6.2 ppm (6.2 μL/L) \(^ {44}\). Another study showed that *Pinus kesiya* leaf's essential oil was rich in germacrene D as a majority compound and proved a significant larvicidal activity against the mosquito larvae of *Anopheles stephensi*, *Aedes aegypti* and *Culex quinquefasciatus* with an LC50 of 52μg /mL (52ppm), 57μg/mL (57ppm) and 62μg/mL (62ppm) respectively \(^ {45}\).

The results that we found indicate that *A. pyrethrum*’s essential oil has an excellent larvicidal potential against the larvae of *C. pipiens* due to the percentage of mortality, the LC50 and LC90 recorded. For this, we can consider that this oil may be used for mosquito control as a botanical insecticide in the future.

Recent studies have also considered the sublethal effects of essential oils on several critical biological characteristics of insects. A sublethal concentration is defined as inducing no apparent mortality in the experimental population but causes biological, physiological, demographic or behavioral effects on individuals exposed to this concentration. In general,
the concentration below lethal concentration (LC50) is considered to be sublethal \(^{46}\). Sublethal effects can be manifested by reductions in life span, development rates, population growth, fertility, deformities and behavioral changes \(^{46}\). Several studies report the sublethal effects of essential oils and their compounds on the biology of insects. The essential oils of *Eucalyptus staigeriana*, *Ocimum gratissimum* and *Foeniculum vulgare* demonstrated several sublethal effects on the biology of *S. frugiperda* by reducing larval and pupal weights in the sublethal concentrations of LC10, LC20 and LC40 \(^{45}\). Also, the essential oils of *A. khorassanica* and *A. sieberi* demonstrated several sublethal effects on the biology of *S. cerealella* at LC30. Indeed, the parameters of the adult life cycle, such as the longevity of females and males and fertility have considerably reduced in the insect treated \(^{47}\). For future works, it is also essential to target a broader perspective on the effect of sublethal concentrations of essential oils on the life cycle of insects.

4. Conclusion

The essential oil of *A. pyrethrum* containing spathulenol, germacrene d, carophyllene oxide, 4(14)-Salvial-1-one and carophyllene-(4(14), 8(15)-dien-5-ol as major compounds showed an interesting insecticidal activity against the larvae of the *C. pipiens*. Due to the exciting results that we have obtained, investigations should be conducted on the insecticidal activity of this essential oil to optimize its use as a bioinsecticide. In prospects of this work, we look to evaluate the insecticidal activity of this essential oil on other mosquito species as well to test the insecticidal activity of the aqueous extracts of this plant.

Conflict of interest

The authors declare that they have no conflict of interest.

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