Research Article

Chemical Composition and Larvicidal Activity of Lavandula angustifolia Subsp. angustifolia and Lavandula dentata Spp. dentata Essential Oils against Culex pipiens Larvae, Vector of West Nile Virus

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The Culex pipiens mosquito (Diptera: Culicidae) is highly suspected to be the vector responsible for the spread of several parasitic and viral diseases. The use of synthetic insecticides is generally the preferred method of controlling these mosquitoes’ proliferation. However, it has led to resistance problems in target mosquitoes and environmental damage. Hence, diverse plant extracts could be considered as an alternative and potential source as mosquito control agents. In this study, essential oils of Lavandula angustifolia subsp. angustifolia and Lavandula dentata spp. dentata that are growing in Morocco were examined for their insecticidal effects on Culex pipiens larvae. The bioassay was performed according to a methodology inspired by the standard protocol of the World Health Organization. The mortality rate was determined after 24 hours of exposure, and probit regression analysis was used to calculate LC50 and LC90. The chemical analysis revealed that the principal compounds of L. angustifolia subsp. essential oils include linalool, linalyl acetate, geraniol, lavandulyl acetate, camphor, β-caryophyllene, terpinen-4-ol, β-myrcene, and 1,8-cineole, while the essential oil of L. dentata spp. was mainly composed of 1,8-cineole, camphor, α-pinene, trans-pinocarveol, linalool, and borneol. These volatile compounds have shown a toxic effect against Culex pipiens larvae, with lethal concentrations LC50 and LC90 being, respectively, 140 µg/ml and 450 µg/ml, for the L. angustifolia subsp. essential oil. Meanwhile, they were estimated at 2670 µg/ml and 7400 µg/ml, respectively, for the L. dentata spp. essential oil. These results suggest using essential oils of two species of Lavandula to control the Culex pipiens mosquito. It could be useful for the study of new natural larvicidal compounds.
1. Introduction

Culex are among the most important mosquito genera, well known for their public health interest [1]. Having a blood-sucking characteristic, *Culex pipiens* L. 1758 (Diptera: Culicidae) plays an important role in the transmission of arboviruses that infect humans, noting West Nile virus. *Culex pipiens* mosquito causes allergic responses that include local skin and systemic reactions such as angioedema and urticaria [2]. According to the WHO, the virus could cause fatal neurological disease in humans [3].

The prevention of these vector-borne diseases depends considerably on a program of vector control [4], with the WHO (2016) supporting the development of effective and sustainable vector control [5]. Thus, various approaches have been adopted [6], including the use of chemical, physical, and biological methods. Indeed, chemical methods are the most utilized to control *Culex pipiens* using synthetic chemicals with insecticidal properties [7]. Nevertheless, these chemicals have numerous negative effects; they cause damage to the environment, to other nontarget organisms, and to human health. Besides, the overuse of these substances induces mosquitoes’ risk of developing resistance against the insecticides [8–10], which considerably reduces treatment effectiveness [11]. Therefore, the acute damage caused by synthetic insecticides has created the need to develop alternative approaches to control nasty mosquitoes [12], with minimized toxic effects on the environment and human health [13, 14].

It is recognized that the plant extracts contain a wide range of components that may have toxicity against insects through different larval and adult instars [15]. Generally, many researchers have been interested in essential oils as an environmentally important natural resource and as a new vector control agent [16]. Various studies had reported the effectiveness of essential oils against *Culex* mosquitoes, particularly those obtained from plant species belonging to the Lamiaceae family, used against *Culex pipiens* larvae [17, 18].

Therefore, in this study, essential oils extracted from *L. angustifolia* subsp. and *L. dentata* spp., which are growing in Morocco, were evaluated for their larvicidal action against *Culex pipiens* larvae. To our knowledge, the larvicidal activity of the *Lavandula* species on *Culex pipiens* larvae has not been previously documented in Morocco.

2. Materials and Methods

2.1. Plant Material. In this study, both *Lavandula angustifolia* subsp. *angustifolia* (*L. angustifolia* subsp.) and *Lavandula dentata* subsp. *dentata* (*L. dentata* ssp.) species were used for testing their larvicidal activity; they were collected between April and June 2017, from the region of Taounate, in the mountainous area, which is in the rural community of Timezgana (northeastern Morocco), at an altitude of approximately 800 m. The specimens were deposited and stored in the Herbarium of the National Agency for Medicinal and Aromatic Plants in Taounate, Morocco.

2.2. Essential Oils’ Extraction and Chemical Characterization. The essential oils (E. Oils) were extracted by hydrodistillation for 3 hours using a Clevenger device. The E. Oils were then dried on anhydrous sodium sulfate to remove residual water and stored in an opaque container at 4°C before use. The chemical characterization of E. Oils was carried out by gas chromatography coupled with mass spectrometry (GC-MS), which allows the identification of compounds according to their mass-to-charge ratio and to precisely quantify the composition of E. oils. The analytical chemical analysis was performed using a Hewlett-Packard instrument equipped with the HP1 fused silica column (30 m × 0.25 mm, film thickness: 0.25 μm) and interfaced with a quadrupole detector (GC-quadrupole MS system, model 5970). The column temperature was programmed from 70 to 200°C at 10°C/min, and the injector temperature was 200°C. Helium was used as the carrier gas with a flow rate of 0.6 ml/min, and the mass detector operated at 70 eV.

2.3. Collection of Culex pipiens Larvae and Morphological Identification. Larvae of *Culex pipiens* mosquito were collected from a breeding site called Oued El-Mehraz (altitude: 423 m; 34°02′13″N, and 4°59′59.279″W). This site is rich in organic substances, favoring overgrowth of the Culicidae species, especially *Culex pipiens* larvae. Larvae were collected using a rectangular plastic plate; they were then kept inside the breeding site at the same conditions with water temperature 22.6°C ± 2°C and relative humidity 70% ± 5%. Only mosquito larvae (fourth and third instars) were selected for experimental testing after a two-day rearing period, according to the recommendations of the WHO protocol. Morphological character larval identification was performed using the Moroccan identification key [19] of Culicidae and Mediterranean African mosquito identification software [20].

2.4. Larvicidal Bioassays. The larvicidal tests were conducted following a methodology inspired by the standard WHO protocol. Preliminary experiments were used to select a range of E. oil concentrations. For this purpose, the concentrations tested were as follows: 50, 100, 200, 400, and 800 μg/ml of *L. angustifolia* subsp. *angustifolia* and 1000, 2000, 4000, 8000, and 16000 μg/ml of *L. dentata* ssp. *dentata* using ethanol as the solvent. The test was performed by placing 1 ml of each prepared suspension in a beaker containing previous 99 ml of distilled water and twenty larvae at the 4th or final 3rd instar. A control test was simultaneously carried out by adding 1 ml of ethanol to 99 ml of distilled water placed in a beaker with twenty larvae. Three replicates were carried out for each of the larvicidal bioassays and the control assay. The percentage of mortality rate for all concentrations was determined after 24 hours of treatment.

If the mortality rate in the control is higher than 5%, the mortality rate of the larvae exposed to the E. Oils must be corrected using Abbott’s formula (1) [21]. If the control assay mortality exceeds 20%, the test is invalid and should be repeated.
2.5. Statistical Processing of Data. The data were analyzed using probit analysis by software developed by CIRAD-CA/ MABIS [22]. The lethal concentration (LC50 and LC90) values were obtained according to Finney’s mathematical methods, with 95% confidence limits and by Chi2 test.

3. Results

3.1. Chemical Characterization. In our study, characterization by chemical analysis revealed that the E. Oil extracted from Lavandula species was mainly composed of compounds with terpene properties. From Table 1, it is clear that the main chemical components identified within L. angustifolia subsp. E. oils were linalool (32.23%), linalyl acetate (14.23%), geraniol (5.8%), lavandulol acetate (4.8%), camphor (4.21%), β-caryophyllene (4.2%), terpinen-4-ol (3.4%), β-myrcene (2.75%), myrtalen (2.62%), 1,8-cineole (2.25%), carophyllene oxide (2.12%), and borneol (2.01), whereas the L. dentata spp. E. Oil was mostly composed of 1,8-cineole (49.82%), camphor (6.31%), α-pinene (4.12%), trans-pinocarveol (2.84%), linalool (2.24%), and borneol (2.01).

3.2. Larval Mortality. After preliminary tests, Figures 1 and 2 present the effectiveness of E. Oils on Culex pipiens larvae after 24 h of exposure. As shown, both E. Oils showed significant larvicidal effect when tested against 4th instar and late 3rd instar larvae of Culex pipiens. The mortality rate, expressed as a percentage, varies according to the concentrations of each E. Oil tested, which means that it was dose dependent. For L. angustifolia subsp. E. Oils (Figure 1), the highest mortality percentage (100%) occurred at a concentration of 800 µg/ml. Simultaneously, it was evaluated at 16000 µg/ml for L. dentata spp. E. Oils (Figure 2), versus the control results.

3.3. LC50 and LC90. According to Table 2 and Figures 1 and 3, obtained LC50 and LC90 confirm the larvicidal activity of both E. Oils tested. It can be seen that the L. angustifolia subsp. E.Oils have the lowest LC50 which was 140 ± 0.1 µg/ml (70–200) and LC90 = 450 ± 0.05 µg/ml (350–610). A larvicidal effect was also attributed to L. dentata spp. E. Oils with LC50 2670 ± 0.07 µg/ml (1750–3480) and LC90 7400 ± 0.05 µg/ml (5990–9870) (Figures 2 and 4). The Chi-square test was not significant at 5% for both E. Oils, which means a good adjustment of the model.

4. Discussion

This study showed that the chemical composition of E. Oil extracted from L. angustifolia subsp. and L. dentata spp. was mainly composed of camphor, linalool, 1,8-cineole, linalyl acetate, and α-pinene, belonging to the monoterpenic fraction. L. angustifolia subsp. E. Oil’s main compounds were linalool (monoterpenol: 32.23%) and linalyl acetate (monoterpenic ester: 14.23%). The high level of these two compounds was widely documented during the characterization of the E. Oil of this plant. Indeed, Smigielski et al. reported significant levels of linalool (26.50–34.70%) and linalyl acetate (19.70–23.4%) as major compounds of the E. Oil obtained from flowers and aerial parts of L. angustifolia subsp. from Poland [23]. Similarly, another study conducted in India showed the same results with slightly elevated levels of linalool (36.10%) and linalyl acetate (19.90%) as the main compounds [24]. de Rapper et al. also noted similar results but with a high level of linalool acetate (36.70%) followed by linalool (31.40%) as the main compounds of the E. Oil of L. angustifolia subsp. from South Africa [25]. These studies also demonstrated the presence of other compounds in L. angustifolia subsp. E. Oil such as α-terpinene, 1,8-cineole, geranyl acetate, terpinen-4-ol, bornyl acetate, and β-caryophyllene with a variation in their levels from one country to another. About the E. Oil of L. dentata spp. characterized in our study, the major compounds were 1,8-cineole (49.82%) and camphor (6.31%). Nevertheless, other studies revealed some differences in the chemical profile of this plant. Dris et al. reported that the main chemical constituents identified in the E. Oil content of the leaves of this plant were α-terpinolene (51.13%), camphor (13.43%), and eucalyptol (3.62%) [26]. Moreover, Martins et al. revealed that the E. Oil of L. dentata spp. from Brazil obtained by steam distillation of aerial parts was mainly composed of monoterpenes, with higher concentrations of eucalyptol (46.30%), fenchone (15.80%), camphor (15%), limonene (3.20%), and linalool (0.30%) [27]. In climatic conditions similar to our country, a recent study conducted by Dammak et al. on the characterization of E. Oil extracted from the leaves of L. dentata spp. grown in northern Tunisia revealed the presence of camphor (35.0 ± 1.90%) and 1,8-cineole (32.02 ± 0.50%) as major compounds [28]. The differences in the relative amounts of some chemical substances may be related to several factors such as geographical and climatic factors, the physiological age of the plant, the genotype, the location and characteristics of the relief on the cultivated land, the harvest period, and the part of the plant used [27–29].

The results of the larvicidal activity showed that the E. Oil of L. angustifolia subsp. induced the highest percentage of toxicity (100%) at a concentration of 800 µg/ml, whereas the E. Oils of L. dentata spp. exerted high toxicity at 16000 µg/ml. The values obtained for LC50 and LC90 were, respectively, between 140 µg/ml (70–200) and 450 µg/ml (350–610) for the E. Oil of L. angustifolia subsp. and 2670 µg/ml (1750–3480) and 7400 µg/ml (5990–9870) for the E. Oil of L. dentata spp. The observed variability in the chemical composition of these E. Oils, thanks to mixtures of components differentiated by the identity and quantity of the main constituents, could be responsible for their insecticidal efficacy and the variability of
The toxicity could also be attributed to the minor constituents or probably the synergistic effects of many chemical components [30, 31].

Other researchers had revealed the larvicidal effect of *L. angustifolia* E. Oil and some of its chemical constituents. Indeed, Pavela [32] showed that E. Oils of *L. angustifolia* subsp. had larvicidal activity against *Culex quinquefasciatus*, with LC 50 and LC 90 at 121.60 µg/ml and 337.20 µg/ml, respectively. Tabari et al. confirmed that linalool had a significant toxic effect on larvae and eggs of *Cx. pipiens* with LC50 values of 14.87 µg/ml and 1.27 µg/ml, respectively [33]. Other studies confirmed that linalol acetate had excellent larvicidal activity against *Cx. pipiens* larvae with the LC50 value of 24.30 µg/ml, while others confirmed its effectiveness against *Aedes aegypti* [34, 35].

Furthermore, a study conducted by Pavela Roman demonstrated an individual larvicidal effect of 30 compounds on *Culex quinquefasciatus* larvae. His study revealed that α-pinene (LD50 95 µg/ml) exerted a particular larvicidal influence compared to other terpene compounds, mainly

### Table 1: Chemical composition of *L. angustifolia* subsp. and *L. dentata* spp. essential oils.

| Peak No | Compound | RI | *L. angustifolia* subsp. (%) | *L. dentata* spp. (%) |
|---------|----------|----|-----------------------------|-----------------------|
| 1       | α-Thujene | 931| 0.09                        | 0.11                  |
| 2       | α-Pinene | 933| 0.21                        | 4.12                  |
| 3       | Camphene | 953| 0.2                         | 1.12                  |
| 4       | β-Pinene | 980| 0.14                        | 0.32                  |
| 5       | β-Myrcene | 991| 2.75                        | —                     |
| 6       | α-Cymene | 1026| 0.25                       | 0.11                  |
| 7       | Limonene | 1031| 0.62                        | 0.41                  |
| 8       | 1,8-Cineole | 1033| 2.25                       | 49.82                 |
| 9       | Linalool oxide E | 1075| 0.9                        | 1.32                  |
| 10      | Fenchone | 1078| 0.21                        | 0.74                  |
| 11      | Linalool | 1098| 32.23                       | 2.24                  |
| 12      | α-Campholenal | 1125| 0.14                        | 0.25                  |
| 13      | *trans*-Pinocarveol | 1139| 1.25                       | 2.84                  |
| 14      | *cis*-Verbenol | 1142| 0.12                        | 0.32                  |
| 15      | Camphor | 1143| 4.21                        | 6.31                  |
| 16      | Lavandulol | 1148| 1.6                         | —                     |
| 17      | Terpinen-4-ol | 1155| 3.4                         | Tr                    |
| 18      | Borneol | 1165| 2.01                        | 2.01                  |
| 19      | *p*-Menth-1,3-dien-8-ol | 1172| —                           | 0.20                  |
| 20      | *cis*-Pinocarvone | 1183| 0.25                        | 1.10                  |
| 21      | *p*-Cymen-8-ol | 1184| 0.09                        | 1.21                  |
| 22      | Cryptone | 1188| 0.12                        | 0.71                  |
| 23      | α-Terpineol | 1189| 0.92                        | 0.43                  |
| 24      | Myrtenal | 1193| 2.62                        | 1.61                  |
| 25      | Myrtenol | 1194| 1.44                        | 1.62                  |
| 26      | Verbenone | 1204| 0.85                        | 0.25                  |
| 27      | *trans*-Carveol | 1217| 0.09                        | 0.27                  |
| 28      | Cuminaldehyde | 1239| —                           | 0.25                  |
| 29      | Geranol | 1241| 5.8                         | 0.22                  |
| 30      | Linalyl acetate | 1241| 14.23                       | 0.12                  |
| 31      | Carvone | 1242| 0.06                        | 0.44                  |
| 32      | Lavandulyl acetate | 1271| 4.8                         | 0.25                  |
| 33      | Geranyl acetate | 1359| 1.7                         | —                     |
| 34      | β-Caryophyllene | 1405| 4.2                         | 0.58                  |
| 35      | β-Šelinene | 1485| —                           | 1.56                  |
| 36      | Caryophyllene oxide | 1581| 2.12                        | 1.57                  |
| 37      | t-Cadinol | 1616| 1.6                         | —                     |
| 38      | β-Eudesmol | 1649| 0.54                        | 1.12                  |
| 39      | α-Bisabolol | 1683| 0.37                        | 0.27                  |
| 40      | β-Bisabolol oxide A | 1744| 0.33                        | 0.22                  |

*Number of a peak in the order of elution. Components identified based on mass spectra and retention indices. Tr: trace (<0.01%).*

![Figure 1: Mortality rate (%) of *Culex pipiens* larvae according to the concentrations of *L. angustifolia* subsp. E. Oil after 24h of exposure.](image-url)
Table 2: Lethal concentrations (LC₅₀ and LC₉₀) of *L. angustifolia* subsp. E. Oils and *L. dentata* spp. E. Oils.

| Plant species    | The probit model: $a + b \cdot \log(\text{dose})$ | LC₅₀ (µg/ml) (Ll-Ul) | LC₉₀ (µg/ml) (Ll-Ul) | Calculated Chi-square |
|------------------|--------------------------------------------------|-----------------------|-----------------------|-----------------------|
| *L. angustifolia* subsp. | $Y = 2.18341 + 2.60318 \times X$ | $140 \pm 0.1$ (70–200) | $450 \pm 0.05$ (350–610) | 4.31 |
|                    | $X = -8.38749e-01$                                | $X = -3.46378e-01$                |                          |                      |
| *L. dentata* spp.  | $Y = -1.22979 + 2.87581 \times X$               | $2670 \pm 0.07$ (1750–3480) | $7400 \pm 0.05$ (5990–9870) | 2.79 |
|                    | $X = 5.92320e-01$                                 | $X = 1.15369e+00$               |                          |                      |

*LI-UL: lower limit-upper limit; LC₅₀ = lethal concentration that kills 50% of the exposed larvae; LC₉₀ = lethal concentration that kills 90% of the exposed larvae.

Figure 2: Mortality rate (%) of *Culex pipiens* larvae according to the concentrations of *L. dentata* spp. E. Oil after 24 h of treatment.

Figure 3: A graphic representation showing the LC₅₀ and LC₉₀ values of *L. angustifolia* subsp. E. Oil after 24 h of exposure.

Figure 4: A graphic representation showing the LC₅₀ and LC₉₀ values of *L. dentata* spp. E. Oil after 24 h of exposure.
detected in our study, in particular, 1,8-cineol, camphor, and borneol (LD₅₀ > 250 µg/ml) [36]. A synergistic binary effect had also been reported against Culex quinquefasciatus larvae between linoleool and several terpene compounds identified in the E. Oil of L. angustifolia subsp. such as camphor, myrcene, borneol, and cineole [36]. E. Oil extracted from L. dentata spp. had also proven its larvicidal actions that could be explained by the high content of α-terpinelone, camphor, and other compounds [26, 37]. Dris et al. noted that the E. Oil of α-terpinelone, camphor, and other compounds [26, 37]. E. Oil had also been reported against the fourth-instar larvae of Culex ppiiens, and LC₅₀ and LC₉₀ values were estimated at 113.38 µg/ml and 150.38 µg/ml, respectively [26].

The variability in the larvicidal efficacy of the E. Oils extracted from Lavandula specimens in our study and previous studies could be explained by the diversity of the chemical composition of each E. Oil, which is significantly influenced by climate, geographical origin, harvest, and mineral nutrition [38]. The commonalities presented by these studies are primarily to solve the problem of larval and insect resistance, to use these oils as an alternative to synthetic insecticides, and to provide their use in developing countries to control many mosquitoes [15].

5. Conclusion

The results obtained in this research showed that the essential oils extracted from L. angustifolia and L. dentata spp. growing in Morocco proved to be rich in promising larvicidal agents to fight against Culex ppiiens larvae. As perspectives, further studies, taking into account the recommendations of the WHO about developing insecticides based on botanicals, are required on the synergistic effects and toxicity of essential oils’ chemical components to optimize their larvicidal potential and to valorize these natural products as an important insecticidal alternative for the control of Culex species.

Data Availability

The data used in this study are included within the article.

Conflicts of Interest

The authors declare that they have no conflicts of interest.

Authors’ Contributions

Fouad El-Akhal conducted larvicidal activity and statistical data analysis. Amal Ramzi interpreted the results obtained and drafted the manuscript. Yassine Ez Zoubi helped in English writing and rectification process. Mousa Benbouker and Khalid Taghzouti helped in the correction of English language, typographical errors, and grammar with text review and corrections. Abdelah Farah and Abdelhakim El Ouali Lalami contributed to the conception and design of the study and helped in the English writing of the manuscript. All authors read and approved the final manuscript.

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