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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 LC_{50} and LC_{90}. 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 LC_{50} and LC_{90} 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* spp. *dentata (L. dentata* spp.*)* 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, 74″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* spp. *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.

$$\%\ \text{mortality corrected} = \left[\frac{\%\ \text{mortality observed} - \%\ \text{mortality control}}{100 - \%\ \text{mortality control}}\right] \times 100. \quad (1)$$

2.5. Statistical Processing of Data. The data were analyzed using probit analysis by software developed by CIRAD-CA/MABIS [22]. The lethal concentration (LC_{50} and LC_{90}) 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%), lavandulyl acetate (4.8%), camphor (4.21%), β-caryophyllene (4.2%), terpinen-4-ol (3.4%), β-myrcene (2.75%), myrtenal (2.62%), 1,8-cineole (2.25%), caryophyllene 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. LC_{50} and LC_{90}. According to Table 2 and Figures 1 and 3, obtained LC_{50} and LC_{90} confirm the larvicidal activity of both E. Oils tested. It can be seen that the *L. angustifolia* subsp. E.Oils have the lowest LC_{50} which was 140 ± 0.1 μg/ml (70–200) and $LC_{90} = 450 \pm 0.05$ μg/ml (350–610). A larvicidal effect was also attributed to *L. dentata* spp. E. Oils with LC_{50} 2670 ± 0.07 μg/ml (1750–3480) and LC_{90} 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 monoterpene 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 linalyl 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-terpineol, 1,8-cineol, 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 ($35.0 \pm 1.90\%$) and 1,8-cineole ($32.02 \pm 0.50\%$) 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 LC_{50} and LC_{90} 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

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

Peak N^a	Compound	RI[b]	*L. angustifolia* subsp. (%)	*L. dentata* spp. (%)
1	α-Thujène	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	*p*-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*-Mentha-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	Geraniol	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-Selinene	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

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

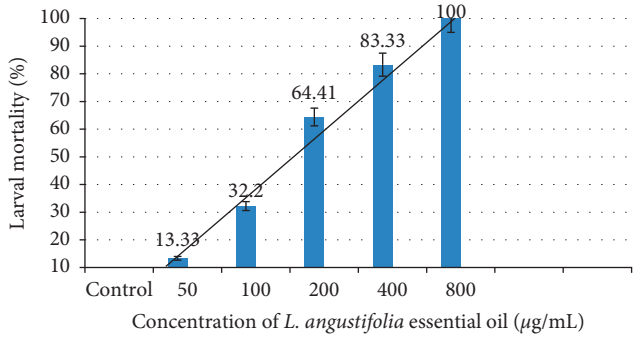

Figure 1: Mortality rate (%) of *Culex pipiens* larvae according to the concentrations of *L. angustifolia* subsp. E. Oil after 24 h of exposure.

toxicity. However, 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 LC_{50} 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 LC_{50} 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. This study revealed that α-pinene ($LD_{50} = 95$ μg/ml) exerted a particular larvicidal influence compared to other terpene compounds, mainly

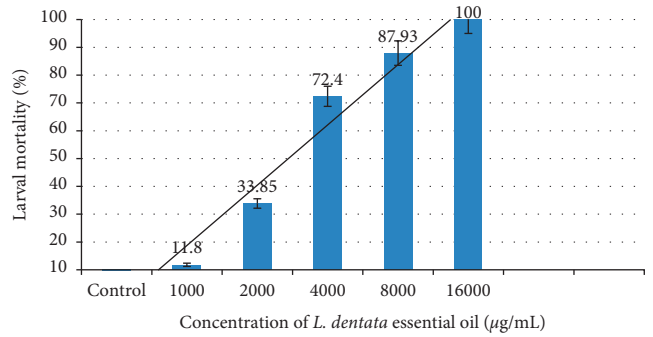

Figure 2: Mortality rate (%) of *Culex pipiens* larvae according to the concentrations of *L. dentata* spp. E. Oil after 24 h of treatment.

Table 2: Lethal concentrations (LC_{50} and LC_{90}) of *L. angustifolia* subsp. E. Oils and *L. dentata* spp. E. Oils.

Plant species	The probit model: $a + b$. log(dose)	LC_{50} (μg/ml) (Ll-Ul)*	LC_{90} (μg/ml) (Ll-Ul)*	Calculated Chi-square
L. angustifolia subsp.	$Y = 2.18341 + 2.60318^{*}X$	140 ± 0.1 (70–200) $X = -8.38749e-01$	450 ± 0.05 (350–610) $X = -3.46378e-01$	4.31
L. dentata spp.	$Y = -1.22979 + 2.87581^{*}X$	2670 ± 0.07 (1750–3480) $X = 5.92320e-01$	7400 ± 0.05 (5990–9870) $X = 1.15369e+00$	2.79

*Ll-Ul: lower limit-upper limit; LC_{50} = lethal concentration that kills 50% of the exposed larvae; LC_{90} = lethal concentration that kills 90% of the exposed larvae.

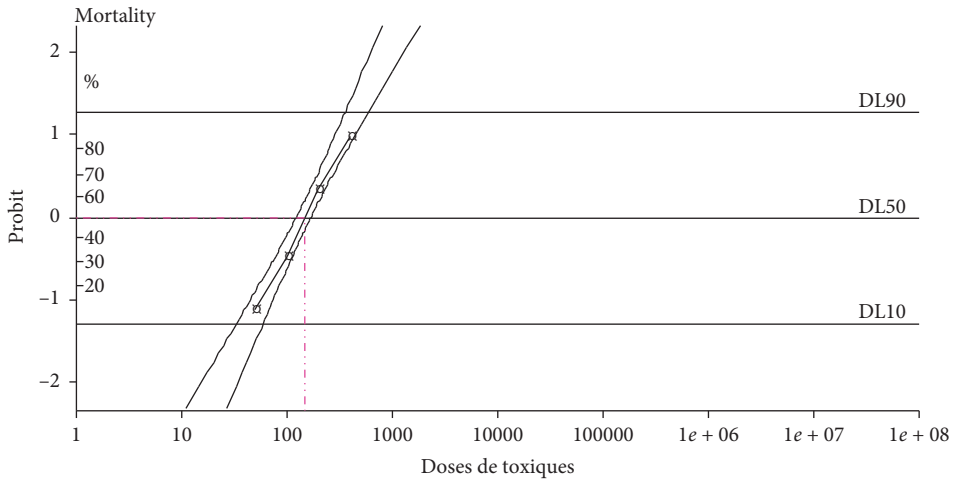

Figure 3: A graphic representation showing the LC_{50} and LC_{90} values of *L. angustifolia* subsp. E. Oil after 24 h of exposure.

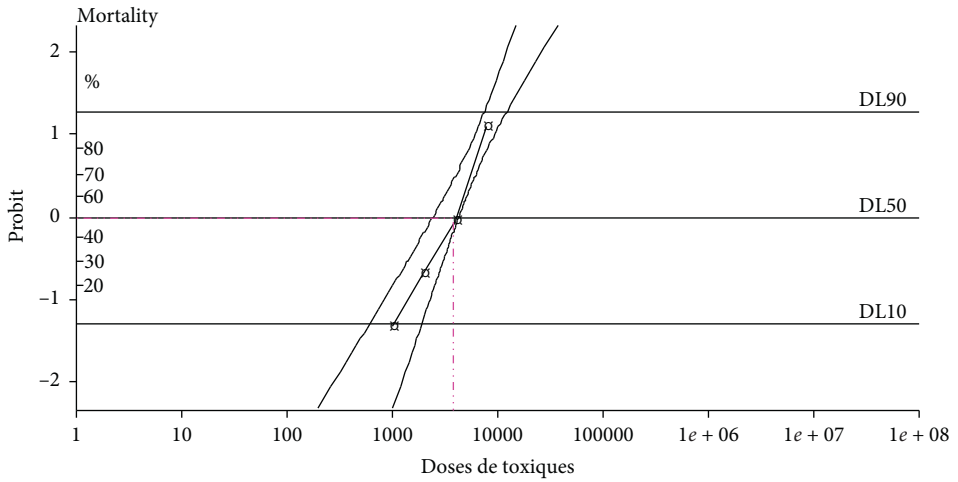

Figure 4: A graphic representation showing the LC_{50} and LC_{90} values of *L. dentata* spp. E. Oil after 24 h of exposure.

detected in our study, in particular, 1,8-cineol, camphor, and borneol ($LD_{50} > 250\,\mu g/ml$) [36]. A synergistic binary effect had also been reported against *Culex quinquefasciatus* larvae between linalool 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-terpinolene, camphor, and other compounds [26, 37]. Dris et al. noted that the E. Oil of *L. dentata* spp. from Algeria exerted a larvicidal effect against the fourth-instar larvae of *Culex pipiens*, and LC_{50} and LC_{90} values were estimated at $113.38\,\mu g/ml$ and $150.38\,\mu g/ml$, 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 pipiens* 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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