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Larvicidal Efficacy of Mentha Pulegium Essential Oil Against Culex Pipiens L. And Aedes Caspius P. Larvae

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ABSTRACT

Mosquitoes are generally controlled by conventional insecticides which poses strong secondary effects on the environment. In this context, the present study examines the chemical composition of the essential oil of Mentha pulegium (Lamiaceae) and its Larvicidal activity against the two mosquito species Culex pipiens and Aedes caspius.
Culex pipiens and Aedes caspius larvae were collected in the year 2015 from untreated areas located at Tébessa (Northeast Algeria). Aerial parts of Mentha pulegium were harvested during April 2015 from the Tebessa area. After the dryness, the plant material was subjected to hydrodistillation using a Clevenger-type apparatus. Chemical composition of the essential oil was determined using a gas chromatography-mass spectrometry analysis. Toxicity test was made on the fourth-instar larvae according to the World Health Organization recommendations. Gas chromatography-mass spectrometry analysis led to the identification of 14 components. Pulegone (72.50%), Eucalyptol (10.44%) and P-menthone 2-ethyl-5-propyle (5.46%), are the major constituents. Biological test revealed that the M. pulegium essential oil exhibits a larvicidal activity and A. caspius is more sensitive than C. pipiens larvae.
M. pulegium essential oils, constitute an alternative to the conventional insecticides for controlling mosquitoes.

Keywords: Mosquitoes, Mentha pulegium, CPG/SM, Chemical composition, Toxicity.

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1. INTRODUCTION

Mosquitoes can transmit serious human diseases such as malaria, dengue, filariasis, and yellow fever, which affect more than 700 million people annually throughout the world (Schaffner et al. 2001; Ghosh et al. 2012). To prevent the proliferation of this arthropod and to improve the environmental quality and public health, more attention has been focused on botanicals, which are effective, eco-friendly, biodegradable and inexpensive, and one of the possible alternatives to synthetic insecticides (Choochate et al. 2005). Previous studies have revealed larvicidal, pupicidal and repellent activities of plant extracts against mosquitos (Shaalan et al. 2005; Pavela et al. 2014; Rocha et al. 2015; El Akhal et al. 2015; El Akhal et al. 2016). More than 2000 plant species have been known to produce secondary metabolites involved in pest control programs (Ghosh et al. 2012). The members of the plant families, Myrtaceae, Asteraceae, Lamiaceae, Miliaceae, Rutaceae, Cupressaceae, Zingiberaceae and Apiaceae, have been examined for anti-insect activities (Onyambu et al. 2015). Essential oils provide a rich source of biologically active Monoterpenes and are well documented for the bioactivities against the insect pests (Govindarajan, 2010). Some of the essential oils with a promising mosquito control potential are plants from genus Tagets spp (Dharmagadda et al. 2005), Ocimum spp (Bhatnagar et al. 1993), Cymbopogon spp (Ansari and Razdan ,1995), Mentha spp (Ansari et al. 2000), Thymus vulgaris (Bouguerra et al. 2017; Dahchar et al. 2016). Mentha is an important genus of Lamiaceae, which recognized for their vital oils, medicinal uses and antimicrobial activity of different species (Skaltsa et al. 2003). It consists of about 25–30 species and most of them are grown in the temperate regions of Australia, South Africa and the Eurasia region. Mentha pulegium L. is a native of temperate Asia, Africa, and Europe (G.R.I.N, 2010). The previous studies have reported that the various Mentha spp. plant extracts exhibited the Larvicidal activity against several mosquito species such as C. pipiens, C. quinquefasciatus, A. aegypti, Anopheles stephensi and An. tesselatus (Amer and Mehlhorn 2006; Koliopoulos et al. 2010; Samarasekera et al. 2008; Sukumar et al. 1991; Traboulsi et al. 2002; Ambindei et al. 2017). C. pipiens and A. caspius are the most interesting mosquito species in Algeria, particularly in the Tebessa area (Tine-Djebbar et al. 2016). This present study aims 1/ to determine the chemical composition of the essential oils of cultivated mint species largely used in Algeria. M. pulegium and 2/ to test their insecticidal activities against C. pipiens and A. caspius.

2. MATERIALS AND METHODS

2.1. Mosquito rearing

Culex pipiens Linnaeus, 1758 and Aedes caspius Pallas, 1771 larvae were collected in the year 2015 from untreated areas located at Tebessa (Northeast Algeria). The larvae specimens were morphologically identified according to the identification keys of Brunhes et al. (2000) and Himmi et al. (1995). The pyrex storage jars (80 by 100mm) containing 150 ml of tap water were maintained at a 25 °C temperature and a photoperiod of 14:10 (L:D). The larvae were daily fed with fresh food consisting of a mixture of Biscuit Petit Regal-dried yeast (75:25 by weight) and water was replaced every four days.

2.2. Plant Material

The Aerial parts of Mentha pulegium Linnaeus, 1753 were harvested during April 2015 from the Tebessa area (Northeast Algeria). Then, the plant parts were washed with tap water, to eliminate soil and other surface contaminants. After the dryness, at laboratory temperature and obscurity, the plant material was cut into small pieces.

2.3. Extraction of the essential oils

100g of the air-dried aerial parts of Mentha pulegium were subjected to hydrodistillation for 3 hours with 500ml distilled water using a Clevenger-type apparatus. The oil obtained was collected and dried over anhydrous sodium sulfate and stored in a screw capped glass vials in a refrigerator at 4 °C prior to the analysis. The yield based on the dried weight of the samples was calculated (Bouguerra et al. 2017).

2.4. Gas chromatography-mass spectrometry

The essential oil of M. pulegium was subjected to a gas chromatography-mass spectrometry (GC-MS) analysis using the Perkin Elmer chromatography Autosystem GC. The column HP-5 (length: 30m with an internal diameter of 0.25mm and a film thickness of 0.25μm) was used with helium as a carrier gas. The GC oven temperature was kept at 60 °C for 1 minute and programmed to 200 °C for 13 minutes. The injector's temperature was set at 250 °C. The sample was dissolved in acetone. A volume of 1μl was injected for the GC-MS analysis. The essential oil was analyzed in the Laboratory of Pharmacognosy (Department of Pharmacy, Faculty of Medicine, Batna).

2.5. Toxicity Bioassay

Bioassays were conducted as previously described (Boudjelida et al. 2005). The essential oil of M. pulegium was added to the treatment beakers at different final concentrations. The newly ecdysed fourth-instar larvae of C. pipiens and A. caspius (< 8 hours old) were exposed to the different concentrations (50, 100, 250, 500, and 1000 ppm) for 24hours in accordance with the World Health Organization (WHO) criteria (Anonym 1983). The positive controls were exposed to ethanol (solvent) while the negative controls were exposed to water only. Mortality was registered after 24hours of treatment. The test was carried out with 4 replicates containing 25 larvae each per concentration. The mortality percentages obtained were corrected **(**Abbott 1925) and the toxicity data subjected to the probity analysis **(**Finney 1971). The Lethal concentrations (LC_{50} and LC_{90}) and 95% confidence limits (95% CL) were estimated, and the slope of the concentration-mortality lines was calculated (Swaroop et al. 1966).

2.6. Statistical Analysis

The number of individuals tested in each series is given with the results. The data are presented as the mean ± standard deviation (SD). The significance of the different series was tested using the Student's t test. All the statistical analyses were performed using the MINITAB Software (Version 17, PA, State College, USA) and $p \leq 0.05$ was considered to be a statistically significant difference.

3. RESULTS AND DISCUSSION

3.1. Extraction yield

From the results of steam distillation, we found that the yield of extraction of the essential oil of M. pulegium was 0.87±0.055 % of the dry matter of the aerial part of the plant. This yield is expressed in milliliters for 100g of dry matter. The obtained yield in our experiments is lower than the EOs extracted from the same species collected in Lavras, Brazil (2.54%) (Silva et al. 2015), in M'rirt (Morocco) (5.2%) (Amalich et al. 2016) and in Reguiba, El-Oued (South-East Algeria) (2.34%) (Ouakouak et al. 2015). Compared to the yield of pennyroyal harvested in Moroccan North (1.9%) (Cherrat et al. 2014) and the one obtained by Boughdad et al. (2011), the yield obtained in this study is also higher. It is relatively comparable to other plants industrially exploited as a source of essential oils like Artemisia herba alba (0.59%) and Artemisia absinthium (0.57%) (Derwich et al. 2009), Thymus vulgaris (1%) (Imelouane et al. 2009), Mentha pulegium and M. spicata (1% and 0.87%, respectively) (Boukhebti et al. 2011). In contrast, Benayad (2008) and Mahboubi and Haghi (2008) reported in the same plant a yield of 2.33% and 0.27 % respectively. However, the EO yield obtained from 7 species of the genus Mentha ranged from 0.69% to 2.33% (Pavela et al. 2014) and the total yield for L. stoechas is around 2.17% (Ben Slimane et al. 2015). Zantar et al. (2015) show a greatest yield for M. pulegium (3.5%) and T. vulgaris (3.6%). The high yield of EOs obtained during the full-flowering period, is probably due to its ecological role in attracting pollinators and in being an antifungal defense mechanism (Verma et al. 2010). These percentages were similar to those reported by Hmiri et al. (2011). In addition, the difference in the yield of the Mentha essential oils with respect to the geographical regions was reported (Abdullah, 2009).

3.2. Chemical composition of essential oil

The percentages and the retention times of the identified compounds of EOs of M. pulegium are listed in Table 1 and Figure 1. Fourteen compounds, representing 99.29% of the total essential oil, were identified. The major component was Pulegone (72.50%), other components present in the appreciable contents were: Eucalyptol (10.44%), Menthone (5.46%), Pentanone (3.78%), L- β Pinene (1.90%), Piperitenone (1.51%) and Isopulegone (1.12%).

The literature data suggest that M. pulegium is a chemical Polymorph species in both the qualitative and/or quantitative composition (Kokkini et al. 2004). Compared to the published data, the chemical profile obtained presented differences, but also some similarities. 38 components were identified in the essential oils of M. pulegium collected from Tunisia (Hafedh et al. 2009) and Iran (Sardashti and Adhami 2013) and 16 from Morocco (Benayad 2008). It has been found that the M. pulegium L. oils from Bulgaria contains major components, Pulegone (42.9–45.4%) (Stoyanova et al. 2005); from Uruguay; pulegone (73.4%), isomenthone (12.9%) (Lorenzo et al. 2002); from Egypt; Pulegone (43.5%), Piperitone (12.2%) (El-Ghorab, 2006); from Tunisia, Pulegone (41.8%), Isomenthone (11.3%) (Mkaddem et al. 2007). Boukhebti et al. (2011) studies showed three chemotypes of M. pulegium with the following major oil components: Pulegone, Piperitenone, and Isomenthone.

The chemical composition of the Mentha pulegium L. oil has been subjected to numbers of studies, which have shown a difference in its constituents depending on the region of cultivation and there have been some variations in the constituents from the different countries (Boukhebti et al. 2011). Studies reported by Mahboubi and Haghi (2008) show clearly that the M. pulegium essential oil contains the following compounds: Piperitenone 33.0%, α-terpineol 4.7%, Pulegone 2.3% from which Piperitone was the major constituent (38.00 %). In contrast, Pulegone (88 %) was the main component in the same plant analysed by Ouraini et al. (2005). In addition, the results from Vian et al. (2008) present that Pulegone (83.70 %) was the main component of the M. pulegium essential oil, whereas, Hilan et al. (2007) found that menthol is the main constituent (50%). The essential oils of M. spicata and M. pulegium mainly consisted of oxygenated monoterpenes and

carvone, menthol and menthone were the principal constituent (Boukhebti et al. 2011).
Several reports revealed that the essential oil contents depend not only on the temperature and relative humidity but also on the duration of sunshine, air movement, and rainfall (Kastner 1969; Vernet et al. 1977). In the leaf essential oil Mentha spicata, 57 compounds and carvone (59.40%) is the major component, with other components which was present in appreciable contents such as: limonene (6.12%), 1.8-cineol, germacrene-D (4.66%), β- caryophyllene (2.969%), β-bourbonene (2.796%), α-terpineol (1.986%) and terpinene-4-ol (1.120%) (Boukhebti et al. 2011). In other studies (Soković et al. 2009), carvone (49.5%) is also the major constituent, followed by menthone (21.9%) and limonene (5.8%) of the extractable essential oils from this plant species.
The GC/MS analysis of the Lavandula stoechas essential oil has led to the identification of 20 components. Camphor (36.14%), 1,8-Cineole (25.16%), camphene (11.44%) and fenchone (9.08%), were the major constituents of which (El Ouali Lalami et al. 2016) 29 components representing 96.43% of the essential oil extracted from Mentha spicata cultivated at Ghardaia were identified with cis-carvone oxide, 1,8-cineole, cis-dihydrocarvone, and limonene (5.80%) as the main components (Laggoune 2016). Various factors as the environmental conditions, the extraction technique, the drying, the period and gathering sites, the plant age (Aberchane et al. 2001; Bourkhiss et al. 2009; Okoh et al. 2007; Bourkhiss et al. 2011), the concentration of the extract and the concentration of its active components, can influence the performance and the chemical composition of the extract.

3.3. Larvicidal Activity

In the present study, the dose-response relationship was determined for the M. pulegium essential oil applied to a newly ecdysed fourth instar larvae of C. pipiens and A. caspius (Table 2). The mortality was scored at 24 hours after treatment. With probit, LC_{50} was calculated as 38.75 and 28.16ppm, and LC_{90} was 85.91 and 53.75ppm for C. pipiens and A. caspius, respectively (Table 3). A. caspius is the most sensitive species. After the treatment, the intoxicated larvae showed a change in their behaviour by sinking to the bottom of the jar and remain there motionless until they died. Our results indicate that the M. pulegium essential oils and their active components could be developed as control agents against the mosquito larvae.
The efficacy of the botanical extracts against the population of mosquitoes can vary depending on the plant species, age and parts of the plant, the physicochemical characteristics and chemical composition of EO, the environmental conditions, the used extraction technique, the drying process, the period and growing environment and the cultivation practices (Sukumar et al. 1991; El Ouali Lalami et al. 2016). The insecticidal properties of EO are very well documented (Traboulsi et al. 2002; Koul et al. 2008; Pavela, 2008a; Pavela, 2008b; Urzua et al. 2010; Rossi et al. 2012), and no development of resistance against the botanicals has yet been reported (Sharma et al. 1992). In some previous investigations, the effect of the essential oil and extracts of the Mentha species of pests was reported (Regnault-Roger and Hamraoui 1994; Pascual-villalobos and Robledo 1998; Mahmoudvand et al. 2011; Michaelakis et al. 2011) especially against the mosquito's species (Sukumar et al. 1991; Ansari et al. 2000; Traboulsi et al. 2002; Amer and Mehlhorn 2006).
The efficacy of the essential oils derived from the three Mentha species, M. pulegium, M. piperita and M. spicata against C. pipiens larvae, revealed that the M. pulegium and M. piperita oils were the most toxic (LC_{50}: 46.97 and 40.28ppm, respectively) and Pulegone was the most effective (27.23mg/1) among the major ingredients (Michaelakis et al. 2011). According to the same author, Pulegone is 5-fold more active than Piperitone, this differentiation probably stands on the account of the location of the C-C double bond that these two molecules contain. We assume that in Pulegone the C-C double bond position on the chain group (isopropylidene versus isopropyl group) enhances the toxicity. Thus, Cetin et al. (2006) concluded that there was a high to the low lethal effect of the extracts of Mentha pulegium against the mosquito larvae, with LC_{50} values of 81.0ppm and Koliopoulos et al. (2010) confirmed the toxicity of Mentha ssp on the Culicidae larvae (C. pipiens).
The petroleum ether extract of Moringa Oleifera exhibited higher activity compared to the other extracts with LC_{50} values of 9.22ppm and 15.82ppm against A. Gambiae and A. aegypti, respectively (Nkya et al. 2014). Sayah et al. (2014) shows that the essential oil of Citrus aurantium (bitter orange), Citrus sinensis (orange) and Pistacia lentiscus presented an interesting larvicidal activity against C. pipiens (LC_{50}= 35, 64 and 62ppm and LC_{90}= 70, 120 and 160 ppm, respectively).
Eight out of the 22 essential oils (aniseed, calamus, cinnamon, clove, lemon, orange, thyme, and tulsi oils) gave promising results on the larvicidal activity against C. quinquefasciatus and the clove oil was found to be the most toxic to the larvae (Ramar et al. 2013). Cinnamon (Cinnamomum cassia), Sabina (Sabina vulgaris), White camphor (Cinnamomum glanduliferum), and Wintergreen (Liex chinensis) oils exhibited a larvicidal activity against the dengue vector Aedes aegypti larvae (Shaalan 2009). Prajapathi et al. (2005) found that the essential oils of the various parts of the 10 medicinal plants showed the different mosquitocidal activity against A. stephensi, A. aegypti and C. quinquefasciatus. The phytoproducts possess different bioactive components that can be used as general toxicants against the various larval stages of mosquitoes (Shaalan et al. 2005).

4. CONCLUSION

In the present study, it can be concluded that the essential oil of M. pulegium with pulegone, and eucalyptol as the major constituents, was found to exhibit a potent larvicidal activity against the C. pipiens and A. caspius larvae. Moreover, the M. pulegium essential oil appeared to be more toxic against the A. caspius larvae in comparison to the C. pipiens larvae. The essential oils can greatly contribute to reducing both the environmental chemicalisation and the population density of the mosquitoes.

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Conflict of Interest

Pr Tine-Djebbar F has received research grants from Ministry of High Education and Scientific Research of Algeria (CNEPRU project, grant number **D01N01UN120120130005**).
Pr. Soltani N is a headmaster of Laboratory of Applied Animal Biology, University of Badji mokhtar, Annaba
Guenez R declares that she has no conflict of interest.

Author's Contribution: All authors are equally contributed to this study.
Guenez Radja, Tine-Djebbar Fouzia and Soltani Noureddine, designed and carried out the experimental study. Tine-Djebbar Fouzia analysed data. Tine-Djebbar Fouzia, Guenez Radja and

Soltani Noureddine wrote the manuscript. All authors approved the manuscript.

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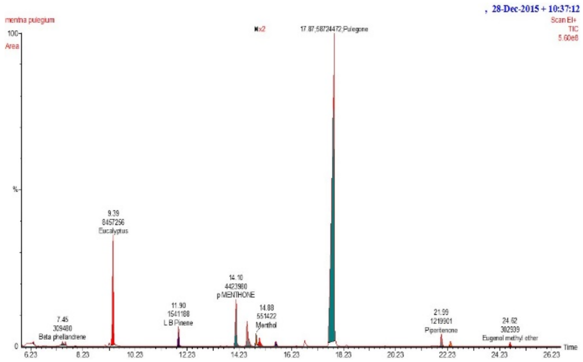

Fig. 1. Typical GC-MS Chromatograms of M. pulegium essential oils showing the separation of chemical components using HP-5MS column (Area as a function of the time in min)

Table 1. Chemical composition of M. pulegium oils: retention time (RT) and concentration (%) of different constituents.

Pic	Compound	RT	%
MONOTERPENES HYDROCARBONS			
1	α- Pinene	6.33	0.26
2	β- Phellandrene	7.44	0.38
3	3-Carene Bicyclo (3.1.0) Hexane.4-Methyl-1-(1-Methylethyl) Didehydro Deriv	7.55	0.32
5	L- β Pinene	11.90	1.90
13	D-Limonene	22.33	0.63
OXYGENATED HYDROCARBONS			
4	Eucalyptol	9.38	10.44
6	P-Menthone 2-Ethyl-5-Propyle	14.09	5.46
7	2- Ethyl-5-Propylcyclo Pentanone	14.53	3.78
8	Menthol	14.87	0.68
9	Isopulegone	14.99	1.12
10	α- Terpineol	15.64	0.63
11	Pulegone	17.87	72.50
12	Piperitenone	21.99	1.51
14	Eugenol Methyl Ether	24.62	0.37
Total (%)			**99.29**

Table 2. Efficacy of essential oil of Mentha pulegium applied on fourth instar larvae of Culex pipiens and Aedes caspius: corrected mortality (m ± SD. n = 4 replicates each containing 25 larvae).

Species	12.5 ppm	25 ppm	50 ppm	75 ppm	100 ppm	P value
C. pipiens	5.38 ± 2.26	24.27 ± 3.59	54.00 ± 5.29	66.22 ± 6.01	95.94 ± 4.00	p≤ 0.001
A. caspius	16.33 ± 6.63	28.77 ± 0.67	57.55 ± 1.34	91.83 ± 3.91	100 ± 0.00	p≤ 0.001

Table 3. Larvicidal activity of Mentha pulegium oils against fourth instar of Aedes caspius and Culex pipiens after 24 hours of treatment

Species	Regression equation	LC_{50}		LC_{90}		Slope
		ppm	95% FL	ppm	95% FL	
C. pipiens	y = 3.706 x - 0.886	38.75	(35.71 - 42.04)	85.91	(70.71 - 104.37)	1.85
A. caspius	y = 3.542 x - 0.534	28.16	(25.86 - 30.65)	53.75	(43.37 - 66.61)	1.65