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Chemical composition and activity of an *Ocimum basilicum* essential oil on *Culex pipiens* larvae: Toxicological, biometrical and biochemical aspects

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ABSTRACT

The present study was undertaken in order to determine the chemical composition of the leaf essential oil of *Ocimum basilicum* (Lamiaceae) cultivated in Tebessa (Algeria) and to assess their potential larvicidal activity against the most abundant and investigated mosquito species, *Culex pipiens* L., 1758 (Diptera, Culicidae). The essential oil extracted from *O. basilicum* was tested at different concentrations ranging between 50 and 150 ppm on newly molted fourth-instar larvae under standard laboratory conditions according to the World Health Organization recommendations. The effects were examined on the mortality, the activities of acetylcholinesterase (AChE) and glutathione-S-transferase (GST), the morphometric measurements and the biochemical composition of body of larvae, pupae and adults, respectively. *O. basilicum* essential oil which is extracted from dried leaves with an output of 1.56 ± 0.15%, is yellow pale. Its chemical composition has been investigated by GC/MS. Thirty eight compounds have been identified. The major compounds were: linalyl acetate (53.89%) and linalool (22.52%). The larval mortality was observed after 24 h of exposure. The LC_{50} and LC_{90} values were 73.45 and 101.20 ppm, respectively. The enzymatic measurements performed in LC_{50} treated larvae revealed a neurotoxic activity and a stimulation of the detoxification system as evidenced by an inhibition of AChE and an increase in GST activity, respectively. The morphometric study shows that the essential oil tested was found to decrease the growth of different developmental stages. Moreover, it reduces significantly the body contents of proteins, carbohydrates and lipids of the different stages studied (larvae, pupae, adult male and female). Overall, our results indicate that *O. basilicum* essential oil has potential for the development of new and safe control products against mosquitoes.

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1. Introduction

Mosquitoes are the major vector of several diseases like malaria, dengue fever, yellow fever, filariasis and chikungunya, causing millions of deaths every year (James, 1992). Mosquitoes also cause allergic responses in humans that include local skin and systemic reactions such as angioedema (Peng et al., 1999). Due to its large geographical distribution and high abundance, *Culex pipiens* called the domestic mosquito, represent the most important species in Algeria and many countries in the world (Boudjelida et al., 2008). The larval stage of mosquitoes is attractive targets for pesticides because mosquitoes breed in water, and thus, it is easy to deal with them in this habitat (Amer and Mehlhorn, 2006). However, the use of conventional pesticides have caused many risks to people and/or the environment. Consequently, there is an urgent need to develop safer, more environmentally friendly and efficient alternatives that have the potential to replace synthetic pesticides and are convenient to use (Tapondjou et al., 2005). Several insect growth disruptors (Tine-Djebbar and Soltani, 2008; Bouaziz et al., 2011; Djeghader et al., 2013; Hamaidia and Soltani, 2016), biological agents such as fish (Bendali et al., 2001), *Bacillus thuringiensis* (Boudjelida et al., 2008; Mansouri et al., 2013), *Metarhizium anisopliae* (Boudjelida and Soltani, 2010; Boudjelida and Soltani, 2011) and plant derivatives (Alouani et al., 2009; Rehimi et al., 2011; Alouani et al., 2013; Dahchar et al., 2016) were tested in our laboratory research. The application of easily degradable plant compounds is considered to be one of the safest methods of control of insect pests and vectors (Alkofahi et al., 1989). An important group of plant secondary metabolites are the essential oils (EO) which exhibit both a repellent and a larvicidal action. Plants EO (or their constituents) have been valued as insecticides owing to their broad spectrum of activity such as toxicity, oviposition and feeding deterrence, and repellency and attraction which appear to result from interaction with the insect nervous system, either by acetylcholinesterase inhibition or antagonism of the octopamine receptors (Rattan, 2010; Pavela et al., 2014). The insecticidal properties of

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EO are very well documented (Traboulsi et al., 2002; Kellouche and Soltani, 2004; Koul et al., 2008; Pavela, 2008; Kellouche et al., 2010; Urzúa et al., 2010; Toudert-Taleb et al., 2014; Hedjal Chebheb et al., 2015) and no development of resistance against the botanical products has yet been reported (Sharma et al., 1992). Basil (*O. basilicum*), belonging to the Lamiaceae family, is one of the most popular plants grown extensively in many continents around the world. The species of *O. basilicum* L., 1753 is the most cultivated in Algeria. It can be used as condiments and insect repellent (Iwu, 1993; Delille, 2007).

In this study conducted under laboratory conditions on *Cx. pipiens*, a medically important mosquito species, we assessed the efficacy of *O. basilicum* essential oil against fourth-instar larvae by determining the lethality parameters. In a second series of experiments, we investigated the biochemical response following *O. basilicum* essential oil exposure by measuring acetylcholinesterase (AChE) and glutathion S-transferase (GST) activities, biomarkers of neurotoxicity and detoxification, respectively. In addition, its effects on morphometric measurements and on main biochemical components (carbohydrates, proteins and lipids) in whole body of the different instars (fourth instar larvae, pupae and adults) were investigated.

2. Materials and methods

2.1. Plant materials and oil extraction

The leaves of *O. basilicum* (Lamiaceae) were collected in April 2015 in Tebessa (Northeast Algeria) and transported to laboratory. Dried leaves of the plants (about 100 g) were cut into small pieces and hydrodistilled in a clevenger type apparatus for 3 h according to the method recommended in the British Pharmacopoeia (1988). The volatile oils were dried over anhydrous sodium sulfate and stored in sealed vials at 4 °C until analysis. The yield of the oils was calculated based on dried weight of plant materials.

2.2. Gas chromatography-mass spectrometry

The essential oil of *O. basilicum* was subjected to GC-MS analysis using Trace GC ULTRA/Polaris Q (GC-MS, Thermo Electron). A VB-5 (5% phenyl/95% dimethylpolysiloxane) column (length 30 m, internal diameter 0.25 mm, film thickness 0.25 μm) was used with helium as carrier gas. The GC oven temperature was kept at 60 °C for 8 min and programmed to 250 °C for 10 min at rate of 2 °C/min. The injector temperature was set at 250 °C. The split flow was adjusted at 50 ml/min. MS were taken at 70 eV. The sample was dissolved in pure hexane. A volume of 0.2 μl was injected for GC-MS analysis. The composition was reported as relative percentage of the total peak area. The components were identified based on their retention times (RT) (determined with reference to a homologous series of normal alkanes), and by comparison of their mass spectral fragmentation patterns with those obtained from the authentic samples and/or the MS library.

2.3. Mosquito rearing

The larvae of *Cx. pipiens* were obtained from a stock colony of the laboratory of Applied Animal Biology and reared as previously described (Rehimi and Soltani, 1999). Larvae were kept in pyrex storage jar (25 larvae/jar) containing 150 ml of stored tap water and maintained at temperature between 25 and 27 °C. 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 two days.

2.4. Larvicidal test

Larval bioassays were conducted as previously described (Boudjelida et al., 2005). The EO of *O. basilicum* was added to treatment beakers at different final concentrations. Newly molted fourth-instar larvae of *Cx. pipiens* were exposed to different concentrations (50, 75, 100, 125 and 150 ppm) for 24 h according to the World Health Organization (WHO) procedure (Anonym, 1983; WHO, 2005). Essential oil was dissolved in 1 ml ethanol solvent, and then diluted in 150 ml of filtered tap water to obtain the desired concentrations. The controls were prepared using 1 ml of ethanol in 150 ml of water for positive controls and no additive with negative ones. After the exposure time (24 h), larvae were removed, washed with untreated water and placed in clean water. The test was carried out with 4 replicates each containing 25 larvae per concentration. The mortality percentage obtained was corrected (Abbott, 1925) and toxicity data were studied by probit analysis (Finney, 1971). Lethal concentrations (LC_{50} and LC_{90}) and 95% confidence limits (95% CL) were estimated, and slope of the concentration-mortality lines were calculated (Swaroop et al., 1966).

2.5. Enzyme assays

The lethal concentration (LC_{50} = 73.45 ppm) was applied on fourth instar larvae and its effects examined on AChE and GST activities measured at various times (24, 48 and 72 h) following treatment. The AChE activity was carried out following the method of Ellman et al. (1961) using acetylthiocholine as previously described (Habes et al., 2006). Pooled heads (each containing four heads per series) were homogenized in the following solution containing 38.03 mg ethylene glycol tetra-acetic acid (EGTA), 1 ml Triton X-100, 5.845 g NaCl, and 80 ml Tris buffer (10 mM, pH 7). After centrifugation (5000 rpm for 5 min), the AChE activity was measured in aliquots (100 μl) of resulting

Table 1
Chemical composition of *O. basilicum* oils: retention time (RT) and concentration (%) of different constituents.

N	RT	Compound	Area
1	10.0636	α-pinene	0.08
2	12.4926	Sabinene	0.14
3	12.6565	2-ß-pinene	0.21
4	13.7168	Beta-Myrcene	0.77
5	16.0012	Benzene, 1-methyl-4-(1-methylethyl)	0.04
6	16.3723	Eucalyptol	3.29
7	16.9988	cis-Ocimene	0.66
8	17.7073	ß- Ocimene	0.42
9	18.3579	γ-Terpinene	0.15
10	19.2061	Trans Sabinene hydrate	0.04
11	20.4255	α-terpinolene	0.07
12	22.2858	**Linalool**	**22.52**
13	22.4785	Octen-1-ol, acetate	0.28
14	23.2449	3-Octanyl Acetate	0.27
15	26.5944	Cyclopentylacetone	0.08
16	27.1004	Terpinene-4-ol	0.05
17	28.4499	α- Terpineol	4.57
18	31.2693	Neral	0.62
19	31.9392	2-Cyclohexen-1-one, 2-methyl-5-(1-methylethenyl)-, (S)-	0.18
20	33.2886	**Linalyl acetate**	**53.89**
21	35.0333	Neryl acetate	0.48
22	38.8840	Terpinyl acetate	2.95
23	39.8865	Neryl acetate	1.22
24	41.1974	Geranyl acetate	2.53
25	41.4817	Beta-elemene	0.09
26	41.7131	Benzene, 1-ethyl-3,5-dimethyl-	0.28
27	42.4601	5-Ethyl-m-xylene	0.08
28	43.1348	Trans-caryophyllene	0.63
29	45.2505	α-Humulene	0.04
30	45.5927	Trans-β-Farnesene	0.18
31	47.0000	Germacrene-D	0.47
32	51.4821	Cyclohexanemethanol	1.89
33	53.7424	Veridiflorol	0.18
34	56.0221	α-Selinene	0.10
35	57.1112	β-Eudesmol	0.10
36	57.2606	α-Eudesmol	0.13
37	58.1474	Cyclohexanemethanol, 4-ethenyl-.alpha.,.alpha.,4-trimethyl-3-(1-methylethenyl)-, (1R-[1.alpha., 3.alpha., 4.beta.])-	0.04
38	59.3089	3-Methyl-2-butenal	0.06

Bold values signifies important component.

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