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Adulticidal, larvicidal and biochemical properties of essential oils against *Culex pipiens* L

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ABSTRACT

In this study, the insecticidal properties of sixteen essential oils isolated from Egyptian plants were evaluated against the fourth instar larvae and adults of *Culex pipiens* L. The inhibitory effects of most active essential oils on the activity of acetylcholinesterase (AChE) and adenosine triphosphatases (ATPases) were also tested. In larvicidal assay against *Cx. pipiens*, the oils of *Artemisia monosperma*, *S. terebinthifolius* and *Origanum vulgare* showed the highest insecticidal activity with LC_{50} values of 7.73, 8.14 and 8.40 mg/l after 24 h of treatment and 2.63, 3.46 and 6.74 mg/l after 48 h of treatment. On the contrary, the oils of *Schinus molle* and *Rosmarinus officinalis* were the less effective. The results of fumigant toxicity assay against the adults of *Cx. pipiens* revealed that the tested oils had remarkable toxicity based on their LC_{50} values which ranged between 0.06 and 12.84 mg/l. The oil of *Artemisia judaica* was the most active with an estimated LC_{50} value of 0.06 mg/l air after 24 h of treatment, while the oil of *Citrus paradisi* was less active. The essential oils of *A. monosperma*, *O. vulgare*, *S. terebinthifolius* and *C. paradisi* elicited marked enzymatic inhibition towards AChE and ATPases isolated from *Cx. pipiens* larvae. These results indicated that the essential oils have potential for the development of natural larvicides and fumigants for *Cx. pipiens* control.

Introduction

Mosquitoes represent a crucial threat for millions of people around the world. They are important vectors of human diseases, such as malaria, filariasis, yellow fever, dengue fever and encephalitis (WHO, 1996; Cheng et al., 2009; Benelli, 2015a; Benelli and Mehlhorn, 2016; Benelli et al., 2016). *Culex* is an important mosquito genus containing well-known vectors of important parasites and pathogens causing disease, such as filariasis, West Nile virus and other encephalitides. *Culex pipiens* are vectors of West Nile virus and an important pest to humans, causing allergic responses that include local skin reaction and systemic reactions such as angioedema, and urticaria (Cheng et al., 2008). In Egypt, eleven *Culex* species are widespread throughout the country with *Cx. pipiens* L., the house mosquito, being the most common (Abd El-Samie and Abd El-Baset, 2012).

Mosquito control worldwide has been relied mainly on the continuous use of conventional insecticides such as organophosphates and pyrethroids. Organophosphate insecticides are commonly used in the mosquito breeding sites as larvicides, while pyrethroids are currently the most widely insecticides for the indoor control of mosquito adults (WHO, 2006; Liu et al., 2012). However, the development of mosquito resistance to these conventional insecticides is a major obstacle encountered effective control programs. Moreover, the increasing public concerns about hazardous effects of conventional insecticides on public health and the environment has increased the interest in developing new effective and environmentally sound alternative products for mosquito control. Plant natural products are effective, environment-friendly, biodegradable and target-specific insect control agents (Hoel et al., 2010; Cantrell et al., 2011; Benelli, 2015b).

Essential oils (EOs) are volatile liquids obtained from various aromatic plant parts. EOs are made from a complex mixture of compounds, mainly monoterpenes, sesquiterpenes, and their oxygenated derivatives (Cowan, 1999). Plant essential oils in general have been recognized as an important natural resource of insecticides (Isman et al., 2007). The insecticidal activity of essential oils against larvae and adults of several mosquito species have been documented (Amer and Mehlhorn, 2006; Ebadollahi, 2013; Manimaran and Cruz, 2014). In addition, the essential oils have also shown to possess oviposition deterrence (Tawatsin et al., 2006; Khandagle et al., 2011), ovicidal activity (Prajapati et al., 2005), repellent activity (Sophia and Pandian, 2009) and growth regulatory activity (Khater and Shalaby, 2007) on

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mosquitoes. In the literature, few studies were described the larvicidal activity (Evergetis et al., 2009; Koliopoulos et al., 2010; Cetin et al., 2011; Kimbaris et al., 2012; Sayah et al., 2014; Zoubiri et al., 2014; Benelli et al., 2015) and fumigant toxicity (Yang et al., 2005; El-Aswad and Abdelgaleil, 2008; Fu et al., 2013) of essential oils against *Cx. pipiens*.

To our knowledge, there is no information concerning the insecticidal and biochemical effects of essential oils isolated from plants growing in North Coast of Alexandria, Behera and Matrouh Governorates, Egypt, on *Cx. pipiens*. Therefore, this study was undertaken to evaluate the larvaicidal and adulticidal activities of sixteen essential oils isolated from Egyptian plants against *Cx. pipiens*. To understand the possible modes of action of essential oils, the inhibitory effects of essential oils on the activity of two key enzymes acetylcholinesterase (AChE) and adenosine triphosphatases (ATPases) were also examined for the first time.

Materials and methods

Test insects

Culex pipiens L. (Diptera: Culicidae) used in the bioassays was maintained in an insectary at the Department of Applied Entomology (Alexandria University, Egypt) for more than decade. Adults were kept in cages (50 × 50 × 50 cm) at 27 ± 1 °C, 70 ± 5% RH, and a photoperiod regime of 14:10 h (light/dark). The 10% sucrose solution, soaked in cotton swab, was provided as food for adult mosquitoes. A pigeon was introduced twice per week for adult blood feeding. Larvae were reared in dechlorinated water under the same temperature and light conditions and were fed daily with baby fish food. Newly emerged fourth instar larvae and 2–3-day-old adults were used for bioassays.

Plant materials

Sixteen plant species were collected from their natural habitats in Alexandria, Behera and Matrouh Governorates, Egypt. The selected plant species were *Artemisia judaica* L., *A. monosperma* Del., *Callistemon viminalis* (Sol.ex Gaertn.) G. Don, *Citrus aurantifolia* (Christm.) Swingle, *C. lemon* (L.) Burm.f., *C. paradisi* Macfad, *C. sinensis* (L.) Osbeck, *Cupressus macrocarpa* Hartw. ex Gordon, *Cup. sempervirens* L., *Origanum vulgare* L., *Pelargonium graveolens* L'Her, *Rosmarinus officinalis* L., *Syzygium cumini* L. Skeels, *Schinus molle* L., *S. terebinthifolius* Raddi and *Thuja occidentalis* L. The collection and taxonomic identification of plant samples were done as previously reported (Abdelgaleil et al., 2016).

Isolation and gas chromatography–mass spectrometry analysis of essential oils

Essential oils were obtained by subjecting plant materials to hydrodistillation using a Clevenger apparatus for 3 h. Quantitative and qualitative analyses of essential oils were performed on a gas chromatography (Hewlwett Packard 5890)/mass spectrometry (Hewlwett Packard 5989B) (GC–MS) apparatus. The isolation and identification of essential oils (Table S1) were carried out as described by Abdelgaleil et al. (2016).

Larvicidal bioassay

The larval mortality bioassay was carried out using the recommended method of the World Health Organization (WHO, 1996). Stock solutions of the tested essential oils were prepared in dimethyl sulfoxide (DMSO). Groups of 20 *Cx. pipiens* larvae were separately put into 200-ml plastic cups containing 100 ml of distilled water. The tested essential oil solutions in 0.1 ml DMSO were added to each cup and suspended with Tween-20 (0.1 ml), with gentle shaking to ensure a homogeneous test solution. A preliminary experiment was conducted at concentrations of 10, 100 and 200 mg/l. A series of at least eight concentrations (ranged between 1 and 200 mg/l) for each essential oil was tested. The control was prepared with distilled water containing the same amount of DMSO and Tween-20. Malathion 95% (Kafr Elzayat Pesticides and Chemicals Co., Egypt) was used as a reference insecticide. There were three replicates for each concentration. Treated and control larvae were held in the same conditions used for colony rearing. Larval mortalities were recorded 24 and 48 h post-treatment. Larvae were considered dead when they did not respond to stimulus or did not rise to the surface of the solution. Mortality data were subjected to probit analysis to estimate the lethal concentration values (LC_{50}) of essential oils (Finney, 1971).

Fumigant toxicity assay

The fumigant toxicity of the essential oils was performed with 2–3 days old *Cx. pipiens* adults. Glass jars of 0.4 l capacity with screw caps were used as exposure chambers. The essential oils were applied on Whatman no. 1 filter paper pieces (3 × 3 cm) attached to the lower surface of the jar screw caps at concentrations ranging between 1 and 50 mg/l (air). Then, the caps were screwed tightly onto the jars containing 20 mosquito adults. Similar treatment without essential oils was served as control. Three replicates of each treatment and control were set up. The number of dead insects and mortality percentages were determined after 24 and 48 h of treatment. The lethal concentration causing 50% mortality (LC_{50}) expressed as mg/l air were calculated from log-concentration mortality regression lines (Finney, 1971).

Acetylcholinesterase (AChE) inhibition assay

The fourth instar larvae of *Cx. pipiens* (6 g) were homogenized in 20 ml of 50 mM ice-cold phosphate buffer (pH 7.4) using a Polytron Kinemetica. The homogenates were filtered through two layers of cheesecloth. The filtrates were centrifuged under cooling (5000 rpm for 30 min at 4 °C), and the supernatants were used as the enzyme source. The inhibition of AChE was determined by the colorimetric method of Ellman et al. (1961) using ATChI as substrate. Enzyme aliquots (50 μl) and dithiodinitrobenzoic (DTNB) (100 μl of 0.01 M) were added to 0.1 M phosphate buffer (pH 8.0; 2.8 ml). To this mixture, the essential oil solutions (20 μl) prepared in acetone and Triton-X 100 (at concentration of 0.01%) were added. The essential oils were tested at a series of concentrations (2.5, 5, 10, 20, 30, 50, 60, 70, 80, 90 and 100 mg/l). The control treatments were prepared by adding 20 μl of acetone without essential oil. The mixtures were incubated at 37 °C for 15 min. The reactions were started by adding ATChI (30 μl of 15 mM) followed by incubation at 37 °C for 10 min. The change in absorption at 412 nm was monitored on Sequoia-Turner Model 340 spectrophotometer. All the experiments were done in triplicate. Protein was measured by the method of Lowry et al. (1951). Specific activity of AChE (ΔOD/mg protein/min) was calculated for each concentration and control. Inhibition percentage of AChE activity was calculated as follows: $\text{AChE inhibition } \% = [1 - SA_T / SA_C] \times 100$, where SA_T is specific activity of the enzyme in the treatment and SA_C is specific activity of the enzyme in the control. The concentrations of the tested essential oil that inhibited the hydrolysis of substrate by 50% (IC_{50}) were determined by a linear regression analysis.

Adenosine triphosphatases (ATPases) inhibition assay

The fourth instar larvae of *Cx. pipiens* were homogenized in an appropriate volume (10% *w*/*v*) of TSE buffer (40 mM Tris-HCl, 320 mM sucrose, 1 mM EDTA, pH 7.4). The homogenates were centrifuged at 5000 rpm for 30 min at 4 °C using IEC-CRU 5000 cooling centrifuge. The supernatants were re-centrifuged at 15000 rpm using Cryofuge 20–3, Heraeus Christ centrifuge for 15 min at 4 °C. The particulate pellets were re-suspended in the homogenizing TSE buffer solution to