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Toxicity of β-citronellol, geraniol and linalool from *Pelargonium roseum* essential oil against the West Nile and filariasis vector *Culex pipiens* (Diptera: Culicidae)

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ABSTRACT

Insect vectors are responsible for spreading devastating parasites and pathogens. A large number of botanicals have been suggested for eco-friendly control programs against mosquito vectors, and some of them are aromatic plants. *Pelargonium roseum*, a species belonging to the Geraniaceae family, due to its pleasant rose-like odor may represent a suitable candidate as mosquito repellent and/or larvicide. In this research, we evaluated the toxicity of the essential oil from *P. roseum* and its major constituents against the West Nile and filariasis vector *Culex pipiens*. The chemical composition of *P. roseum* essential oil was analyzed by gas chromatography-mass spectroscopy. Major constituents were citronellol (35.9%), geraniol (18.5%), and linalool (5.72%). The bioactivity of *P. roseum* essential oil and its three major compounds on larvae and egg rafts of *Cx. pipiens* was evaluated. The essential oil had a significant toxic effect on larvae and egg rafts of *Cx. pipiens*, with 50% lethal concentration (LC_{50}) values of 5.49 and 0.45 μg/mL, respectively. Major constituents, geraniol, citronellol and linalool resulted in LC_{50} values of 6.86, 7.64 and 14.87 μg/mL on larvae, and 0.8, 0.67 and 1.27 μg/mL on egg rafts. Essential oil and two of its constituents, citronellol and geraniol showed moderate knock-down on *Cx. pipiens* adults. Overall, the present investigation revealed that the major components of *P. roseum* and specially the whole essential oil could be helpful in developing novel and safe mosquito control tools and also offer an environmentally safe and cheap tool for reducing *Cx. pipiens* mosquito populations.

1. Introduction

Insect vectors, especially mosquitoes, are responsible for spreading devastating parasites and pathogens causing serious diseases, including malaria, yellow fever, dengue, filariasis and, more recently Zika virus (Mehlhorn, 2008; Mehlhorn et al., 2012; Benelli, 2016a, b; Benelli et al., 2016). The numerous preparations and devices designed to reduce or prevent such vectors, including pesticides and microbial formulations (Benelli, 2015a; Benelli and Mehlhorn, 2016), have not been completely successful due to increased resistance developed by a number of mosquito species (Hemingway and Ranson, 2000) and also side effects on the environment and human health (Benelli et al., 2017a).

In recent years, research attention has been devoted to the arsenal of nature (Govindarajan et al., 2008, 2016a, b, c; Benelli, 2015b; Pavela, 2015a, b). A large number of botanicals, including plant extracts, essential oils, and plant derived bioactive compounds, has been suggested for eco-friendly and safe control programs against mosquito vectors (Pavela and Benelli, 2016a, b). Young instar populations of mosquitoes are the targets of the majority of control programs, since focus on killing adults may only temporarily reduce the population and has higher operational costs (Pavela, 2015a). Notably, targeting mosquito egg and larvae is more efficacious and would help preventing vector outbreaks (Benelli, 2015b).

The *Culex pipiens* complex has a global geographic distribution and can be found across the world in all urban and sub-urban temperate and tropical regions, where they are often vectors of key diseases. Indeed, the *Cx. pipiens* complex play important roles in the transmission of several pathogens and parasites that infect humans, including West Nile virus, St. Louis encephalitis virus, and filarial worms, and other wildlife pathogens such as bird malaria (*Plasmodium* spp.) (Farajollahi et al., 2011; Ward and Benelli, 2017).

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Essential oils (EOs) from plants are widely used as alternative sources of pest management tools (Govindarajan et al., 2011; Govindarajan and Sivakumar, 2014; Pavela and Benelli, 2016a, b). Several studies have reported the effectiveness of EOs against *Culex* mosquitoes (Amer and Mehlhorn, 2006; Koliopoulos et al., 2010; Traboulsi et al., 2002; Michaelakis et al., 2009; Govindarajan and Benelli, 2016a, b, c; Govindarajan et al., 2016d, e; Benelli et al., 2017b; Benelli and Govindarajan, 2017). Some of the most promising botanical larvicides are aromatic plants. Within the huge literature available on plant EOs as larvicides, a recent review by Pavela (2015a) identified 7 plants with EOs showing LC_{50} lower than 10 ppm. It is also noteworthy that most of the studies available on the topic tested only raw EOs, while only a limited number focused on the toxicity exerted by their main molecules, as well as on possible synergistic or antagonistic effects among them (Benelli et al., 2017b, c).

Pelargonium roseum Willd, a species belonging to the Geraniaceae family, is indigenous of Southern Africa. This species, due to its strong pleasant rose-like odor, is widely grown as ornamental plant nearly in all parts of the world. *P. roseum* has woody straight stems, and leaves covered with short rough hairs, which give the plant a sweet scent (Carmen and Hancu, 2014; Lis-Balchin et al., 1996). The *P. roseum* EO has been studied for antimicrobial, antifungal and anti-inflammatory activities (Carmen and Hancu, 2014; Lis-Balchin et al., 2003; Rezaie et al., 2008.

However to the best of our knowledge, this species has not been studied as a potential source of mosquitocides. To our mind, the pleasant odor of *P. roseum* EO makes it an interesting candidate to develop both eco-friendly pesticides and repellents. In this scenario, the present study shed light on the chemical composition of *P. roseum* EO. Furthermore, the *P. roseum* EO and its major constituents (i.e. β-citronellol, geraniol and linalool) were evaluated as toxic agents against eggs, larvae and adults of *Cx. pipiens*.

2. Material and methods

2.1. Essential oil extraction and GC–MS analysis

Fresh leaves of *P. roseum* were collected during Jun 2015 from a commercial medicinal plants garden (Kashan, 34.0351° N, 51.0671° E, Iran). The species taxonomic identity was confirmed by Sari University of Agriculture and Natural Resources (Sari, Iran). The *P. roseum* EO was obtained by hydro-distillation and GC–MS analysis was performed to reveal the chemical constituents of the EO. GC–MS was carried out on an Agilent 789, a gas chromatography with 5975C mass selective detector and a HP5M5 column (30 m × 0.25 mm × 0.25 μm). The samples (2 μL) were diluted to 1% with *n*-hexane and the carrier gas was helium at flow rate of 1.0 mL/min. Analysis was carried out in the oven, while temperature was held at 60 °C for 3 then 3 °C/min to 150 °C (held for 1 min), and after that programmed at 3 °C/min to 260 °C (held for 3 min). The injector and the temperature of detector were at 230 and 250 °C, respectively. The identification of the *P. roseum* EO compounds was based on the comparison of their retention indices and mass spectra with those in commercial libraries NIST 98.1 and Mass Finder 3.1. The concentration of each essential oil component was calculated from the integration area of the chromatographer (Govindarajan and Benelli, 2016a, b).

2.2. Mosquito rearing

Egg and larval stages of *Cx. pipiens* were obtained from a laboratory-bred colony at Babol Medical University, Babol, Iran. Adults were kept in cages (40 × 40 × 40 cm) at temperature of 25 ± 2 °C, 80 ± 2% relative humidity and light: dark period of 12:12 h. Females laid eggs in dishes (9 cm diameter × 1.5 cm depth) filled with approximately 20 mL of tap water. Rafts were removed daily and, in order to hatch, placed in cylindrical enamel pans (with diameter of 35 cm and 10 cm deep). An aqueous yeast suspension (5% w:v) was used as food source.

2.3. Larvicidal assays

Larvicidal activity of the *P. roseum* EO and its three major compounds, β-citronellol, geraniol and linalool, was evaluated according to WHO (2005). Larvae were reared under the same temperature and light conditions as adults and were fed with dog biscuits and yeast powder in the 3:1 ratio (Govindarajan and Benelli, 2016a). *P. roseum* EO and the three main compounds at the concentrations of 1, 2, 5, 10, and 15 μg/mL diluted in tap water were used for assessment of larvicidal activity. The *P. roseum* EO and each of the tested compounds were dissolved in 1 mL DMSO (dimethyl sulfoxide) as emulsifier and then diluted in 249 mL of tap water to obtain each of the desired concentrations. The control was prepared using 1 mL DMSO in 249 mL of water. Twenty 3rd instar larvae of *Cx. pipiens* were stored into each test solution replicate belonging to a selected concentration. Five replicates were made for each concentration. Larval mortality was recorded after 24 h.

2.4. Ovicidal assays

Ovicidal activity was evaluated according to the method described by Govindarajan et al. (2011) and Benelli and Govindarajan (2017) with slight modifications. Freshly laid egg rafts were collected using overpass in mosquito cages. Egg rafts of *Cx. pipiens* were exposed to different concentrations of *P. roseum* EO, i.e., 0.25, 0.5, 1, and 1.5 μg/mL in 5 mL tap water containing 0.001% DMSO. The tests for citronellol, geraniol and linalool were carried out at the same doses. Control vials contained only 0.001% DMSO in 5 mL tap water. Five replicates were run for all tested compounds as well as for the control and EO. Egg mortality was calculated after 24 h of exposure (Benelli et al., 2017c).

2.5. Adulticidal bioassays

In order to test the toxicity of the *P. roseum* EO and its three main components on adults of *Cx. pipiens*, 20 adults (4–6 days old) were placed in a specially designed box for vapor toxicity (Prajapati et al., 2005). Pads with size of 22 × 35 × 2 mm, the same size as commercially available mosquito mats, were loaded with different concentration of test compounds equal to 1, 2 and 5 μg/L of box. The relatively low doses mentioned above were selected in order to test the products at doses safely employable for indoor use of these compounds. DMSO at 0.01% concentration was used as emulsifier. Control pads received only 0.01% DMSO. Treated pads were dried in a fume hood for 5 min, then placed on a mosquito mat machine and the machine was kept on for 15 min. Three replicates were run for all tested compounds, as well as the control, under the same experimental conditions. Adult mortality rates were determined after 1, 2 and 4 h.

2.6. Statistical analysis

The Statistical Package for the Social Sciences 16.0 software was used for all the analyses. The 50 and 90% lethal concentrations (LC_{50} and LC_{90}) were calculated by probit analysis (Benelli, 2017). One-way analysis of variance (ANOVA) and Tukey's HSD test were used to analyze data from the multiple concentration tests. Results with $P < 0.05$ were considered to be statistically significant.

3. Results

The chemical constituents of *P. roseum* EO were identified by GC–MS analysis and are shown in Table 1. The *P. roseum* EO was characterized by a high amount of citronellol (35.9%), geraniol (18.5%) and linalool (5.72%). The bioactivity of *P. roseum* EO and its three major compounds on larvae of *Cx. pipiens* is presented in Table 2. *P. roseum* EO showed significant larvicidal activity with LC_{50} and LC_{90} values of 5.49 and 12.05 μg/mL, respectively. Among *P. roseum* EO major components, the highest larvicidal activity was observed for geraniol, followed by