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Insecticidal and repellence activity of the essential oil of *Pogostemon cablin* against urban ants species

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ABSTRACT

Ants are highly abundant in neotropical regions, with certain species adapted to the urban environment, where they can cause damage to human health. The main method for controlling ants consists of using organosynthetic insecticides, which are potentially toxic to the environment. Essential plant oils are considered a viable alternative to the use of conventional insecticides. In this study, we analyze the bioinsecticidal activity and repellence of patchouli essential oil (*Pogostemon cablin*) against three species of urban ants: *Camponotus melanoticus*, *Camponotus novograndensis*, and *Dorymyrmex thoracicus*. The chemical composition of the essential oil was analyzed by GC–MS and GC-FID. The major compounds were patchoulol (36.6%) followed by α-bulnesene (13.95%), and α-guaiene (11.96%). Toxicity and repellency bioassays were performed using the essential oil over the ants, and mortality evaluations were performed at 4, 24, and 48 h after performing the bioassays. Mortality percentage of the ants on 7 μg/mg was on average 84%. The essential oil of *P. cablin* displayed toxicity against all three species of urban ants, with the lowest LD_{50} being observed for *D. thoracicus* (2.02 μg oil/mg insect) after 48 h of exposure compared to *C. melanoticus* (2.34 μg oil/mg insect) and *C. novogranadensis* (2.95 μg oil/mg insect). The essential oil of *P. cablin* was strongly repellent to the three species of ants in all concentrations tested (0.01% and 1% v/v). Considering the potential toxicity and repellency of the *P. cablin* essential oil to the urban ants, future studies could investigate the practical application of this oil to control of this insects.

1. Introduction

Ants (Hymenoptera: Formicidae) display a high degree of dominance and diversity in tropical environments (Hölldobler and Wilson, 1990), with certain species being considered agricultural and urban pests (Bueno et al., 2008; Jaffe, 2004).

Numerous ants' species colonize and exploit environments altered by human activity, most notably the genera *Camponotus* and *Dorymyrmex* (Campos-Farinha et al., 2002). *Camponotus* ants normally nest in diverse environments (Campos-Farinha et al., 2002), and possess broad ecological adaptations with opportunistic habits and an elevated capacity for invasion (Fonseca et al., 2010). *Dorymyrmex* is highly diversified genus with distribution restricted to the neotropical regions of the Americas. Several species demonstrate a large degree of endemism with preferential nesting in dry or disturbed habitats (Cuezzo and Guerrero, 2012). These organisms reside in houses, commercial establishments, and hospitals with the ability to cause discomfort and structural damage and to be vectors for pathogenic organisms (Campos-Farinha et al., 2002; Fonseca et al., 2010). Among the pathogens carried by ants, the bacteria *Escherichia coli* and *Enterobacter* spp. are causative agents of severe intestinal disorders and are responsible for hospital infections (Pesquero et al., 2008).

Although several forms of control have been employed against urban ants, they are often inefficient. The use of conventional methods, in addition to resulting in the contamination of the environment, may lead to fragmentation of the colonies, increasing the number of nests and consequently the population of these insects (Fonseca et al., 2010). Other factors that impede the control of urban ants are inappropriate management practices, such as the use of agricultural insecticides that are not specific to the pests in question (Oliveira and Campos-Farinha, 2005).

In recent years, a series of studies have shown that plants are potential sources of insecticides (Dugassa et al., 2009; Nerio et al., 2010; Odalo et al., 2005), thereby representing future alternatives for new methods for control of insect pests. Of the products with

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insecticidal activity derived from plants, essential oils have proven to be toxic to different pests (Rattan, 2010). Due to their volatility, these essential oils have low persistency in the environment which contributes to its selectivity for the non-target organisms (Isman, 2006). Essential oils of plants are formed by complex mixes of compounds structurally different which can act synergistically increasing its action potential and efficacy (Berenbaum, 1985). This blend can contribute to hamper the development of resistance by target organisms (Machial et al., 2010).

Pogostemon cablin Benth. (Lamiaceae) is a medicinal and aromatic plant extensively cultured in several countries for extraction of its essential oil (patchouli oil) rich in terpene, which have great commercial value to perfumery and cosmetic industry (Sant'ana et al., 2010). Essential oil of patchouli already has demonstrated insecticidal activity (Park and Park, 2012), including repellency against mosquito species (Trongtokit et al., 2005), whitefly (Yang et al., 2010) and termites (Zhu et al., 2003). The toxicity of patchouli oil and their major compound patchouli alcohol appear to have multiple actions against termites including neurotoxicity, and internal tissue damage (Zhu et al., 2003).

Products with activity of repellency could be the most effective in controlling urban ants since it could avoid their contact in unwanted places. Because of the ease in which urban ants introduce in domestic environments (Kamura et al., 2007) and mainly because it have nests unstructured (Solis et al., 2009) the effective control over the nests would be difficult (e.g. using fumigation). However, to date, no study has evaluated the activity of the essential oil of *P. cablin* against ants.

In this study we assessed chemical composition and bioinsecticidal activity and the repellence of the essential oil of *P. cablin* against urban ants: *Camponotus melanoticus*, *Camponotus novogranadensis*, and *Dorymyrmex thoracicus*.

2. Materials and methods

2.1. Plant matter and the extraction of the essential oil

The species *P. cablin* was cultivated at the Rural Campus of the Federal University of Sergipe, São Cristóvão, SE, Brazil (10°56′ S, 37°05′ W). The average annual temperature and precipitation of the region are 27 °C and 1590 mm, respectively. A voucher specimen was deposited in the Herbarium of the Federal University of Sergipe, Biology Department, São Cristóvão, Sergipe, Brazil, 49100-000. The leaves of *P. cablin* were collected and dried at 40 ± 1 °C for 4 days in a kiln (Marconi MA 037) (Sant'ana et al., 2010). The leaves of *P. cablin* were dried at 40 ± 1 °C in circulating air drying oven (Marconi MA 037) for four days (Sant'ana et al., 2010). The essential oils were extracted by hydrodistillation with Clevenger-type equipment for 140 min (Ehlert et al., 2006). Subsequently, the essential oil was separated from the aqueous phase and stored in a freezer until it was used.

2.2. Analysis of the essential oil in GC–MS and GC-FID

The chemical composition of the essential oils was analyzed using a gas chromatograph coupled to a mass spectrometer (GC–MS) (Shimadzu, model QP 5050A) equipped with an AOC-20i auto injector (Shimadzu) and a fused-silica capillary column (5% phenyl/95% dimethylpolysiloxane, 30 m × 0.25 mm id., 0.25 μm film, J&W Scientific). Helium was used as the carrier gas at a flow rate of 1.2 mL/min. The temperature program was as follows: 50 °C for 1.5 min, an increase of 4 °C/min to 200 °C, an increase of 15 °C/min to 250 °C and 250 °C for 5 min. The injector temperature was 250 °C, and the detector (or interface) temperature was 280 °C. The injection volume of ethyl acetate was 0.5 μL, the partition rate of the injected volume was 1:87, and the column pressure was 64.20 kPa. The mass spectrometer conditions were as follows: ionic capture detector impact energy, 70 eV; scanning speed, 0.85 scans/s; and range, 40–550 Da.

The chemical constituents were quantified by gas chromatography with flame ionization detection (GC-FID), using a Shimadzu GC-17A system (Shimadzu Corporation, Kyoto, Japan) equipped with a ZB-5MS (5% phenyl-arylene/95% dimethylpolysiloxane) fused silica capillary column (30 m × 0.25 mm i.d. × 0.25 μm film thickness) from Phenomenex (Torrance, CA, USA) under the same conditions described for GC–MS. The amount of each constituent was determined by area normalization (%). The concentrations were calculated from the GC peak areas and arranged in order of GC elution.

The essential oil components were identified by comparison of mass spectra with mass spectra available on database (NIST05 and WILEY8) libraries. Additionally, retention indices values were compared with those in the literature (Adams, 2007) and the relative retention indices (RRIs) were determined according to Van Den Dool and Kratz (1963) using and a homologous series of *n*-alkanes (C_8–C_{18}) injected under the chromatography conditions described above.

2.3. Urban ants

Adult workers of the species *C. novogranadensis*, *C. melanoticus*, and *D. thoracicus* were directly obtained from four nests on the campus of the Federal University of Sergipe, São Cristóvão, SE, Brazil. Workers were kept in nest fragments in plastic containers (50 cm diameter × 20 cm height) under ambient conditions (temperature 25–27 °C and a relative humidity of 60 ± 5%) for 24 h before performing the tests for ambience.

2.4. Insecticidal activity bioassays

2.4.1. Lethal dose

Preliminary toxicity tests were performed using three doses (1, 4, and 7 μg oil/mg insect) of *P. cablin* essential oil for workers of the species *C. novogranadensis*, *C. melanoticus*, and *D. thoracicus*. From these tests, between seven and fourteen doses of the compounds were determined for use in subsequent bioassays to obtain the dose–mortality curves.

The treatments consisted of the essential oil from patchouli and the control sample (acetone solvent, Panreac PAI-ACS 99.9%). Preliminary tests were performed by topically applying acetone to ensure that this solvent did not affect the survival of the workers.

The tests were performed in Petri dishes (9 cm in diameter × 2 cm in height) that contained 7 ants per dish for each species. These dishes were kept in a freezer for 1 min to reduce the activity of the ants and to allow for the topical application of the treatments on the individuals. Each ant received, in the pronotum region, 1 μL of solution of patchouli essential oil in different concentrations, which was diluted in acetone and applied by means of a microsyringe (Hamilton, 10 μL). For the control treatment, 1 μL of acetone was applied to each individual. The experimental design was completely randomized with four replicates.

The dishes containing the treated insects were covered with plastic film and stored in a B.O.D. oven at a temperature of 25 °C ± 1 °C and a 70% relative humidity 75%. Evaluations of the mortality of the insects were performed at 4, 24, and 48 h after performing the bioassays to verify the increase in toxicity over time. In all cases, individuals were considered to be dead when they remained immobile and did not respond to stimulation applied with a brush.