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Selection of an essential oil from *Corymbia* and *Eucalyptus* plants against *Ascia monuste* and its selectivity to two non-target organisms

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ABSTRACT

Due to an increase in environmental and health issues and with the excessive use of synthetic pesticides, many studies are investigating plant essential oils (EOs) for pest control. These compounds are considered safe control agents since they have low toxicity to non-target organisms. Thus, this study aimed to select potential EOs from the Myrtaceae family and their constituents for the control of *Ascia monuste* (Godart) and to evaluate those EOs selectivity to two non-target organisms (*Solenopsis saevissima* Smith and *Tetragonisca angustula* Latreille). Twelve EOs, extracted by hydrodistillation from *Corymbia* and *Eucalyptus* plants, were tested in this study. All toxicity bioassays were performed by topical application. *C. citriodora* EO had the highest insecticidal activity against *A. monuste* (LD_{50} = 20.61 μg/mg) and also presented a fast action (LT_{50} < 10 min). Citronellal was the main compound of *C. citriodora* EO (86.8% of the oil constitution) and exhibited toxicity similar to this EO. The other compounds caused no significant mortality. Hence, the toxicity of the *C. citriodora* EO is mostly explained by the citronellal activity. This EO was selective in favor of the predatory ant *S. saevissima* but caused high mortality of the pollinator bee *T. angustula*. Therefore, *C. citriodora* EO is a promising model in the development of insecticides against *A. monuste*. However, to mitigate its impact over pollinators, its application must rely on the principles of ecological selectivity.

1. Introduction

Plant essential oils (EOs) are volatile secondary metabolites from aromatic plants that may act in their direct defenses against herbivores and pathogens, or in indirect defenses as communicative substances to attract other organisms (Franz and Novak, 2009; Zuzarte and Salgueiro, 2015). EOs are complex mixtures of organic substances and their constitution differs largely among plant species and are generally classified as terpenes, terpenoids and phenolic compounds (Pavela, 2015; Pavela and Benelli, 2016). This constitution is decisive because the EO toxicity varies according to their number of components and the interactions between them (i.e., synergism and antagonism) (Akhtar and Isman, 2012).

Due to an increase in environmental and health issues, the effect of the indiscriminate use of synthetic pesticides, and the growing demand for safer food and products by consumers, the adoption of EOs has become more attractive in agriculture (Ebadollahi, 2011). Several studies have reported their larvicidal, adulticidal and antifeedant activity, deterrent effects, repellent action and insect cycle alteration (Tripathi et al., 2009). Especially on horticulture crops, EOs are desirable in pest management programs since they are non-persistent in the environment, they usually are selective to non-target insects and, they have low toxicity to humans (Dey and Gupta, 2016).

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The Brassicaceae family is one of the major horticulture crops worldwide. In America, the butterfly *Ascia monuste* (Godart) (Lepidoptera: Pieridae) is an important pest of crops from this family, causing losses up to 100% (Alam, 1992). Therefore, considering the suitability of the uses of EOs in horticulture crops, they have a high potential to be incorporated in *A. monuste* Integrated Pest Management (IPM) programs.

In agroecosystems, *A. monuste* populations are regulated by natural control agents like the generalist predatory ant *Solenopsis saevissima* (Smith) (Ramos et al., 2012). Hence, an *A. monuste* IPM might consider the EOs selectivity to *S. saevissima*. Furthermore, during the flowering growth stage, some Brassicaceae plants, such as canola (*Brassica napus*, *B. rapa* or *B. juncea*), are important nectar and pollen sources for pollinator insects (Westcott and Nelson, 2001; Woodcock et al., 2013). This fact enhances the necessity to assess the selectivity of EOs to these insects, including the bee *Tetragonisca angustula* (Latreille), an important generalist pollinator in tropical regions (Iwama and Melhem, 1979; Morgado et al., 2011).

Currently, many studies report the effects of EOs from plants of the Myrtaceae family (which includes both *Corymbia* and *Eucalyptus* genera) on insect pests (Batish et al., 2008; Ebadollahi, 2013). However, no study has yet tested the effect of Myrtaceae plants EOs in *A. monuste*, nor their selectivity to *S. saevissima* and *T. angustula*. Thus, this study was carried out (*i*) to select potential EOs from plants of the Myrtaceae family and to evaluate the toxicity of their constituents against *A. monuste* and (*ii*) to assess the toxicity of the selected EOs against two non-target organisms (*S. saevissima* and *T. angustula*).

2. Material and methods

2.1. Plant material

EOs were extracted from fresh leaves of three species of *Corymbia* (*C. citriodora*, *C. henryi* and *C. maculata*) eight of *Eucalyptus* (*E. andrewsii*, *E. resinifera*, *E. sphaerocarpa*, *E. phaeotricha*, *E. cinerea*, *E. pyrocarpa*, *E. punctata* and *E. siderophloia*) and a hybrid (*E. alba* x *E. tereticornis*). The plants were around 30 years old and grown at the campus of the Federal University of Viçosa (UFV), Viçosa, Minas Gerais State, Brazil (20°48′45″ S, 42°56′15″ W, 600 m above sea level and tropical weather). The leaves used for EO extractions were collected at the end of the dry season in November 2013.

2.2. Extraction and analysis of EOs

The EOs were extracted by hydrodistillation for 3 h, in triplicate, using a modified Clevenger apparatus. Each replicate was 100 g of leaves (cut into small pieces) mixed in 1 L of distilled water. The plant material was stored in a freezer at −15 ± 4 °C until the completion of the extraction procedure. The EOs were stored in glass amber vials and maintained at −5 °C.

Chromatography analysis of EOs components was performed by gas chromatography (Shimadzu GC-17A) coupled to flame ionization detection (quantification) and mass spectrometry (identification). The quantification of the components of the EOs was accomplished using a RTX-5 fused silica column (30 m × 0.25 mm, film thickness of 0.25 μm) at a constant nitrogen flow rate of 1.8 mL/min. The oven's initial temperature was programmed to be 40 °C (isothermal for 4 min), followed by an increase of 3 °C/min up to 240 °C, remaining isothermal at this temperature for 15 min. The sample injection volume was 1.0 μL (10 mg/mL in dichloromethane) with a split ratio of 1:10 and column pressure of 115 kPa. The concentration of each component was calculated as the percentage of its corresponding peak area in relation to the total area of all peaks observed in the chromatogram.

The identification of EOs components was performed at the same chromatographic conditions used for the quantification process except for the carrier gas and column pressure: helium and 100 kPa, respectively. The EOs components retention indexes (RI) (Adams, 2007) were compared to a standard alkane series (C9-C26) and their mass spectrum compared with those on record in the Wiley library database (Wiley 7.0 and NIST 11) or in the literature.

2.3. Bioassays

Toxicity bioassays were conducted with second-instar larvae of *A. monuste* and adults of the predatory ant *S. saevissima* and the pollinator bee *T. angustula*. The larvae of *A. monuste* were obtained from a rearing maintained in a greenhouse located in the UFV campus following the methodology described in Bastos et al. (1997) and Neves, 1996 The adult stage of ants and pollinator bees were collected from natural nests located at the UFV Campus and acclimated in laboratory controlled conditions for 2 h before treatment. During this acclimation period, both insects were fed with a mixture of water (50%) and honey (50%) soaked in cotton pieces. Pure water was also provided.

The experimental design of the bioassays was completely randomized. Each replication was a Petri dish (9 cm in diameter x 2 cm in height) containing 10 insects and food. Disks (7.5 cm in diameter) of cabbage plants (*Brassica oleracea* var. capitata) were daily provided as a food source to the larvae of *A. monuste*. The ants and the bees were fed with the 50% honey solution and pure water. Each individual insect was exposed to 0.5 μL of solution (each EO diluted with acetone as solvent), topically applied, via a Hamilton microsyringe, at the dorsal thorax. The EOs and solvent quantities for each treatment varied accordingly to the dose tested and the average weight of each insect. The average weight of each insect was estimated, previously to the bioassay, by weighing 30 insects on an analytical balance (Gehaka, AG200). The solvent acetone (99.5%, Vetec) was used as negative control. Petri dishes were placed in a biochemical oxygen demand (B.O.D.) incubator at 25 ± 5 °C, relative humidity of 75± 5% and a photoperiod of 12 h. The insects were considered dead when they did not move while touched by a fine brush.

A total of six bioassays were conducted. In the first bioassay, a screening toxicity bioassay was performed to select the most lethal EOs against *A. monuste*. The second bioassay was conducted to determine the lethal doses of the *C. citriodora* EO and azadirachtin against *A. monuste*. In the third, the lethal time of this EO against *A. monuste* was determined. The toxicity of the *C. citriodora* EO constituents against *A. monuste* was evaluated in the fourth and fifth bioassays. Finally, the effect of the *C. citriodora* EO to two non-target organisms was assessed in the sixth bioassay.

2.3.1. Screening toxicity bioassay

Each treatment consisted of an EO in the dose of 30 μg of EO/mg of insect, with four replicates. This dose was adopted based on previous study that recommends doses from 10 to 50 μg of EO/mg of insect to select substances with insecticidal activity (Alvarenga, 2012; Moreira et al., 2007). The insecticidal activity of a commercial neem oil formulation (azadirachtin A/B 12 g/L, E.I.D. Parry Limited) was evaluated as a positive control with four replicates in the dose of 30 μg/mg of insect. Insect mortality was recorded 72 h after exposure to treatments. This moment was used for evaluations because we previously verified that the mortality stabilized after this period.

The EOs that caused mortality higher than 80% were selected for subsequent bioassays, since it is the criteria used in Brazil to consider an insecticide efficient to its registration (Bacci et al.,

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