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Apis mellifera (Insecta: Hymenoptera) in the target of neonicotinoids: A one-way ticket? Bioinsecticides can be an alternative

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ABSTRACT

The recent decline of *Apis mellifera* populations around the world has been subject of intense research due to ecological and economic damages resulting from the loss of pollination services. The intensive use of insecticides from the neonicotinoids group is among the possible causal factors of this decline, including also sub-lethal effects. However, the use of synthetic insecticides has been increased on a global scale in the recent decades. In order to evaluate an alternative to the use of neonicotinoids, this work investigated the effects of a bioinsecticide and its major compound on *A. mellifera* (Apidae: Hymenoptera), one of the main pollinators of crop plants. For this, bees were exposed, by contact and ingestion, to the essential oil of *Cymbopogon martinii* (Poaceae: Poales), to geraniol (major compound) and the insecticide imidacloprid to evaluate the toxicity and behavioral effects as well as the locomotion changes and immune responses of bees treated with these compounds. In general, toxicity was greater through ingestion and the insecticide imidacloprid was more toxic to *A. mellifera* compared to the essential oil and its major compound. The individual and collective behaviors (*i.e.* trophallaxis, grooming, avoidance) as well as the immune responses of bees were not significantly affected by bioinsecticides. However, the locomotion response and flight orientation of the bees were significantly altered by insecticide when administered by ingestion. Our results highlight the potential of *C. martinii* essential oil and its major compound as a possible alternative to mitigate the harmful effects of neonicotinoids on bees.

1. Introduction

The maintenance of biodiversity ensures ecosystem services, which provides a range of benefits for humans (Dirzo et al., 2014). Among these services, insect pollination – mainly carried out by bees (Klein et al., 2007) – represents a crucial service for maintenance of the genetic diversity of wild plants (Knight et al., 2005) and for the world's agricultural productivity (Ricketts et al., 2008). The recent global decline in *Apis mellifera* populations – known as Colony Collapse Disorder (CCD) - is considered threatening because of the huge economic damage from reduced pollination in different crops (Potts et al., 2010). CCD has been attributed to multiple factors that appear to act synergistically, including: loss of natural habitat, incidence of parasites and diseases and intensification of agriculture (Staveley et al., 2014). Although the relative importance of these factors is not yet known, the use of insecticides from the neonicotinoids group has been reported as an important factor, mainly due to their sublethal effects on bees (Pisa et al., 2017; Sánchez-Bayo et al., 2016). However, some recent studies have also showed that minor doses of neonicotinoids present non sublethal effects on honeybees (Byrne et al., 2014; Dively et al., 2015).

Neonicotinoids can contaminate bees directly during the application in the field and, mainly, through the consumption of resources such as pollen and nectar from contaminated plants (*e.g.* oral exposure), since they are systemic pesticides (*i.e.* once absorbed by plants, they are diffused in tissues of bees) (Farooqui, 2013). These insecticides act on arthropods, causing physiological and behavioral effects by directly interfering in the acetylcholine receptors – neurotransmitter receptors responsible for triggering the depolarization of the postsynaptic membranes in the central nervous system (Gbylik-Sikorska et al., 2015). Although the effects of acute lethal toxicity are not always observed,

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chronic sublethal effects (*i.e.* effects from doses that do not cause mortality directly) may contributes to CCD (Henry et al., 2012; Pisa et al., 2017). Among such sublethal effects is reduction of immune response of bees contaminated with neonicotinoids (Brandt et al., 2016), which makes them more susceptible to infection by pathogens (Sánchez-Bayo et al., 2016) [*e.g. Nosema ceranae* (Aufauvre et al., 2012) and *Varroa destructor* (Barron, 2015)]. In addition, other important sublethal effects include the reduction of the learning abilities and memory of the bees. These changes may interfere in the orientation ability, navigation and consequently in the forage efficiency as well as in the return of foragers to their colonies (Henry et al., 2012), culminating in the reduction of population size and colony productivity (Pisa et al., 2017).

A range of studies have been developed to obtain efficient products against insect pests that have reduced negative effects on non-target organisms, such as bees (Furlan et al., 2018). The essential oils from plants (EOs), for example, consist of a complex mixture of volatile components that can interact, triggering different functions in the plant, such as: protection against pathogens, herbivores and/or attraction of pollinator insects and seed dispersers (Bakkali et al., 2008). Therefore, due its bioactivity, the EOs and its constituents isolated – mainly monoterpenes – may consist of potential bioinsecticides. The EOs are considered an alternative to the use of synthetic insecticides to pest control because several desirable characteristics, such as: efficiency in herbivore control, low toxicity to non-target organisms, reduced persistence in the environment and slow induction of insect resistance due the complexity of compounds (Koul et al., 2008).

Although the EOs are natural compounds considering environmentally safe, they are toxic to different insects and they may also cause undesired effects in non-target organisms (Xavier et al., 2015). The EO of *Cymbopogon martinii* plants has as major compound the geraniol, a monoterpene that is also present in the attraction and aggregation pheromone (*i.e.* during foraging) synthetized by bees (Trhlin and Rajchard, 2011). The effects of EO from plants of *Cymbopogon* genus and the compound geraniol have been showed to control a range of insect pest groups (Hernandez-Lambraño et al., 2015; Lima et al., 2013; Tak and Isman, 2016), including sucking insects [EOs: (Costa et al., 2013; Deletre et al., 2015) and geraniol: Baldin et al., 2014; Deletre et al., 2015) for which the neonicotinoids are widely used (Qu et al., 2015). However, the possible effects (*i.e.,* toxicity, behavior and immunity) of this EO on bees has not been investigated.

As *Apis mellifera* are considered the most important pollinators due to their management in different agricultural crops worldwide (Potts et al., 2010), in the present study, we evaluated the toxicity, behavioral, locomotion changes and the immune response of these bees under the effect of the neonicotinoid imidacloprid, the EO of *C. martinii* and its major compound geraniol.

2. Material and methods

2.1. Collection of bees

The individuals of *A. mellifera* used in the bioassays were obtained from four colonies held at Experimental Apiarium of Federal University of Sergipe, São Cristóvão, Sergipe, Brazil (10°55'S, 38°6′W). Forage bees were collected with a flexible nylon funnel (80 cm), which had one end attached to a plastic pot and other inserted at the entrance of the colony, maintaining a slope toward the light. The captured individuals were kept in B.O.D incubator with food supply (50% sucrose solution) for a maximum of 3 h before the experiments.

2.2. Compounds and chemical analysis of essential oil of C. martinii

The EO of *C. martinii* and the compound geraniol (98% of purity) were acquired from Raros Naturals® (Macaíba, Rio Grande do Norte, Brazil) and Sigma-Aldrich® (Steinheim, Germany) companies, respectively. The commercial insecticide used was the neonicotinoid imidacloprid (Bayer CropScience®, São Paulo, SP, Brazil) in form of granules dispersible in water (700 g a.i/kg).

The analysis of the EO components was performed by Gas Chromatography coupled to Mass Spectrometry (GC/MS) and Flame Ionic Detector (GC/MS/FID) using the equipment GCMSQP2010 Ultra (Shimadzu Corporation, Kyoto, Japan) equipped with AOC-20i automatic injector (Shimadzu Corporation, Kyoto, Japan). The separations of components were performed on 30 m, Rtx®-5MS Restek fused-silica capillary column (5% diphenyl–95% dimethylpolysiloxane) with a 0.25 mm internal diameter and 0.25 mm film thickness. Helium 5.0 was used as the carrier gas at a flow rate of 1.0 mL min^{-1}. A one microliter (μL) of the EO sample was injected at a temperature of 280 °C, in a split ratio of 1:30. The oven temperature started with 50 °C (isotherm for 1.5 min), increasing 4 °C min^{-1} until reaching 200 °C and then an increase of 10 °C min^{-1} up to 300 °C, which was maintained for 5 min.

In the GC/MS, the molecules were ionized by electrons with energy of 70 eV and the fragments were analyzed by a quadrupolar system programmed to filter fragments/ions with m/z from 40 to 500 Da, detected by an eletron multiplier. The ionization process for GC/FID was realized by the flame coming from hydrogen gases 5.0 (30 mL min^{-1}) and synthetic air (300 mL min^{-1}). The chemical compounds collected and the electric current generated was amplified and processed in GCPostrun Analysis software (Labsolutions- Shimadzu).

The identification of constituents from EO of *C. martinii* was performed based on the comparison of retention indices of the literature (Adams, 2007). For the retention index, the Van Den Dool and Kratz (1963) equation in relation to a homologous series of *n*-alkanes (nC_9-nC_{31}) was used. Three libraries from the equipment (WILEY8, NIST107 e NIST21) were also used to compare spectra data obtained with those from libraries, using a similarity index of 80%.

2.3. Bioassays

Treatments used in the bioassays were the EO of *C. martinii*, geraniol and imidacloprid. Bioassays were performed in a completely randomized design with four replicates (*i.e.* colonies). It was performed toxicity, behavioral, locomotion, flight orientation and immune response bioassays. Tested bees were submitted to treatments by two exposure routes: contact (by topic application) and ingestion. In the behavioral and locomotion/flight orientation bioassays, the variables were analyzed after 1 and 24 h from exposure of bees to treatments in both exposure routes.

2.3.1. Toxicity

To obtain the dose-mortality curves, it was used initially for each route of exposure, doses which resulted in mortalities between 0% and 100% of individuals. Posteriorly, immediate doses were used to determine the curves. In all bioassays, a replicate consisted of a group composed by eight forager bees previously anesthetized at −8 °C for 2 min to allow the application of treatments. Treated individuals were placed in a Petri dish (9 × 2 cm) covered with filter paper and food supply (50% sucrose solution). Petri dishes were maintained in B.O.D. incubator under controlled conditions (26 ± 2 °C, RH 70 ± 5%, darkness) and mortality observations were performed after 24 h. Preliminary tests indicated that methodology used did not affect the survivorship of bees.

For contact exposure route, 1 μL of treatments were applied in the prothorax for each individual using a 10 μL microsyringer (Hamilton®, Renon, NV, USA). To determine the applied doses (μg/individual), the body mass of 40 individuals were determined using a precise analytical balance (AUW220D, Shimadzu). In all treatments, acetone (Panreac, UV-IR-HPLC-GPC PAI-ACS, 99.9%) was used as solvent. Preliminary tests showed that acetone do not affect the survival and behavior of honeybees.

For ingestion exposure route, each bee was individually placed in a