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Corymbia spp. and *Eucalyptus* spp. essential oils have insecticidal activity against *Plutella xylostella*

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ABSTRACT

Plutella xylostella (L.) is the main brassica pest worldwide and is difficult to control even with commercial insecticides. In this study, assessments were made of the contact toxicity of essential oils (EOs) from ten Myrtaceae species. For this, the leaves of four *Corymbia* species (*C. citriodora*, *C. intermedia*, *C. maculata*, and *C. ptychocarpa*) and six *Eucalyptus* species (*E. andrewsii*, *E. crebra*, *E. punctata*, *E. pyrocarpa, E. siderophloia*, and *E. sphaerocarpa*) were collected during the Brazilian dry season and subjected to hydrodistillation for extraction of EOs. For *C. citriodora*, another sample was also collected during the rainy season. The essential oil (EO) content of all samples ranged from 0.07% w/w to 3.3% w/w and GC–MS analyses allowed the identification of 52 compounds. For *E. andrewsii*, *E. pyrocarpa*, and *C. ptychocarpa* there was no previous report on EO chemical composition. For *C. citriodora*, a higher EO content for the samples collected during the dry season was found (2.72% versus 1.53% for the rainy season). The insecticidal activity of all eleven EOs was evaluated *via* topical application on the second-instar larvae of *P. xylostella*. The most active EOs were produced by *C. citriodora* collected during the dry season, with citronellal (86.8%) and isopulegol (4.7%) being the main constituents. At 30 μg/mg of insect, these EOs caused 80% *P. xylostella* mortality, being more active than neem oil used as positive control. These EOs presented low toxicity for *Solenopsis saevissima*, a natural predator of *P. xylostella*. Bioassays also demonstrated a synergistic effect between citronellal and (-)-isopulegol at all tested proportions. The results described here suggest that *C. citriodora* EOs can be an environmentally friendly alternative method to control *P. xylostella*.

1. Introduction

Corymbia and *Eucalyptus* are among the principal genera cultivated from the Myrtaceae family. There are 900 species and subspecies belonging to these genera and they are mainly used for timber production. *Corymbia* and *Eucalyptus* trees have perennial leaves that are odorous due to the presence of essential oils (EOs) which are produced and stored in secretory cells (Vuong et al., 2015). These EOs are mixtures of volatile compounds that have found pharmaceutical (Hassine et al., 2012) and perfumery applications (Bizzo et al., 2009). The EOs utilized as pharmaceuticals are rich in 1,8-cineole. In the perfumery industries, EOs rich in citronellal, citral, and geranyl acetate are the most used (Pino et al., 2002). In terms of toxicity, eucalyptus oils have been categorized as GRAS (Generally classified as safe) by the Environmental Protection Agency (US EPA) due to the high LD_{50} of their major components for rats (Batish et al., 2008).

The biological activities of EOs from *Corymbia* and *Eucalyptus* species have been recently reviewed (Barbosa et al., 2016; Vuong et al., 2015; Zhang et al., 2010). Some of these activities include antimicrobial (Sebei et al., 2015; Mekonnen et al., 2016; Luis et al., 2016; Salem et al., 2016; Said et al., 2016), herbicidal (Tomaz et al., 2014), and anticancer (Vuong et al., 2015) activities. Several investigations also reported on

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the insecticidal activities of these EOs (Karemu et al., 2013; Bossou et al., 2013, 2015). The EOs from *E. saligna* SM. have been shown to efficiently control major insect pests of stored food grains (Bett et al., 2016), while the oils from *E. cinerea* F.Muell. ex Benth effectively control *Musca domestica* L. (Rossi and Palacios, 2015). The EOs from *E. citriodora* Hook, rich in citronellal, showed potent insecticidal activity against third and fourth instar lavae of *Aedes aegypti* L. (Vera et al., 2014) and have also been shown to possess repellent activity against *Tribolium castaneum* Herbst (Olivero-Verbel et al., 2010). Such bioactivities are related to the chemical composition of the EOs (Souza et al., 2005), which may be influenced by a number of factors, including plant age, harvest time, location where the plant is cultivated, and soil fertility (Geocze et al., 2013; Filomeno et al., 2016; Martins et al., 2007).

Insects represent an important problem for humans. There is a myriad of insects that cause severe losses to farmers by devouring crops (Ware, 1999). It can be affirmed that insects compete with man for food and fiber sources around the world. Additionally, insects are vectors for several important diseases such as dengue, malaria, and leishmaniasis. In light of these problems, the control of insect species is highly desirable.

The most widely used method to control insects is the employment of conventional synthetic insecticides. However, the intensive application of these chemicals on crops can result in harmful effects to the environment, damage to food security, and selection of resistant populations. For example, one-third of cultivated plants depend on honeybees for pollination (Klein et al., 2007). *Tetragonisca angustula* Latreille (Hymenoptera: Apidae: Meliponinae) is a stingless species and an important pollinator of plants in the Neotropical region (Moreno et al., 2009; Braga et al., 2012). One problem of concern to the global community is the decline of bee populations in several parts of the world (Bernal et al., 2010; Neumann and Carreck, 2010). One hypothesis for this decline is the use of some groups of conventional insecticides, which eliminate undesirable insects but also probably the pollinators (Goulson, 2013). The use of natural products either as insecticide *per se* or as prototype models for the development of newer, more effective, and environmentally safer agrochemicals can be an alternative strategy to overcome such problems (Copping and Duke, 2007; Cantrell et al., 2012; Moreira et al., 2007; Sparks et al., 2016).

The development of resistance by pest populations can occur with both conventional and natural pesticides. However, the use of the latter, in rotation with insecticides presenting different modes of action, is an important measure in the management of pest resistance to insecticides (Denholm and Rowland, 1992).

The cabbage moth *Plutella xylostella* (L.) (Lepidoptera: Plutellidae) is the main brassica pest worldwide (Mohan and Gujar, 2001; Chaudhary et al., 2011; Bandeira et al., 2013; Abro et al., 2013; Grzywacz et al., 2010; Bacci et al., 2009b). This species causes severe losses in *Brassica oleracea* L. var. *italica* (broccoli), *Brassica oleracea* L. var. *capitata* L. (cabbage), *Brassica napus* L. (canola), *Brassica oleracea* L. var. *botrytis* (cauliflower), and *Brassica nigra* (L.) Koch (black mustard) crops. Its larvae feed on the leaves of the crops, causing a reduction in photosynthetic area, leading to delayed growth that, in extreme cases, can result in plant death (Bacci et al., 2009b). In the 1990s, the worldwide annual cost to control this pest was US$ 1 billion (Talekar and Shelton, 1993); nowadays this cost has reached US$ 4–5 billion per year (Abro et al., 2013; Kumrungsee et al., 2014).

Currently, the major strategy employed to control *P. xylostella* involves the use of synthetic organic insecticides (Sarfraz and Keddie, 2005). Despite their effectiveness, the continuous use of these products resulted in the development of insect populations resistant to even the most active compounds (Abro et al., 2013; Chaudhary et al., 2011; Mohan and Gujar, 2001; Grzywacz et al., 2010). Additionally, traditional insecticides can have a major impact on populations of natural enemies of *P. xylostella*, thereby, causing a resurgence of populations of this pest in greater numbers in many parts of the world (Lingathurai et al., 2011; Bandeira et al., 2013).

In integrated pest management programs, it is important that insecticides are effective in controlling pests and that they have a low impact on non-target organisms, such as natural enemies and pollinators (Bacci et al., 2009a; Abro et al., 2013). One of the natural enemies of *P. xylostella* is the predatory ants from the *Solenopsis* genus, including *Solenopsis saevissima* (Smith) (Hymenoptera: Formicidae). These ants prey on eggs, larvae, and pupae of Lepidoptera (Harvey and Eubanks, 2004; Ramos et al., 2012).

Within this context, in the present investigation we describe the results concerning the evaluation of the insecticidal activity on *P. xylostella* of EOs extracted from *Corymbia citriodora* (Hook.) K.D.Hill & L.A.S.Johnson, *C. intermedia* (R.T.Baker) K.D.Hill & L.A.S.Johnson, *C. maculate* (Hook.) K.D. Hill & L.A.S.Johnson, *C. ptychocarpa* (F.Muell.) K.D.Hill & L.A.S.Johnson, *Eucalyptus andrewsii* Maiden, *E. crebra* F. Muell., *E. punctata* DC., *E. pyrocarpa* L.A.S.Johnson & Blaxell, *E. siderophloia* Benth., *E. sphaerocarpa* L.A.S.Johnson & Blaxell. In addition, the most active EOs, namely from leaves of *C. citriodora* collected during the dry season in Brazil, were also evaluated on *S. saevissima*, a natural *P. xylostella* predator, as well as on the *T. angustula* bee.

2. Material and methods

2.1. Plant material

The EOs were extracted from four species of *Corymbia* (*C. citriodora*, *C. intermedia*, *C. maculata*, and *C. ptychocarpa*) and six species of *Eucalyptus* (*E. andrewsii*, *E. crebra*, *E. punctata*, *E. pyrocarpa*, *E. siderophloia*, and *E. sphaerocarpa*). The plants were approximately 30 years old and grown on the campus of the Universidade Federal de Viçosa, Viçosa, Minas Gerais State, Brazil (20°48′45″ S, 42°56′15″ W, 600 m above sea level and tropical climate). The leaves used for essential oil (EO) extraction were collected from plants in November 2013. In the case of *C. citriodora*, leaves were also collected in January 2014. The leaves of *C. citriodora* were collected during two seasons in order to verify whether the chemical composition and biological activity of EOs would be influenced by the season of harvesting. As the most intense rains in Viçosa begin in November and end in late March (Minuzzi et al., 2007), the harvest of November 2013 corresponded to the end of the dry season and the one in January 2014 corresponded to the rainy season.

2.2. Extraction and chemical analysis of the essential oils

The leaves of each plant species were separated into three portions of 100 g each. This material was stored in a freezer at -15 ± 4 °C until extraction. The leaves were cut into small pieces and mixed with 1 L of distilled water. Subsequently, the material was submitted to extraction by hydrodistillation, for a period of three hours and in triplicate, using a modified Clevenger-type apparatus. The EOs were collected and weighed, and the percentage of extracted EOs was determined in relation to dry matter mass. The EOs were stored in glass flasks and maintained in a nitrogen atmosphere at -5 °C until the moment of analysis by gas chromatography.

The determination of the dry matter content was performed in triplicate using 3 g of leaf sample, which were placed in an oven at 100 °C for 24 h. After this period, the mass of the dried material was weighed and the average water content of the samples calculated (ASAE, 2000).

The quantification of the EO components was carried out with Shimadzu GC-17 equipment fitted with a flame ionization detector (FID) and RTX-5 fused silica capillary column (30 m × 0.25 mm, film thickness of 0.25 µm). The following chromatographic conditions were employed: N_2 carrier gas under a flow of 1.8 mL min^{-1}; injector temperature of 220 °C; detector temperature of 240 °C; initial temperature of the column of 40 °C, isothermal for 4 min followed by heating at 3 °C min^{-1} to 240 °C, remaining isothermal for 15 min; sample injection volume of 1.0 μL (10 mg mL^{-1} in CH_2Cl_2); split ratio of 1:10; and