Bioassay

Toxicity of Corymbia citriodora essential oil compounds against Ascia monuste (Linnaeus, 1764) (Lepidoptera: Pieridae) and Plutella xylostella (Linnaeus, 1758) (Lepidoptera: Plutellidae)

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Abstract. Essential oils (EO’s) have been investigated as a safe alternative to pest management. The toxicity of an EO can vary due to abiotic and biotic factors. The individual compounds of different EO’s have shown promise to insect control and they may present toxicity similar to or greater than the EO’s. In this study, we determined the toxicity of Corymbia citriodora EO compounds against Ascia monuste (Linnaeus, 1764) and Plutella xylostella (Linnaeus, 1758). Citronnellal, trans-caryophyllene, and citronellol (LD₅₀ = 23.24, 24.17 and 27.84 µg/mg, respectively) were the most toxic compounds to A. monuste. On the other hand, α-pinene and β-pinene presented low toxicity to this pest. For P. xylostella, citronellol and citronellone were the most toxic compounds (LD₅₀ = 22.36 and 25.53 µg/mg, respectively). The other compounds presented lower toxicity with similar doses. Thus, the individual compounds of C. citriodora EO can be an alternative for A. monuste and P. xylostella control.

Keywords: botanical insecticide, crop protection, Myrtaceae, terpenes.

Essential oils (EO’s) have been extensively studied as a safe alternative to pest management. These substances contain a diversity of compounds with concentrations varying in the EO’s due to abiotic and biotic factors such as harvest time (Filomeno et al. 2017) and region of the plant where the material has been collected (Alves et al. 2016). This difference in EO’s composition may result in different toxicity among plants of the same species (Alves et al. 2016; Filomeno et al. 2017).

Some studies indicated that the individual compounds from EO’s have the potential to control insects, with toxicity similar to or greater than the EO’s (Tak et al. 2016). In previous works, we found that Corymbia citriodora (Myrtaceae) EO is promising for the control of Ascia monuste (Linnaeus, 1764) (Lepidoptera: Pieridae) (Ribeiro et al. 2018) and Plutella xylostella (Linnaeus, 1758) (Lepidoptera: Plutellidae) (Filomeno et al. 2017), pests of the brassica crops in temperate and tropical areas. However, changes can occur in the composition of C. citriodora EO (e.g., the harvest time), resulting in different insecticidal activity (Filomeno et al. 2017). Thus, the individual compounds of this EO can be an alternative use in pest control. Therefore, we determined the toxicity of C. citriodora EO compounds against A. monuste and P. xylostella.

Larvae of A. monuste and P. xylostella were obtained from a rearing maintained in the laboratory. Both larvae were fed with leaves of kale and adults with a mixture of honey and water. Bioassays were performed with second-instar larvae of both insect species. The chemical composition of C. citriodora essential oil is: citronellol (86.8%), citronellon (3.3%), isopulegol (4.7%), trans-caryophyllene (1.2%), α-pinene (0.6%) and β-pinene (0.9%) (Filomeno et al. 2017). Thus, citronellol, citronellon, (-)-isopulegol, trans-caryophyllene, α-pinene, and β-pinene were obtained from Sigma Aldrich and used as received.

In the lethal dose bioassay, we determined the lethal dose of each compound for both species to kill 50% of population (LD₅₀). The treatments consisted of the isolated compounds and acetone (99.5%, Vetec) as solvent. The doses tested (µg/mg) for A. monuste were as follows: citronellol (10, 15, 20, 25, 30, 35, and 40), citronellon (5, 15, 20, 25, 30, 35, and 45), (-)-isopulegol (15, 20, 25, 30, 35, and 45), trans-caryophyllene (15, 25, 35, 40, 45, and 50), α-pinene (10, 15, 20, 25, 30, 35, and 50), and β-pinene (10, 15, 20, 25, 30, 35, and 50). For P. xylostella, we tested: citronellol (10, 20, 30, 40, and 50), citronellon (10, 20, 40, and 50), (-)-isopulegol (10, 20, 30, 40, 50, and 70), trans-caryophyllene (20, 40, 80, and 90), α-pinene (15, 30, 40, 60, and 80), and β-pinene (20, 30, 40, 60, and 80) The mass of the 30 larvae of each species was obtained using an analytical balance (Gehaka, AG20200). Four replicates were used for each tested dose. Each replicate consisted of 10 larvae of A. monuste or P. xylostella in a Petri dish (9 cm of diameter x 2 cm in height) with a leaf disk (7.5 cm in diameter) using leaves of kale as food source.

The treatments were applied (0.5 µL/larvae) in the dorsal thorax of larvae using a microsyringe (Hamilton®, 10 µL). After the application, Petri dishes were placed in an incubator at 25 ± 2°C, relative humidity of 75 ± 5% and photoperiod of 12 h. The mortality was evaluated after 48 h, and larvae were considered dead when they did not move while touched by a fine brush. Mortality in treatments was corrected to that occurred in the control (acetone) by Abbott formula (Abbott 1925). Data were submitted to Probit analysis (PROC PROBIT SAS) and models with a probability of acceptance of the null hypothesis higher than 5% (P > 0.05) were accepted by the chi-square goodness-of-fit test. The lethal doses and their respective fiducial limits at 95% probability (Fl₉₅) were obtained.

The compounds of C. citriodora EO were toxic to A. monuste and P. xylostella. Citronellol, trans-caryophyllene, and citronellon were the most toxic compounds to A. monuste (Fig. 1). On the other hand, α-pinene and β-pinene showed low toxicity to this pest at the highest tested dose i.e., 50 µg of compounds/mg of larvae ([mean ± SE]; α-pinene (26.67 ± 7.07%); β-pinene (28.33 ± 7.03%)]. Thus, the lethal doses of these two compounds were not determined for A. monuste. For P. xylostella, citronellol and citronellone were the most toxic compounds (Fig. 2). The other compounds presented lower toxicity with similar doses for this pest.
In conclusion, *C. citriodora* EO compounds are toxic to both *A. monuste* and *P. xylostella*. Thus, the use of the individual compounds of *C. citriodora* EO is an alternative for the development of new insecticides for both pests.

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**Authors’ contribution**

AAS and MCP experimental design. RCS and JSP performed the bioassays and analyzed data. AAS and AVR wrote the manuscript. All authors read and approved the final version.

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