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Quantifying the harmful potential of ten essential oils on immature *Trichogramma pretiosum* stages

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HIGHLIGHTS

- Side-effects of ten essential oils were evaluated on *Trichogramma pretiosum* immatures.
- Eight essential oils affected the emergence, longevity, parasitism and sexual ratio of *T. pretiosum*.
- The F_1 generation was more susceptible to essential oils than the F_2 generation.
- Only *Allium sativum* and *Citrus sinensis* oils were not harmful to *T. pretiosum*.

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ABSTRACT

The use of chemical insecticides and non-selective natural products authorized for use in organic farming may reduce the effectiveness of egg parasitoids. The side-effects of ten plant essential oils on immature stages of *Trichogramma pretiosum* were evaluated. *Carapa guianensis*, *Origanum vulgare* and *Zingiber officinalle* during the F_1 generation, and Azadirachtin and *Mentha piperita* in the F_2 generation were slightly harmful (class II: 30–79%) to the emergence of this parasitoid. All essential oils affected the longevity of females of the F_1 and F_2 generations. *Thymus vulgaris* and *Z. officinalle* were the oils most harmful to female longevity. *Carapa guianensis* proved slightly harmful (class II: 30–79%) to parasitism in the F_1 generation when applied during the egg-larval and pre-pupal stages and *O. vulgare* in the F_1 generation in the pre-pupal stage alone, of this parasitoid. The sex ratio was lower than 0.5 during the pre-pupal stage of the F_1 generation with Azadirachtin, *C. guianensis, O. vulgare, Piper nigrum* and *Syzigium aromaticum,* but this parameter was not affected for the other biological stages of *T. pretiosum* in the F_1 and F_2 generations. The Azadirachtin, *C. guianensis*, *M. piperita*, *O. vulgare, T. vulgaris* and *Z. officinalle* oils revealed a mild toxic effect to the immature stages of *T. pretiosum* and, therefore, it should be used according to patterns of ecological selectivity. *Allium sativum* and *Citrus sinensis* essential oils were not harmful to *T. pretiosum,* and can be used in Integrated Pest Management.

1. Introduction

Plants produce secondary metabolites, secreted for defense, possessing potential insecticidal properties (Akhtar et al., 2010), exerting sublethal effects on oviposition, feeding activity (Ribeiro et al., 2015) and repelling herbivores (Isman, 2006), as well as attracting natural enemies (Miresmailli and Isman, 2014).

Essential oils are complex mixtures of these secondary metabolites such as monoterpenes, sesquiterpenes and oxygenate compounds (alcohols, ethers, aldehydes, ketones, lactones, and phenols), obtained from seeds, stems, leaves and flowers (Nerio

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et al., 2010). Hydrocarbon monoterpenes such as α-pinene, β-pinene, limonene, monoterpene and terpinene and oxygenated as carvacrol, 1,8-cineol, eugenol, fenchone, linalool, menthone, terpineol and thymol have been found to adversely affect Diptera, Coleoptera, Lepidoptera and Hemiptera (Isman, 2006; Chang et al., 2009).

Essential oils, used as bioinsecticides to control pests, have as their main market niche organic food production in integrated production systems (Isman et al., 2011), being an economically viable alternative for small farmers (Mkenda et al., 2015). The essential oils registered for pest control in Europe and the United States include thymol for two parasitic mites of honey bees (*Apis mellifera*) (Rice et al., 2002) and eugenol, a broad spectrum insecticide (EcoPCO® D) (Wilson and Isman, 2006). Clove, thyme and cinnamon essential oils are exempt from toxicity requirements and are listed as 'safe' by the Environmental Protection Agency of the United States of America (2017).

Pest control in integrated production systems is conducted with natural enemies, both parasitoids and predators, but insecticide applications, either chemical or organic, are often needed to complement the control. The insecticides used should not cause side effects on non-target insects (Khan et al., 2015; Zanuncio et al., 2018); however, there is evidence that some essential oils utilized in integrated production can be harmful to these organisms (Ndakidemi et al., 2016; Parreira et al., 2017). For example: the emergence of *Trichogramma pretiosum* (Riley) (Hymenoptera: Trichogrammatidade) and *Telenomus remus* (Nixon) (Hymenoptera: Scelionidae) was lower with the Asteraceae extracts (Tavares et al., 2009).

Trichogramma species rank among the most important parasitoids worldwide used to control forest, fruit, grain and vegetable pests (Parra and Zucchi, 2004). The widespread use of *Trichogramma* species as biological control agents is due to their ease of breeding on alternative hosts, highly aggressive parasitism and their wide geographic distribution (Souza et al., 2016). The host egg size is the major factor affecting the resources available during the immature stages, and the size and fitness of the emerging adults, bowever, xenobiotic agents can affect these and other fitness parameters (Boivin, 2009).

In Brazil, *T. pretiosum* is the most abundant parasitoid species (Altoé et al., 2012), but chemical insecticides and non-selective natural products authorized for use in organic farming, may reduce the effectiveness of egg parasitoids (Silva and Bueno, 2015; Rampelotti-Ferreira et al., 2017). The aim was to evaluate the toxicity of ten essential oils on the biological and reproductive parameters of *T. pretiosum* when applied during immature stages.

2. Materials and methods

2.1. Developing the experiment

Experiments were performed in the Laboratory of Biological Control of Insects (LCBI) at the Institute of Applied Biotechnology to Agriculture (BIOAGRO), Universidade Federal de Viçosa, Brazil, in climate chambers (BOD) at a temperature at $25 \pm 2\,^{\circ}C$, relative humidity of $70 \pm 10\%$ and a photoperiod of 12 h.

Trichogramma pretiosum females were obtained from the mass rearing of the LCBI. The *Carapa guianensis* essential oil was obtained from Embrapa Amazônia Oriental (CPATU) in Belém, Pará, Brazil, while the essential oils of *Allium sativum*, *Citrus sinensis*, *Mentha piperita*, *Origanum vulgare*, *Piper nigrum*, *Syzygium aromarticum*, *Thymus vulgaris* and *Zingiber officinale* were acquired from Viessence Trade in Natural Products Ltda. (Porto Alegre, Rio Grande do Sul, Brazil). These oils were extracted at industrial scales by hydrodistillation and water vapor dragging (Dapkevicius et al., 1998). The chemical composition of the oils was provided by the manufacturer who individually analyzed each batch manufactured (Table 1). Neem oil with 1.8–2.2% of Azadirachtin (Bioneem Tecnologia Consultoria Ind. e Comércio Ltda, Brazil) was used as a reference bioinsecticide because it is already widely used for pest control.

The essential oil solutions used were prepared at the acute median lethal dose (LC_{50}) estimated previously for *Anticarsia gemmatalis* (Hübner) (Lepidoptera: Noctuidae) (Table 1) (Ribeiro et al., 2015).

2.2. Bioassay

Newly emerged *T. pretiosum* females were placed in glass tubes (8 cm high x 2 cm diameter) coated with honey drops on the inner wall and closed with Polyvinyl Chloride (PVC) film. *Anagasta kuehniella* (Zeller) (Lepidoptera: Pyralidae) eggs (±125) glued onto paper card strips (5 cm × 0.5 cm) with gum Arabic diluted in distilled water (50:50, v/v) were exposed to *T. pretiosum* females for 24 h. The paper cards with *A. kuehniella* eggs parasitized by *T. pretiosum* (at 0–24, 72–96 and 168–192 h after parasitism, corresponding to the egg-larval, pre-pupal and pupal stages of the parasitoid, respectively) were immersed in the essential oil solutions and ethanol (control) for 5 s, shade dried for 30 min to evaporate the solvent (Carvalho et al., 2010), and placed in a BOD at $25\,^{\circ}C$, relative humidity of $70 \pm 10\%$ and photoperiod of 12 h until the F_1 generation larvae emerged. Then, 594 newly emerged females of this generation were placed in glass tubes to parasitize *A. kuehniella* eggs again, in the conditions described, until the F_2

Table 1
Scientific name, active ingredient (AI) or major component percentage (%MC), and LC_{50} ($\mu L\ mL^{-1}$) tests with *Anticarsia gemmatalis* (Lepidoptera: Noctuidae) eggs in the laboratory.

Scientific Name	AI and/or %MC	LC_{50} (CI95)[a]
Allium sativum	Diallyl disulfide (40%), diallyl thiosulfate (30%), diallyl sulphide (8%), metialyl disulfide (4%) and metialyl trisulfite (10%)	0.12 (0.08–0.16)
Carapa guianensis	Limmonoids (2–5%), comoandirobine, 6α-acetoxyhepoxyazadadione, 6α-acetoxygedunin, 6β-acetoxygedunin, 11β-acetoxygedunin, 6α, 11β-acetoxygedunin, 6β, 11β- diacetoxy gedunin, 6α-hidroxygedunin, e 7-desacetoxy-7-oxogedunin	16.30 (13.60–19.00)
Syzygium aromaticum	Eugenol (92,3%) e β-caryophyllene (5,50%)	1.88 (0.36–4.14)
Zingiber officinale	Zingiberene (33%), β-sesquifelandreno (12%), β-bisabolene (10%), camphene (8%), myrcene (7%)	54.80 (39.20–70.40)
Citrus sinensis	Limonene (95.48%), myrcene (2.10%)	14.90 (12.10–17.70)
Mentha piperita	Menthol (55%), menthone (25%), methyl acetate (10%)	4.20 (2.00–6.70)
Origanum vulgare	Carvacrol (70%), p-cimene (15%), thymol (4,3%)	16.50 (13.50–19.50)
Piper nigrum	α-pinene (30%), caryophyllene (30%), limonene (10%), e-nerolidol (6%)	40.20 (32.90–47.50)
Thymus vulgaris	Thymol (50%), p-cymene (40%), linalool (6.0%)	2.10 (0.27–3.93)
Neem	Azadirachtin	0.17 (0.07–0.27)

[a] CI values are the upper and lower limits (±) of the 95% confidence intervals ($n = 18$).