



Essential oils cause detrimental effects on biological parameters of *Trichogramma galloi* immatures

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Received: 10 August 2017 / Revised: 28 November 2017 / Accepted: 7 December 2017 / Published online: 12 December 2017
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Abstract

Essential oils from botanical extracts used for insect pest control should be both effective and have low impact on natural enemies. The objective was to evaluate the effects of ten essential oils on the biological and reproductive parameters in two *Trichogramma galloi* Zucchi (Hymenoptera: Trichogrammatidae) generations. The emergence F_1 generation of *T. galloi* was reduced by more than 30% with *Allium sativum*, *Carapa guianensis*, *Citrus sinensis*, Neem and *Syzygium aromaticum*. *Zingiber officinale* reduced the *T. galloi* emergence of the F_1 and F_2 generations by between 30 and 99%. The longevity of the F_1 generation was reduced by more than 50% with *Z. officinale* at the pre-pupae and pupae stages. All oils reduced the parasitism rate of the F_1 generation in the egg-larva and pre-pupa stages. *Allium sativum*, *C. guianensis* and *C. sinensis*, Neem and *Origanum vulgare* reduced parasitism by between 30 and 79%. *Zingiber officinale* was slightly and moderately harmful to the F_1 and F_2 generations, respectively, for the *T. galloi* parasitism. The sex ratio of the two *T. galloi* generations was not affected by the essential oils. The low side effects of the oils on the F_2 generation, except for *Z. officinale* (the most harmful oil), suggest that *T. galloi* developed some post-transgenerational tolerance/resistance mechanisms to these oils and/or their components. The *A. sativum*, *C. guianensis*, *C. sinensis*, Neem, *O. vulgare*, *S. aromaticum* and *Z. officinale* oils were not selective for *T. galloi*. *Mentha piperita*, *Piper nigrum* and *Thymus vulgaris* essential oils can be included in integrated pest management programs for this parasitoid.

Keywords Immature stages · Natural products · Parasitoids · Reproductive performance · Selectivity

Key message

Communicated by M. B. Isman.

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- *Trichogramma* species are important parasitoids of agricultural and forest pest eggs. However, knowledge of side effects of botanical insecticides on these species is scarce.
- Side effects of ten essential oils were evaluated in immature *T. galloi*, and seven of them were not selective for this parasitoid by reducing the parasitism and emergence rate of the F_1 generation.
- *Zingiber officinale* oil was the most harmful for two generations of *T. galloi*.
- *Mentha piperita*, *Piper nigrum* and *Thymus vulgaris* oils were compatible with *T. galloi*.

Introduction

Parasitoids of the *Trichogramma* genus (Hymenoptera: Trichogrammatidae) parasitize eggs of agricultural and forest pests, preferably Lepidoptera (Soares et al. 2007; Wang et al. 2014a). The *Trichogramma* parasitism prevents the hosts reaching the larval stage and damages the plants (Wang et al. 2014b). Moreover, the wide geographical distribution and easy laboratory rearing facilitate the use of these natural enemies in biological control programs (Khan et al. 2015).

Trichogramma galloi Zucchi (Hymenoptera: Trichogrammatidae) is the main biological control agent of *Diatraea saccharalis* Fabricius (Lepidoptera: Crambidae) in sugarcane cultures (Isas et al. 2016). However, *Trichogramma* spp. releases are often insufficient to control the target pest, making other management practices, including pesticide applications necessary (Khan et al. 2015).

Pesticides have adverse side effects on natural enemies (Desneux et al. 2007; Plata-Rueda et al. 2017). Plant protection strategies should focus on minimizing chemical pesticide use, compatible with biological control agents (Khan et al. 2015; Mkenda et al. 2015; Alcántara-de la Cruz et al. 2017). Essential oils from botanical extracts present different biological activities, such as insecticides and antimicrobials (Tavares et al. 2010; Luiz et al. 2017), representing an alternative to chemical pesticides (Tavares et al. 2011; Cantrell et al. 2012; Gerwick and Sparks, 2014; Miresmailli and Isman 2014).

Studies into botanical pesticides highlight their compatibility with other management strategies, including biological control (González et al. 2013; Campos et al. 2016). However, some botanical pesticides are non-specific and toxic to natural enemies (Regnault-Roger et al. 2012; Ndakidemi et al. 2016). This makes the evaluation of their use in integrated pest management (IPM) programs necessary, given that botanical pesticides from citronella, eucalyptus, garlic, pyrethrum and Neem products cause malformations and mortality in beneficial insects (Raguraman and Kannan 2014; Ndakidemi et al. 2016; Zanoncio et al. 2016).

The effects of pesticides on insects are mainly evaluated using the acute median lethal dose (LD₅₀) or lethal concentration (LC₅₀) (Wang et al. 2017). However, knowledge of the side effects of botanical insecticides on natural enemies, especially *Trichogramma* species, is scarce and requires further study that takes into account their reproductive parameters (Tavares et al. 2009). The objective was to evaluate the effect of ten essential oils on the biological and reproductive parameters of *T. galloi*.

Materials and methods

Botanical essential oils and parasitoids

Trichogramma galloi females were obtained from the mass rearing of the Laboratory of Biological Control of Insects (LCBI) at the Universidade Federal de Viçosa (UFV) in Viçosa, Brazil.

The *Carapa guianensis* essential oil was obtained from EMBRAPA Amazônia Oriental (CPATU) in Belém, Pará, Brazil, while that of *Allium sativum*, *Citrus sinensis*, *Mentha piperita*, *Origanum vulgare*, *Piper nigrum*, *Syzygium aromarticum*, *Thymus vulgaris* and *Zingiber officinale* from Viessence Trade Natural Products Ltda (Porto Alegre, Brazil) extracted 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 of them manufactured (Table 1). Neem oil with 1800–2200 ppm of azadirachtin (Bioneem Tecnologia Consultoria Ind. e Comércio Ltda, Brazil) was also used.

Bioassays

Newly emerged *T. galloi* females were placed in glass tubes (8 cm high × 2 cm in diameter) coated with honey drops on the inner wall and closed with a plastic film. *Anagasta kuehniella* Zeller (Lepidoptera: Pyralidae) eggs (± 125) glued onto paper card strips (5 cm long × 0.5 cm wide) with Arabic gum diluted in distilled water (50:50) were exposed to the *T. galloi* females for 24 h. Paper cards with parasitized *A. kuehniella* eggs were immersed in essential oil solutions and ethanol (control) for 5 s and shade dried for 30 min to evaporate the solvent (Carvalho et al. 2010) at 0–24, 72–96 and 168–192 h after parasitism, corresponding to the egg-larva, pre-pupa and pupa stages of *T. galloi*, respectively. Then, the paper cards were placed in a climatic chamber (BOD) at 25 °C, relative humidity of $70 \pm 10\%$ and photoperiod of 12 h until the F₁ generation larvae emerged.

The essential oil solutions were prepared with the acute median lethal dose (LC₅₀), estimated in previous tests on *A. gemmatilis* eggs up to 48 h old (Ribeiro et al. 2015), parasitized with *T. galloi* females (Table 1). These eggs were glued onto paper cards (15 × 5 cm) sprayed with 50 mL of each essential oil or the ethanol (control) with a precision micropipette at concentrations of 1, 5, 10, 15 and 20% (v/v) (mortality from 0 to 100%).

Females from the F₁ generation were placed in glass tubes to parasitize *A. kuehniella* eggs again, in the conditions described, until the F₂ generation larvae emerged.

The experiments had a completely randomized design with 18 replications (one female and a paper card carrying

Table 1 Scientific name (Name), active ingredient (AI) and/or major component percentage (%MC), and LC₅₀ (μL mL⁻¹) tests with eggs *Anticarsia gemmatilis* (Lepidoptera: Noctuidae) in the laboratory

Name	AI and/or %MC	LC ₅₀ (CI95) ^a
<i>Allium sativum</i>	Diallyl disulfide (40%), diallyl thiosulfate (30%), diallyl sulfide (8%), metialyl disulfide (4%) and metialyl trisulfite (10%)	0.12 (0.08–0.16)
<i>Carapa guianensis</i>	Limmonoids (2–5%), comoandirobine, 6α-acetoxyhepoxazadione, 6α-acetoxygedunin, 6β-acetoxygedunin, 11β-acetoxygedunin, 6α, 11β-acetoxygedunin, 6β, 11β- diacetoxy gedunin, 6α-hidroxygedunin, e 7-desacetoxy-7-oxogedunin	16.3 (13.6–19.0)
<i>Syzygium aromarticum</i>	Eugenol (92.3%) e β-caryophyllene (5.50%)	1.88 (0.36–4.14)
<i>Zingiber officinale</i>	Zingiberene (33%), β-sesquifelandreno (12%), β-bisabolene (10%), camphene (8%), myrcene (7%)	54.8 (39.2–70.4)
<i>Citrus sinensis</i>	Limonene (95.48%), myrcene (2.10%)	14.9 (12.1–17.7)
<i>Mentha piperita</i>	Menthol (55%), menthone (25%), methyl acetate (10%)	4.2 (2.0–6.7)
<i>Origanum vulgare</i>	Carvacrol (70%), p-cimene (15%), thymol (4.3%)	16.5 (13.5–19.5)
<i>Piper nigrum</i>	α-pinene (30%), caryophyllene (30%), limonene (10%), e-nerolidol (6%)	40.2 (32.9–47.5)
<i>Thymus vulgaris</i>	Thymol (50%), p-cymene (40%), linalool (6.0%)	2.10 (0.27–3.93)
Neem	Azadirachtin	0.17 (0.07–0.27)

^aCI values are the upper and lower limits (±) of the 95% confidence intervals ($n = 18$)

parasitized eggs per tube) and were repeated twice. The emergence, longevity, parasitism and sex ratio of the F₁ generation females and those of the F₂ were then evaluated.

Essential oils were classified in toxic classes (TC) based on the reduction rates of the emergence and parasitism in the F₁ and F₂ generations: TC1 = harmless (< 30% reduction), TC2 = slightly harmful (30–79% reduction), TC3 = moderately harmful (80–99% reduction) and TC4 = harmful (> 99% reduction) according to the IOBC International Organization for Biological and Integrated Control of Noxious Animals and Plants-IOBC (Sterk et al. 1999). The parasitism and emergence reductions were calculated by the equation: % reduction = 100 – mean [(% mean of the treatment/% mean of the control) × 100].

Statistical analysis

Development and reproduction data of *T. galloi* were submitted to ANOVA. The Student–Newman–Keuls test at 5% probability was used to separate means. Statistical analysis was performed with Statistix software (version 9.0; Analytical Software, USA).

Results

Emergence of the F₁ and F₂ generations

The impact on the *T. galloi* emergence differed between the botanical oils. The pupae were the most sensitive stage for the essential oils. The lowest *T. galloi* emergence of the F₁ generation at the egg-larva stage was caused by *Citrus sinensis*, and this essential oil was classified as toxic class 2 (TC2). In the pre-pupa stage, *A. sativum*, *C. guianensis* *O.*

vulgare and *Z. officinale* oils reduced the emergence rate; however, they were classified as TC1. The emergence of *T. galloi* treated at the pupae stage was lower with *A. sativum*, *C. guianensis*, *C. sinensis*, Neem and *S. aromarticum*, and these oils were classified as TC2, while the *Z. officinale* oil as TC3. In the F₂ generation, the emergence of *T. galloi* was reduced by more than 30% by *Z. officinale* in all biological stages of this parasitoid evaluated, being the most harmful oil (TC2) (Table 2).

Longevity of the F₁ generation females

The longevity of *T. galloi* females from the F₁ generation ranged from 7.3 to 10.8, 4.7 to 10.9 and 3.0 to 10.3 days in the egg-larva, pre-pupae and pupae stages, respectively. The lowest longevity rate of *T. galloi* females was caused by *Z. officinale* oil in the pre-pupae and pupae stages. This parameter was not affected by the other nine essential oils compared to the control (Table 3).

Parasitism of females of the F₁ and F₂ generations

Parasitism of the F₁ *T. galloi* generation treated during the egg-larva and pre-pupa stages was lower with all oils tested compared to the control. The egg-larva and pre-pupae were the developmental stages most susceptible to essential oils in this generation. *Carapa guianensis*, *C. sinensis* and *Z. officinale* reduced parasitism rates of *T. galloi* females by more than 30% (TC2) in the egg-larva and pre-pupa stages, and *A. sativum*, Neem and *O. vulgare* oils in the pre-pupa stage of this parasitoid. In the F₂ generation, the *Z. officinale* oil reduced the parasitism by the *T. galloi* females, being more harmful during the pupa (TC3) than in egg-larva and pre-pupa stages (TC2) (Table 4).

Table 2 Emergence percentage and toxic class (TC) of different immature stages of the F₁ and F₂ *Trichogramma galloi* (Hymenoptera: Trichogrammatidae) from *Anagasta kuehniella* (Lepidoptera: Pyralidae) eggs

Treatments	Generation F ₁						Generation F ₂					
	Egg-larva	TC	Pré-pupae	TC	Pupae	TC	Egg-larva	TC	Pré-pupae	TC	Pupae	TC
Control	98.2 ± 1.2 ^A	–	97.5 ± 1.3 ^A	–	98.9 ± 1.6 ^A	–	99.1 ± 1.3 ^A	–	99.1 ± 1.2 ^A	–	99.7 ± 1.5 ^A	–
<i>Allium sativum</i>	84.0 ± 2.1 ^{Aa}	1	75.0 ± 1.9 ^{Bab}	1	61.9 ± 2.2 ^{BCb}	2	88.6 ± 1.2 ^A	1	92.6 ± 1.9 ^A	1	84.6 ± 2.2 ^A	1
<i>Carapa guianensis</i>	76.4 ± 1.7 ^{ABa}	1	74.9 ± 1.2 ^{Ba}	1	55.7 ± 1.5 ^{Cb}	2	92.6 ± 1.6 ^A	1	93.4 ± 1.4 ^A	1	85.7 ± 2.0 ^A	1
<i>Citrus sinensis</i>	69.3 ± 2.9 ^{Bb}	2	88.2 ± 1.8 ^{Aa}	1	56.2 ± 2.0 ^{Cb}	2	86.7 ± 2.1 ^A	1	82.8 ± 2.2 ^A	1	75.0 ± 2.4 ^A	1
<i>Mentha piperita</i>	93.6 ± 1.2 ^{Aa}	1	84.1 ± 2.0 ^{ABa}	1	76.4 ± 1.8 ^{Bb}	1	85.7 ± 1.9 ^A	1	81.5 ± 2.3 ^A	1	90.2 ± 3.3 ^A	1
Neem	88.6 ± 1.4 ^{Aa}	1	82.3 ± 1.8 ^{ABa}	1	53.8 ± 2.0 ^{Cb}	2	84.8 ± 1.3 ^A	1	84.6 ± 2.0 ^A	1	92.5 ± 1.6 ^A	1
<i>Origanum vulgare</i>	88.5 ± 2.2 ^{Aa}	1	73.4 ± 1.7 ^{Bb}	1	72.2 ± 1.4 ^{BCb}	1	90.3 ± 2.3 ^{Aa}	1	86.7 ± 2.2 ^{Aa}	1	70.1 ± 3.7 ^{ABb}	1
<i>Piper nigrum</i>	91.9 ± 1.2 ^{Aa}	1	86.9 ± 2.6 ^{Aa}	1	73.7 ± 1.4 ^{BCb}	1	86.3 ± 3.1 ^{Aab}	1	89.8 ± 2.1 ^{Aab}	1	97.6 ± 1.5 ^{Ab}	1
<i>Syzygium aromaticum</i>	92.9 ± 2.0 ^{Aa}	1	79.5 ± 2.1 ^{ABb}	1	65.0 ± 1.6 ^{BCc}	2	90.2 ± 2.7 ^{Aa}	1	75.3 ± 3.2 ^{Ab}	1	80.7 ± 3.5 ^{Ab}	1
<i>Thymus vulgare</i>	93.7 ± 1.1 ^{Aa}	1	89.0 ± 1.5 ^{Aa}	1	70.1 ± 2.0 ^{BCb}	1	83.5 ± 2.3 ^{Aab}	1	90.3 ± 2.2 ^A	1	85.9 ± 2.4 ^A	1
<i>Zingiber officinale</i>	81.4 ± 1.7 ^{Aab}	1	73.1 ± 2.2 ^{Ba}	1	17.3 ± 1.3 ^{Db}	3	53.1 ± 2.5 ^{Ba}	2	58.5 ± 2.7 ^{Ba}	2	26.5 ± 2.6 ^{Bb}	2

Means followed by the same uppercase per column or lowercase per row does not differ by Student–Newman–Keuls test ($p < 0.05$). Toxic class (Sterk et al. 1999): TC1 = innocuous; TC2 = slightly harmful; TC3 = moderately harmful; TC4 = harmful. ± Standard error ($n = 18$)

Table 3 Longevity (days) of *Trichogramma galloi* (Hymenoptera: Trichogrammatidae) F₁ generation females exposed to essential oils in different immature stages

Treatments	Egg-larva	Pré-pupae	Pupae
Control	10.8 ± 0.5	10.9 ± 0.6 ^A	9.1 ± 0.5 ^A
<i>Allium sativum</i>	9.9 ± 0.5	10.3 ± 0.6 ^A	10.3 ± 0.6 ^A
<i>Carapa guianensis</i>	9.2 ± 0.9	9.9 ± 0.6 ^A	9.7 ± 0.9 ^A
<i>Citrus sinensis</i>	7.4 ± 0.8	8.7 ± 0.8 ^A	6.9 ± 1.0 ^A
<i>Mentha piperita</i>	8.7 ± 0.9	7.5 ± 1.1 ^A	7.0 ± 1.0 ^A
Neem	10.3 ± 0.6	10.1 ± 0.8 ^A	10.3 ± 0.9 ^A
<i>Origanum vulgare</i>	9.2 ± 0.8	9.1 ± 0.6 ^A	7.8 ± 1.0 ^A
<i>Piper nigrum</i>	9.2 ± 0.6	7.6 ± 0.8 ^A	7.4 ± 0.7 ^A
<i>Syzygium aromaticum</i>	10.3 ± 0.5	9.7 ± 0.8 ^A	7.9 ± 0.6 ^A
<i>Thymus vulgare</i>	7.3 ± 0.9	8.2 ± 0.8 ^A	7.0 ± 0.8 ^A
<i>Zingiber officinale</i>	8.4 ± 0.9 ^a	4.7 ± 1.2 ^{Bb}	3.0 ± 1.0 ^{Bb}

Means followed by the same uppercase per column or lowercase per row do not differ by Student–Newman–Keuls test ($p < 0.05$). ± Standard error ($n = 18$)

Sex ratio in the F₁ and F₂ generations

The F₁ and F₂ generation sex ratio of *T. galloi* showed no reduction when the *A. kuehniella* eggs with the egg-larva, pupa and pre-pupa stages of this parasitoid were dipped into the essential oils, except for *O. vulgare* and *S. aromaticum* in the egg-larva stage of the F₂ generation individuals (Table 5).

Discussion

The reduction in the emergence of the *T. galloi* F₁ generation with *A. sativum*, *C. guianensis*, *C. sinensis*, Neem, *O. vulgare*, *S. aromaticum* and *Z. officinale*, especially when applied during the pupa stage demonstrated the side effects of these oils on *T. galloi* immatures. Some compounds of these oils can penetrate the host corion, acting upon the nervous system by inhibiting the acetylcholinesterase enzyme and causing involuntary movements such as convulsions, followed by paralysis and death (Agarwal et al. 2001; González et al. 2013). In addition, compounds such as 6a-acetoxiepoxiazadiradione, 6a-acetoxypedunin, 6b-acetoxypedunin, 11b-acetoxypedunin, 6a, 11b-diacetoxypedunin, 6b, 11b-diacetoxypedunin, 6a-hydroxyl-gedunin and 7-deacetoxyl-7-oxogedunin of *C. guianensis* (Prophiro et al. 2012), carvacrol and p-cymene of *O. vulgare* (Khalfi et al. 2008), and dehydrozingerone and dehydroshogaol and the mixture of dehydrozingerone and zingerone of *Z. officinale* oils (Agarwal et al. 2001) act as growth regulators that can also explain the emergence reduction in the *T. galloi* F₁ generation. The compounds from *Z. officinale* (dehydrozingerone, dehydroshogaol and zingerone) increased the duration of each larva and pupa stage of *Ceraeochrysa claveri* Navás (Neuroptera: Chrysopidae) (Scudeler et al. 2016), impeding molting and causing morphological anomalies and mortality, as well as the nymphal *Nezara viridula* Linnaeus (Hemiptera: Pentatomidae) stages, particularly during molting (González et al. 2011). Similar developmental abnormalities were observed for *Aedes aegypti* Linnaeus (Diptera: Culicidae) with *C. guianensis* (Prophiro et al. 2012); *Musca domestica* Linnaeus (Diptera: Mucidae) with *M. piperita* (Kumar et al. 2011) and *Spodoptera litura* Fabricius

Table 4 Number of eggs of *Anagasta kuehniella* (Lepidoptera: Pyralidae) parasitized by females of the F₁ and F₂ of *Trichogramma galloi* (Hymenoptera: Trichogrammatidae) treated in different immature stages and toxic class (TC)

Treatments	Generation F ₁						Generation F ₂					
	Egg-larva	TC	Pré-pupae	TC	Pupae	TC	Egg-larva	TC	Pré-pupae	TC	Pupae	TC
Control	29.9 ± 1.3 ^{Aab}	–	33.7 ± 1.3 ^{Aa}	–	34.2 ± 1.6 ^a	–	31.1 ± 1.3 ^A	–	32.4 ± 1.3 ^A	–	32.3 ± 1.5 ^A	–
<i>Allium sativum</i>	22.2 ± 2.3 ^{BCb}	1	22.7 ± 2.0 ^{Bb}	2	29.2 ± 2.1 ^a	1	29.5 ± 1.6 ^A	1	28.8 ± 1.9 ^A	1	27.6 ± 2.3 ^A	1
<i>Carapa guianensis</i>	16.4 ± 1.8 ^{Db}	2	22.4 ± 1.8 ^{Ba}	2	26.5 ± 1.6 ^a	1	31.0 ± 1.5 ^A	1	26.6 ± 1.3 ^A	1	27.2 ± 2.2 ^A	1
<i>Citrus sinensis</i>	19.1 ± 3.0 ^{CDb}	2	21.9 ± 2.0 ^{Bb}	2	27.8 ± 2.8 ^a	1	30.9 ± 1.9 ^A	1	27.9 ± 2.7 ^A	1	26.0 ± 2.6 ^A	1
<i>Mentha piperita</i>	24.9 ± 1.3 ^{AB}	1	24.6 ± 1.7 ^B	1	25.7 ± 2.1	1	28.8 ± 1.9 ^A	1	25.9 ± 2.3 ^A	1	25.3 ± 3.4 ^A	1
Neem	22.9 ± 1.4 ^{BC}	1	23.4 ± 1.8 ^B	2	27.8 ± 1.9	1	30.5 ± 1.8 ^A	1	28.8 ± 2.2 ^A	1	27.6 ± 1.6 ^A	1
<i>Origanum vulgare</i>	22.4 ± 2.5 ^{BCb}	1	22.1 ± 2.1 ^{Bb}	2	27.9 ± 1.5 ^a	1	24.9 ± 2.4 ^A	1	30.0 ± 2.3 ^A	1	23.4 ± 3.8 ^A	1
<i>Piper nigrum</i>	31.0 ± 1.1 ^{Aa}	1	24.7 ± 2.7 ^{Bb}	1	29.8 ± 1.0 ^a	1	26.3 ± 3.2 ^A	1	28.8 ± 2.1 ^A	1	32.9 ± 1.5 ^A	1
<i>Syzygium aromaticum</i>	25.9 ± 2.1 ^{ABab}	1	24.0 ± 2.4 ^{Bb}	1	30.4 ± 1.7 ^a	1	32.3 ± 2.6 ^A	1	24.9 ± 3.3 ^A	1	27.9 ± 2.9 ^A	1
<i>Thymus vulgare</i>	26.0 ± 1.0 ^{AB}	1	26.1 ± 1.4 ^B	1	27.1 ± 2.2	1	29.4 ± 2.6 ^A	1	29.8 ± 1.9 ^A	1	24.2 ± 2.5 ^A	1
<i>Zingiber officinale</i>	18.9 ± 1.8 ^{CDb}	2	23.3 ± 2.1 ^{Bab}	2	26.2 ± 2.2 ^a	1	16.3 ± 3.0 ^{Ba}	2	14.3 ± 2.7 ^{Ba}	2	6.7 ± 2.6 ^{Bb}	3

Means followed by the same uppercase per column or lowercase per row does not differ by Student–Newman–Keuls test ($p < 0.05$). Toxic class (Sterk et al. 1999): TC1 = innocuous; TC2 = slightly harmful; TC3 = moderately harmful; TC4 = harmful. ± Standard error ($n = 18$)

Table 5 Sex ratio of the F₁ and F₂ individual of *Trichogramma galloi* (Hymenoptera: Trichogrammatidae) coming from *Anagasta kuehniella* (Lepidoptera: Pyralidae) treated with parasitoids in different immature stages

Treatments	Generation F ₁			Generation F ₂		
	Egg-larva	Pré-pupae	Pupae	Egg-larva	Pré-pupae	Pupae
Control	0.67 ± 0.07 ^A	0.69 ± 0.07	0.67 ± 0.08	0.50 ± 0.08 ^{AB}	0.56 ± 0.07	0.51 ± 0.08
<i>Allium sativum</i>	0.48 ± 0.07 ^{AB}	0.51 ± 0.08	0.47 ± 0.08	0.61 ± 0.06 ^{ABa}	0.61 ± 0.05 ^a	0.48 ± 0.08 ^b
<i>Carapa guianensis</i>	0.45 ± 0.07 ^{AB}	0.47 ± 0.08	0.46 ± 0.09	0.63 ± 0.06 ^{Aa}	0.52 ± 0.08 ^b	0.63 ± 0.05 ^a
<i>Citrus sinensis</i>	0.47 ± 0.06 ^{AB}	0.43 ± 0.08	0.48 ± 0.08	0.46 ± 0.09 ^{AB}	0.50 ± 0.07	0.46 ± 0.07
<i>Mentha piperita</i>	0.48 ± 0.09 ^{AB}	0.44 ± 0.09	0.45 ± 0.08	0.55 ± 0.06 ^{AB}	0.40 ± 0.07	0.46 ± 0.06
Neem	0.48 ± 0.07 ^{AB}	0.44 ± 0.08	0.43 ± 0.09	0.60 ± 0.04 ^{ABa}	0.49 ± 0.07 ^b	0.46 ± 0.08 ^b
<i>Origanum vulgare</i>	0.69 ± 0.05 ^{Aa}	0.57 ± 0.07 ^{ab}	0.52 ± 0.07 ^{ab}	0.45 ± 0.08 ^{Bb}	0.63 ± 0.06 ^a	0.45 ± 0.06 ^b
<i>Piper nigrum</i>	0.41 ± 0.07 ^{AB}	0.45 ± 0.08	0.48 ± 0.08	0.47 ± 0.07 ^{AB}	0.45 ± 0.07	0.46 ± 0.08
<i>Syzygium aromaticum</i>	0.49 ± 0.07 ^{AB}	0.50 ± 0.07	0.52 ± 0.07	0.41 ± 0.08 ^{Bb}	0.49 ± 0.08 ^{ab}	0.65 ± 0.04 ^a
<i>Thymus vulgare</i>	0.42 ± 0.08 ^{AB}	0.48 ± 0.08	0.47 ± 0.08	0.53 ± 0.06 ^{AB}	0.55 ± 0.06	0.56 ± 0.06
<i>Zingiber officinale</i>	0.45 ± 0.08 ^{AB}	0.47 ± 0.08	0.51 ± 0.09	0.48 ± 0.06 ^{ABab}	0.61 ± 0.07 ^a	0.43 ± 0.05 ^{ab}

Means followed by the same uppercase per column or lowercase per row does not differ by Student–Newman–Keuls test ($p < 0.05$). ± Standard error ($n = 18$)

(Lepidoptera: Noctuidae) with Neem (Packiam and Ignaci-muthu 2012). Reduction in emergence due to essential oils was also reported for *Spodoptera frugiperda* Smith (Lepidoptera: Noctuidae) (Tavares et al. 2009) with Neem; *Ephestia kuehniella* Zeller (Lepidoptera: Pyralidae), *Sitophilus zeamais* Motschulsky (Coleoptera: Curculionidae) and *Tribolium castaneum* Herbst (Coleoptera: Tenebrionidae) with *A. sativum* and *Z. officinale* (Agarwal et al. 2001; Mikhael 2011); *Alphitobius diaperinus* Panzer (Coleoptera: Tenebrionidae) and *Rhipicephalus microplus* Canestrini (Acari: Ixodidae) with *O. vulgare* (Mendes et al. 2011).

The lowest longevity of F₁ generation *T. galloi* females with *Z. officinale* agrees with the lowest value of the

parasitoid emergence treated during pre-pupa and pupa stages. Terpenoids, such as sesquiterpene hydrocarbons (50–60%), oxygenated sesquiterpenes (17%), monoterpene hydrocarbons and oxygenated monoterpenes of *Z. officinale* may not kill (Agarwal et al. 2001); however, they induced sublethal effects by acting on the nervous and neuromuscular systems or caused deformations in immature and adult insects. These compounds may alter the morphological and physiological characteristics, such as reducing the size of parasitoids and predators (Ahmad et al. 2003; Charleston et al. 2005), and causing deformities or abnormalities in the adult gut (Singh et al. 2008), which could jeopardize the *T. galloi* longevity. The side effects caused by essential oils

are very variable among non-target species. For example, *Z. zerumet* was at least ten times more toxic for *Anisopteromalus calandrae* (Howard) than *T. deion* (Pinto and Oatman) (Suthisut et al. 2011). *Ferula assafoetida* essential oil reduced drastically the longevity of *T. embryophagum* (Hartig) and *T. evanescens* (Westwood) (Poorjavad et al. 2014), so it was strongly suggested not to apply this oil and make wasp release simultaneously.

The lower parasitism rate of F_1 *T. galloi* generation females by the *A. sativum*, *C. guianensis*, *C. sinensis*, Neem, *O. vulgare* and *Z. officinale* essential oils in the egg-larva and pre-pupa stages, and *Z. officinale* in the egg-larval, pupa and pre-pupa stages of the F_2 generation resulted from the sublethal effects on the immature stages of this parasitoid. *Carapa guianensis*, *C. sinensis* and *O. vulgare* have ovicidal and larvicidal actions inducing malformations in *A. aegypti*, *M. domestica* and *Amblyomma cajennense* Fabricius (Ixodidae: Ixodida) adults (Mendes et al. 2011; Kumar et al. 2012; Prophiro et al. 2012), as well as reducing egg laying and increasing the number of infertile eggs. Malformation in the adult reduces efficiency, physical condition and reproduction as observed for *Rhynocoris kumarii* Ambrose and Livingston (Hemiptera: Reduviidae) (George and Ambrose 2004). Azadirachtin acts as an insect growth regulator (Zanuncio et al. 2016), interfering in biochemical and physiological functions (Campos et al. 2016) and affecting the parasitism capacity of *Uscana lariophaga* Steffan (Hymenoptera: Trichogrammatidae), *Dinarmus basalis* Rondani (Hymenoptera: Pteromalidae) (Boeke et al. 2003), *Eretmocerus rui* Zolnerowich and Rose (Hymenoptera: Aphelinidae) (Hammad and McAuslane 2006) and *T. pretiosum* Riley (Hymenoptera: Trichogrammatidae) (Correia et al. 2013). For *Z. officinale*, the low parasitism rate of *T. galloi* in the two generations was the most important characteristics and explained the reduced emergence rate with this oil. Compounds such as gingerols, dehydroshogaol and phenolic alkenones of *Z. officinale* disturb the metabolism of the epithelial membrane by affecting the ecdysone-dependent production on the Cyt-P450 and monooxygenases actions (Singh et al. 2008; Cui et al. 2016). This inhibits the chitin synthesis system and the antioxidant enzyme by affecting insect growth and reproduction with intestinal and endocrinal disorders (Agarwal et al. 2001).

The parasitism rates of *Dinarmus basalis* (Rond.) (Hymenoptera: Pteromalidae) were reduced by more than 80% with sublethal doses of *Artemisia herba-alba* and *A. campestris* oils, whereas for *Triaspis luteipes* (Thompson) (Hymenoptera: Braconidae), this parameter was only reduced by 20–35% under the same experimental conditions (Titouhi et al. 2017). Already, *Piper aduncum* oil had no side effects in *Telenomus podisi* (Ashmead) (Hymenoptera: Platygasteridae) and *Trissolcus urichi* (Crawford) (Hymenoptera: Platygasteridae) (Turchen et al. 2016), evidencing different

susceptibility levels and toxicity among parasitoid species and essential oils, respectively.

Allium sativum, *C. guianensis*, *C. sinensis*, Neem, *O. vulgare* and *S. aromaticum* reduced the emergence and parasitism of the F_1 *T. galloi* generation females by 30–79%. However, these parameters were not affected in the F_2 generation, suggesting that *T. galloi* individuals developed some post-transgenerational tolerance/resistance mechanism to these oils and/or their components as observed for resistance to Neem oil (Feng and Isman 1995). The resistance mechanisms are at least as diverse as those involved with chemical insecticides (Siegwart et al. 2015), and they could be detoxified mainly by the Cyt-P450 (Cui et al. 2016). Further research is required to determine the possible mechanism involved in *T. galloi* post-transgenerational tolerance to these oils. In addition, given that the essential oils of these plants did not lead to evident side effects for subsequent *T. galloi* generations, the possibility of assuming a performance reduction in the F_1 generation of this parasitoid caused from the essential oils following the first application should be evaluated.

A similar sex ratio in the F_1 and F_2 generations of *T. galloi* agrees with the finding that this parameter varies more commonly due to food and host quality or the bacterium *Wolbachia* in the *Trichogramma* parasitoids (Heimpel and Boer 2008). The sex ratio above 0.5 in the F_1 and F_2 generations is important to conserve the parasitoid *T. galloi* in classical biological control programs (Vianna et al. 2009).

The main market niche of plant essential oils is the organic food production (Isman et al. 2011), and these products are an economically viable alternative for small farmers to control pests (Mkenda et al. 2015). However, the use of non-selective natural products, authorized in these cropping systems, may not be compatible with biological control agents (Silva and Bueno, 2015), as well as most essential oils used here that were not selective for *T. galloi* immatures. In addition, the possible phytotoxic effects on the crop(s) of interest should also be evaluated, because for example, *P. nigrum* did not cause damage on *T. galloi* immatures, but there is evidence that sarmentine from *Piper* species has potential bioherbicidal effects (Dayan et al. 2015).

Conclusions

Allium sativum, *C. guianensis*, *C. sinensis*, Neem, *O. vulgare* and *S. aromaticum* reduced the emergence and parasitism of the F_1 *T. galloi* generation by 30–79%. *Zingiber officinale* oil was the most harmful to this parasitoid, reducing the longevity of the F_1 females and the emergence and parasitism of the F_1 and F_2 generations by 30–99%, showing that this oil is not compatible with this parasitoid in IPM programs. *Mentha*

piperita, *Piper nigrum* and *T. vulgaris* exerted a low impact on the biological aspects of immature *T. galloi*.

Author contribution statement

DSP, JCZ and JES designed the research; DSP and RAC performed the experiments; DSP, RAC and JES analyzed the data and wrote the draft. DSP, RAC, GSR, GLDL, JCZ, LRB, PGL and JES wrote, corrected and approved the manuscript.

Acknowledgements We thank to the Dr. Phillip Villani (University of Melbourne) revised and corrected the English language used in this manuscript.

Funding This study was funded by the “Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq)”, “Coordenação de Aperfeiçoamento de Pessoal de Nível Superior (CAPES)”, “Fundação de Amparo à Pesquisa do Estado de Minas Gerais (FAPEMIG)” and the “Programa Cooperativo sobre Proteção Florestal/PROTEF do Instituto de Pesquisas e Estudos Florestais/IPEF”.

Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

Human and animal rights This article does not contain any studies with human participants or animals (other than insects) performed by any of the authors.

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