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ORIGINAL ARTICLE

Effect of *Eucalyptus citriodora* and *Schinus terebinthifolius* essential oils on the diamondback moth and its parasitoid *Trichogramma pretiosum*

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Abstract

Essential oils (EOs), from plants' secondary metabolism, present bioactive compounds that may have insecticidal activity. Their use associated with parasitoid hymenoptera can be an alternative, however, little is known about the impact of EOs on the diamondback moth, *Plutella xylostella* and parasitoids. This work aimed to evaluate the effect of oils from *Eucalyptus citriodora* (EOEC) and *Schinus terebinthifolius* (EOST) (0.5% and 1%) on eggs and larvae of *P. xylostella* and on *Trichogramma pretiosum*. *P. xylostella* eggs were immersed in solutions containing EOs and in water (control). Treated cabbage leaf discs were offered to larvae. Eggs with EOs were offered to *T. pretiosum* females to evaluate parasitism and emergence. Already parasitized *P. xylostella* eggs were immersed in the treatments to observe the effect on the emergence of parasitoids. The survival of *T. pretiosum* adults exposed to EOs was recorded. The average number of inviable eggs was higher in treatments with EOs, ranging from 6.9 ± 1.31 to 13.0 ± 1.12 than in the control (3.5 ± 0.32) ($p < 0.05$). The average number of dead caterpillars was higher in treatments with EOs, ranging from 1.4 ± 0.24 to 2.2 ± 0.19 than in the control (0.1 ± 0.06) ($p < 0.05$) Leaf consumption in 48 h did not differ between the EOST treatment (0.05) (5.42%) and the control (9.7%). For the other treatments it was significantly lower: 3.8% (EOEC 0.5), 1.6 (EOEC 1), and 3.4 (EOST 1). Eggs treated with EOs prior to exposure to the parasitoid had lower parasitism rates than eggs that were already parasitized and subsequently treated with EOs. The mortality of adults exposed to EOs was 3% to 6%. The EOs of *E. citriodora* and *S. terebinthifolius* were toxic to eggs and larvae of *P. xylostella*, acted as feeding deterrents, and had minimal impact on parasitoids. As a result, they are promising for use in IPM.

Keywords: bioactive plants, biological control, *Plutella xylostella*, Trichogrammatidae, botanical insecticides

Introduction

The diamondback moth, *Plutella xylostella* (Linnaeus) (Lepidoptera: Plutellidae), is an important pest of brassicas, originating from the Mediterranean region (Yang *et al.* 2015). Due to its migratory capacity and plasticity in adapting to different climatic conditions, it has become a cosmopolitan pest (Saeed *et al.* 2010). Chemical control of *P. xylostella* typically involves using insecticides such as pyrethroids (e.g., deltamethrin), organophosphates, and neonicotinoids (Banazeer *et al.* 2021). One of the major disadvantages is the rapid development of resistance in *P. xylostella* populations. Over time, the consistent use of the same insecticides results in genetic mutations in the moth, reducing their susceptibility to these chemicals. Furthermore, these insecticides can negatively impact non-target organisms, including beneficial insects (Shehzad *et al.* 2023).

Biopesticides have emerged as an alternative because they are less toxic to the environment, have lower persistence, and have higher selectivity, thus they are recognized as ecofriendly (Walia *et al.* 2017). Several

EOs and their components have been researched for their biocidal and repellent properties against insects, arthropods, nematodes, larvae, and other pests, showing promising results (Garrido-Miranda *et al.* 2022; Gupta *et al.* 2023; Ayllón-Gutiérrez *et al.* 2024; Vivekanandhan *et al*. 2024). Regarding *P. xylostella*, Da Câmara *et al*. (2015) observed that the essential oil of *Citrus reticulata* (Blanco) (Rutaceae) showed insecticidal activity for larvae of the moth. Furthermore, exposure to the oil of *Corymbia citriodora* (Hook) (Myrtaceae) resulted in an 80% mortality rate of *P. xylostella* larvae (Filomeno *et al.* 2017). EOs can also result in feeding deterrence of third instar larvae when in contact with cabbage discs treated with oil of *Ocimum basilicum* (Linnaeus) (Lamiaceae) (86.73%) and *Acorus calamus* (Acoraceae) (82.61%) (Song *et al*. 2022).

Trichogramma pretiosum Riley (Hymenoptera: Trichogrammatidae) is an endoparasitoid indicated for the biological control of *P. xylostella*, due to its high parasitism and emergence capacity in the eggs of this moth. In semi-field tests on kale culture, (Massaroli *et al*. 2021) showed that about 57.65% of *P. xylostella* eggs were parasitized by *T. pretiosum*. Few studies have evaluated the effects of essential oils on trichogrammatids. Experiments with the essential oil of *Leptospermum petersonii* (F.M. Bailey) (Myrtaceae) showed low toxicity to adult *T. pretiosum* when exposed to plants treated with this oil. However, a reduction in the longevity of parasitoids emerging from eggs treated with this essential oil compared to the control was observed (Purwatiningsih *et al*. 2012). Parreira *et al*. (2019) found that the integrated use of *T. pretiosum* with the essential oils of *Azadirachta indica* (A. Juss.) (Meliaceae), *Syzygium aromaticum* (Merrill & Perry) (Myrtaceae), *Citrus sinensis* (Osbeck) (Rutaceae), *Piper nigrum* (Linnaeus) (Piperaceae), and *Thymus vulgaris* (Linnaeus) (Lamiaceae) did not affect parasitism and parasitoid emergence.

Among the diversity of plants that produce EOs and have insecticidal and/or repellent action are pink pepper *Schinus terebinthifolius* Raddi (Anacardiaceae) and lemon-scented eucalyptus, *Eucalyptus citriodora* Hook (Myrtaceae) (Filomeno *et al*. 2017; Hussein *et al*. 2017). The insecticidal activity of *S. terebinthifolius* EO has been reported in the cereal weevil *Rhyzopertha dominica* (Fabricius) (Coleoptera: Bostrichidae) (Nascimento *et al*. 2018) and the bean weevil *Callosobruchus maculatus* (Fabricius) (Coleoptera: Bostrichidae) (De Oliveira *et al*. 2017). *Eucalyptus globulus* (Labill) (Myrtaceae) essential oil produces a very similar level of larval mortality of *Anopheles stephensi* (Liston) (Diptera: Culicidae), *Aedes aegypti* (Linnaeus) (Diptera: Culicidae) and *Culex quinquefasciatus* (Say) (Diptera: Culicidae) compared with chemical insecticide Temephos at 24 h and 48 h post-treatment (Vivekanandhan *et al*. 2019). The essential oil of *E. citriodora* leaf was effective in the control and repellency of the maize weevil *Sitophilus zeamais* (Motschulsky) (Coleoptera: Curculionidae) (Mardiningsih and Rizal 2022).

This work aimed to evaluate the effects of the essential oils of pink pepper and lemon-scented eucalyptus on eggs and larvae of *P. xylostella* and its parasitoid *T. pretiosum* under laboratory conditions.

Materials and Methods

The bioassays and insect rearing were conducted under controlled conditions (25 ± 1°C, 60 ± 10% relative humidity, 12:12 photoperiod) at the Laboratory of Biological Insect Control (CBLab), Faculty of Agronomy at the Federal University of Rio Grande do Sul (UFRGS), Brazil, from April 1, 2020, to January 28, 2022.

Cabbage cultivation

Green cabbage seedlings *B. oleraceae* var. *acephala* were commercially obtained and transplanted to a bed in the didactic and experimental vegetable garden of the Department of Horticulture and Forestry at the Faculty of Agronomy (FAGRO) at UFRGS (30°4'28.04"S and 51°8'6.87"W). Management was carried out without chemical inputs and with manual irrigation when necessary. The plants were used to obtain *P. xylostella* in natural infestation and the leaves were for maintaining the rearing in the laboratory and experiments. Green cabbage cultivation occurred continuously, from April 1st, 2020, to January 28th, 2022.

Plutella xylostella rearing

The rearing of P. xylostella began from individuals collected from an organic production of Brassica oleracea var. acephala in the experimental garden of FAGRO/UFRGS, where there was a natural infestation. Larvae were housed in crystal polystyrene boxes Gerbox®, with an opening in the lid covered with voile-type fabric for ventilation (11 × 11 × 3.5 cm) and kept in an incubator, receiving cabbage leaves for feeding. Upon reaching the adult stage, the moths were placed in a wooden cage (51 × 27 × 51 cm), lined with voile fabric. Two Petri dishes (8 cm diameter × 1.5 cm) lined with filter paper moistened with distilled water and cabbage discs (9 cm diameter) (substrate for oviposition) and a plate with cotton moistened with a honey and water solution at 10% (feeding substrate) were placed in each cage. Subsequently, the cabbage discs containing *P. xylostella* eggs were placed in plastic pots (16 × 25 × 8 cm) containing filter paper at the bottom, moistened with distilled water and fresh cabbage leaves for feeding the larvae. The pupae were transferred to

a 250 ml plastic pot until emergence, with the adults being released again into the cage. The rearing of *P. xylostella* occurred concurrently with the cultivation period of green cabbage, with new organisms being introduced throughout the year.

Trichogramma pretiosum rearing

The parasitoids were obtained from Koppert Biological Systems Brazil. *T. pretiosum* adults were kept in 250 ml Becker-type bottles and fed with droplets of pure honey deposited on the walls of the bottles, which were sealed with parafilm and kept in a BOD incubator.

Ephestia kuehniella (Zeller) (Lepidoptera: Pyralidae) eggs, irradiated with ultraviolet light (Philips® UV 15 W) for about 50 min and fixed on light blue cardboard (180 g) with 10% gum arabic, were used for parasitoid multiplication. Daily, *E. kuehniella* egg cards were offered to the parasitoids. The rearing of *E. kuehniella* was maintained using a diet based on wheat flour (97%) and brewer's yeast (3%).

Essential oil extraction

Mature pink pepper fruits (*Schinus terebinthifolius*) were collected in the agronomy neighborhood of Porto Alegre, RS (30°02'48"S and 51°08'17"W). Fresh lemon-scented eucalyptus leaves (*Eucalyptus citriodora*) were collected in the municipality of Ivoti, RS (29°36'00"S and 51°09'13.68"W). Both were taken from plants without phytosanitary treatment. The pink pepper fruits were dried at room temperature (25 ± 3°C) for 72 h, and the extraction was performed immediately after this period. After collection, the lemon-scented eucalyptus leaves were placed in plastic bags and refrigerated (4°C) until the extraction, approximately 1 week.

Extraction was carried out by hydrodistillation (steam distillation) (AOAC 1992), using a Clevenger apparatus from Êxodo Tecnologia® model 3000, coupled to a 3 l round bottom volumetric flask. To increase the surface area of the plant materials, pink pepper fruits (300 g) and fresh lemon-scented eucalyptus leaves (200 g) were previously crushed using a blender. Each material was placed separately inside the flask where 1.5 l of distilled water was added. After 3 h of hydrodistillation (Oliveira Junior *et al.* 2013), the essential oil of each species was separated from the liquid phase (hydrolate) with the aid of a 10 ml graduated glass pipette and suction pump. The essential oils were stored in amber glass containers at 4°C for later use during the experiments. The essential oil extractions occurred between April and May 2020. The chromatographic profiles and chemical compositions of essential oils were analyzed by the researcher Márcia Ortiz M. Marques at the Agronomic Institute of Campinas/(IAC) in São Paulo, Brazil (Tables 1–3). To analyze the EOs, 1 µl of solution (1 µl of the essential oil in 1 ml of ethyl acetate-chromatographic grade) was injected for each oil into a GC-DIC (Shimadzu CG-2010, DB-5 column, i.d. 30 m × 0.25 mm, 0.25 µm film; Jand & W Scientific, Folsom, CA, USA) through an injector (AOS-Shimadzu), split mode (1/20) and helium as carrier gas. The initial ramp temperature was 60°C for 1 min with a gradual increase of 3°C · min^{-1} to 240°C. The detector temperature was 300°C. Data were collected using Class-CG software and processed with Origin 5.0 software (Originlab Coorporation, Northamptom, MD, USA). For qualitative analysis, selected samples were injected into a gas chromatograph coupled to mass spectrometry (Shimadzu QP-5000 GC-MS) with electron impact ionization (ionization energy 70 eV) with a quadrupole analyzer. The extracts were injected in split mode, 1/20, and helium was used as carrier gas. A fused silica capillary column was used: OV-5 (Ohio Valley Specialty Chemical, Inc. 30.0 m × 0.25 mm × 0.25 µm). The temperature program and column were identical to those used in GC analysis. The substances were identified by comparing their mass spectra with the database of the GC-MS system (Nist62.LIB, 288 Wiley139.LIB) and literature and by comparing their calculated linear retention indices (LRIs). A mixture of n-alkanes (C9-C24 Sigma Aldrich 99%), analyzed under the same operational conditions as the essential oils, was used to calculate the linear retention indices (LRIs), applying the equation of Van

Table 1. Chemical composition (relative %) of the essential oil of *Schinus terebinthifolius*

Peak	Retention time [min]	LRI	LLRI	Compound	Relative [%]
1	6.688	931	932	α-pinene	5.38
2	7.800	967	–	un	0.27
3	7.888	970	969	sabinene	0.67
4	8.021	974	974	β-pinene	0.77
5	8.412	988	988	myrcene	42.64
6	8.926	1002	1002	α-phellandrene	4.06
7	9.148	1009	–	un	33.36
8	9.638	1020	1020	*p*-cymene	1.81
9	9.821	1025	1025	β-phellandrene	5.61
10	12.149	1084	1086	terpinolene	0.63
11	26.438	1411	1417	E-caryophyllene	2.78
12	28.968	1472	1478	γ-murolene	1.57
13	30.649	1514	1522	δ -cadinene	0.46
Total identified					66.38

LRI – Linear retention index; LLRI – Literature linear retention index (Adams 2007); un – unidentified

Table 2. Chemical composition (relative %) of the essential oil of *Eucalyptus citriodora*

Peak	Retention time [min]	LRI	LLRI	Compound	Relative [%]
1	6.018	911	908	isobutyl isobutyrate	0.43
2	6.689	932	932	α-pinene	0.15
3	8.022	974	974	β-pinene	0.62
4	9.914	1027	1026	1.8-cineol	0.40
5	10.954	1054	1054	γ -terpinene	0.18
6	12.555	1095	1095	linalool	0.83
7	14.529	1140	1145	isopulegol	6.14
8	14.851	1150	1148	citronelal	79.92
9	15.011	1154	–	un	3.41
10	15.536	1163	1167	*neo-iso*- -isopulegol	0.37
11	18.076	1223	1223	citronellol	7.17
12	26.436	1412	1417	*E*-cariofileno	0.37
Total identified					96.58

LRI – Linear retention index; LLRI – Literature linear retention index (Adams 2007); un – unidentified

Table 3. Average number (± SE) and corrected mortality rates of non-viable *Plutella xylostella* eggs treated with essential oil of *Eucalyptus citriodora* (EOEC) and *Schinus terebinthifolius* (EOST) and control (water)

Treatments	Average number of unviable eggs	Mortality corrected [%]
Control	3.5 ± 0.32 a	17.25
EOEC 0.5%	13.0 ± 1.12 c	58.01
EOEC 1%	10.9 ± 0.64 bc	45.02
EOST 0.5%	6.9 ± 1.31 b	21.15
EOST 1%	8.4 ± 0.54 bc	29.61

Means followed by different letters in the column differ statistically by Dunn test ($p < 0.05$)

den Dool and Kratz. Whenever possible, the synthetic standard was injected.

Bioassays

The EOEC and EOST were tested at concentrations of 0.5% and 1%. To prepare the dilutions, the oils were dissolved in Tween80® at 1% and then in distilled water. The treatments evaluated were: T1 = control treatment, with distilled water + Tween 80® solvent; T2 = EOEC (0.5%); T3 = EOEC (1%); T4 = EOST (0.5%); and T5 = EOST (1%). The bioassays followed the methodology proposed by (Turchen *et al.* 2014) and (Poorjavad *et al.* 2014), with modifications.

Effect of essential oils on *Plutella xylostella* eggs

Twenty *P. xylostella* eggs up to 24 h old were used for each treatment. These were placed in a voile fabric bag (6 × 6 cm), secured with an elastic band at the ends, and immersed with the aid of a sterilized metal tweezer for 5 s in each treatment. After drying at room temperature for approximately 50 minutes, these eggs were placed in Petri dishes (80 × 15 mm), which were kept in a climate-controlled chamber (25 ± 1°C, 65 ± 10% relative humidity, and 12:12 photoperiod). Evaluations were made under a stereoscopic microscope (40 ×), 48, 72, and 96 h after immersion, evaluating the integrity of the eggs, considering whether they shriveled or ruptured, not allowing larval hatching. Twenty repetitions were performed per treatment, totaling 400 eggs per treatment. The corrected mortality of *P. xylostella* eggs was calculated using the Schneider-Orelli formula, (% mortality in the treated plot – % mortality in the control plot / 100 – % mortality in the control plot) * 100 (Püntener 1981).

Effect of essential oils on *Plutella xylostella* larvae

The mortality and leaf consumption by *P. xylostella* larvae were recorded on cabbage leaf discs with a leaf area of approximately 51.125 cm^2 which were submerged for 10 s in one of the treatments already described. After drying at room temperature (about 50 min), the discs were individually placed in Petri dishes (8 cm × 1.5 cm) and kept in a climate-controlled chamber (25 ± 1°C, 65 ± 10% relative humidity, and 12:12 photoperiod). Subsequently, three third instar larvae were introduced into each dish, which were evaluated 24 and 48 h after exposure under a stereoscopic microscope (40 ×). Insects that did not respond to the touch of a fine-bristled brush (No. 00) were considered dead. Leaf consumption tests were performed simultaneously with mortality tests. For this, the leaf area meter model LI-3100C was used. The leaf discs used in this experiment had their leaf area measured before and after the exposure period (24 and 48 h) to *P. xylostella* larvae, with no replacement of the cabbage disc in any repetition until the end of the experiment. Twenty repetitions were performed per treatment.

Chemotaxic responses of *Trichogramma pretiosum* to essential oils

The bioassay was conducted with females up to 24 h old. Each female was inserted individually into a Y-shaped glass olfactometer with a diameter of 2 cm in an initial arena of 20 cm, bifurcated into two arms of 8 cm each. The tested insects were individualized

before the experiments and acclimated for 1 h in the test room. The airflow was conducted into the system with a blower connected to a flow meter at a rate of 0.3 l · min^{-1}. The arena was inverted (rotated 180°) every five repetitions, and every 10 repetitions, it was washed with neutral soap, 70% alcohol, and hexane, then dried in a sterilization oven at 150°C. The tests were performed during the photophase, with fluorescent light (60W, luminance 290 lux).

The essential oils of pink pepper and lemon-scented eucalyptus at concentrations of 0.5% and 1% contrasted with distilled water (control), all with the presence of Tween 80® (10 µl · ml^{-1}), were evaluated. Pieces of filter paper (3 × 6 cm) were immersed in each solution and evaluated after drying at room temperature (about 50 min).

A positive response (first choice) was considered when *T. pretiosum* females remained for at least 15 s at the end of one of the arms. Insects that did not move or did not reach one of the two arms of the olfactometer within 5 min were recorded as non-responsive and excluded from the statistical analysis. Forty repetitions of responsive insects were performed for each contrast.

Trichogramma pretiosum parasitism in *Plutella xylostella* eggs exposed to essential oils

Two bioassays were performed, one applying the oils before parasitism (pre-parasitism) and another with the application of the oils on already parasitized eggs (post-parasitism). Twenty groups of 20 eggs up to 36 h old were immersed in the oil or water dilutions, following the previously described methodology. In the pre-parasitism exposure, after drying (approximately 50 min), the 20 eggs were placed in a glass test tube (8.5 × 2.4 cm) and offered to a *T. pretiosum* female, 24 to 36 h old. After 24 h, the females were removed, and the eggs were stored under controlled conditions (25 ± 1°C, 65 ± 10% relative humidity, and 12:12 photoperiod).

To evaluate the effect of the oils on post-parasitism, *P. xylostella* egg clutches up to 36 h old were exposed to paired *T. pretiosum* females 24 to 36 h old. After 24 h of exposure, the females were removed, and the parasitized eggs were placed in glass test tubes (8.5 × 2.4 cm) and kept under controlled conditions for 3 days. The eggs that showed darkening due to parasitism (third instar to prepupa) (TNAU Agritech Portal 2022) were immersed in the different treatments, as described in both bioassays (pre- and post-parasitism). The eggs were inspected under a stereoscopic microscope (40 ×) every 24 h until the parasitoids emerged or the larvae hatched. The sex ratio of the emerged offspring was recorded.

Toxic potential of essential oils for *Trichogramma pretiosum* adults

Ten *T. pretiosum* females 24 to 36 h old were introduced into glass test tubes (8.5 × 2.4 cm) containing filter paper (1 × 1 cm), immersed for 5 s in one of the oil or water dilutions (control), described previously and dried at room temperature (about 50 min). The tubes with the females were kept in a climate-controlled environment. Twenty repetitions were performed per treatment.

The number of dead parasitoids per tube was quantified after three hours of exposure. Insects that did not respond to the touch of a fine-bristled brush (No. 00) were considered dead.

Statistical analysis

The average mortality data of *P. xylostella* eggs, mortality and leaf consumption of *P. xylostella* larvae, parasitism percentage, emergence rate, sex ratio, and mortality of *T. pretiosum* adults were analyzed for normality using the Lilliefors test. ANOVA followed by the Tukey test compared parametric data, and non-parametric data were analyzed using the Kruskal-Wallis test followed by Dunn's test, at a 5% significance level using the Bioestat 5.3® software (Ayres *et al*. 2007). Mortality was corrected using Schneider-Orelli's formula.

The results of the olfactometry bioassay of *T. pretiosum* were analyzed using the GLM (General Linear Model) test, binomial distribution model, at a 5% significance level using the R Studio® statistical software (version 4.1.1) R Development Core Team (2019). The corrected mortality of *P. xylostella* eggs and larvae was also calculated using the Schneider-Orelli formula (Püntener 1981).

Results

Viability of *Plutella xylostella* eggs treated with essential oils

The essential oils of *E. citriodora* (EOEC) and *S. terebinthifolius* (EOST) caused greater egg inviability of *P. xylostella* at both tested concentrations (0.5% and 1%) than the control treatment. EOEC at the lower concentration (0.5%) was more lethal than EOST ($H = 44.1$; $p < 0.0001$) at the same concentration. Additionally, the highest percentages of inviability (corrected mortality) were observed for lemon-scented eucalyptus oil at a concentration of 0.5% compared to pink pepper oil at the same concentration and water (Table 3).

Effect of essential oils on *Plutella xylostella* larvae

The average number of dead larvae was significantly higher in the groups exposed to cabbage discs with essential oils than the control (H = 43.5; $p < 0.0001$). However, mortality was similar among larvae subjected to different oils and concentrations (Table 4).

Leaf consumption was significantly lower in the EOEC (0.5% and 1%) and EOST (1%) treatments than in the control, both in the first 24 h and after 48 h of evaluation (Table 5). In comparison to the leaf areas consumed between the two periods, this increase was significant only for control treatment, with this difference being greater than those recorded for EOEC 0.5%, EOEC 1%, and EOST 1%.

Table 4. Mean number (± SE) and corrected mortality rates of dead *Plutella xylostella* caterpillars in contact with cabbage discs treated with *Eucalyptus citriodora* essential oil (EOEC), *Schinus terebinthifolius* (EOST) and control (water)

Treatments	Average number of dead caterpillars	Mortality corrected [%]
Control	0.1 ± 0.06 a	3.33
EOEC 0.5%	2.2 ± 0.19 b	70.68
EOEC 1%	2.2 ± 0.19 b	70.68
EOST 0.5%	1.4 ± 0.24 b	44.82
EOST 1%	1.7 ± 0.22 b	53.45

Means followed by different letters in the column differ statistically using Dunn test ($p < 0.05$)

Table 5. Percentage of leaf consumption of three caterpillars of *Plutella xylostella on* cabbage discs treated with essential oil of *Eucalyptus citriodora, Schinus terebinthifolius* at concentrations of 0.5 and 1% and water + tween 80 (control), exposed for 24 to 48 h to insects

		Treatments			
Time	Water	*E. citriodora* [0.5%]	*E. citriodora* [1%]	*S. terebinthifolius* [0.5%]	*S. terebinthifolius* [1%]
242h	5.15% a	1.55% b	0.96% b	3.10% ab	1.94% b
48 h	9.70% a	3.80% bc	1.61% c	5.42% ab	3.41% bc

Values followed by different letter in the line differ statistically using Dunn test ($p < 0.05$)

Olfactometry of *Trichogramma pretiosum* to volatiles from EOEC and EOST

Trichogramma pretiosum females were significantly more attracted to the odor of lemon-scented eucalyptus essential oil (0.5%) ($\chi^2 = 7.3121$ df = 1; $p = 0.006$) than to the control (air) and did not differentiate odors in the other treatments (Fig. 1).

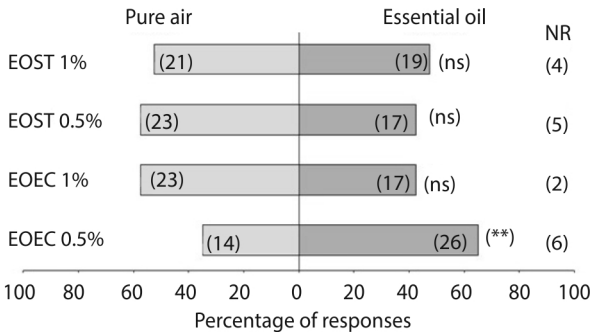

Fig. 1. Chemotaxic responses of *Trichogramma pretiosum* females (up to 24 h of age) in a double-choice olfactometer, subjected to volatiles from the essential oils of pink pepper (EOST) (*Schinus terebinthifolius*) and lemon eucalyptus (EOEC) (*Eucalyptus citriodora*) (0.5% and 1%) versus pure air. Numbers inside the bars indicate the number of responding insects. NR = number of non-responsive insects. ns = non-significant response. Bars followed by an asterisk differ significantly using the Chi-square test ($p < 0.01$)

Parasitism and emergence of *Trichogramma pretiosum* in *Plutella xylostella* eggs exposed to EOEC and EOST before and after parasitism

In the bioassays where the oils were applied to *P. xylostella* eggs before parasitism, the lowest parasitism rate was observed in eggs exposed to *S. terebinthifolius* oil at 1%, while for the other treatments, it was like the control (F = 2.97; $p < 0.0228$). The emergence (H = 8,065; $p = 0{,}0864$) and sex ratio (H = 3.4098; p = 0.4917) of *T. pretiosum* did not differ statistically between treatments (Table 6). Regarding the bioassay where the oils were applied on already parasitized eggs, over 70% mortality of parasitoid immatures was observed for the EOEC 1%, EOST 0.5%, and 1% treatments. Emergence was significantly lower in these treatments than in the control (H = 34.61; $p < 0.0001$). Only the EOEC treatment at a 0.5% dosage behaved similarly to the control treatment (Table 7).

Table 6. Mean percentage (± SE) of emergence of *Trichogramma pretiosum* in *Plutella xylostella* eggs treated with essential oil of *Eucalyptus citriodora* (EOEC), *Schinus terebinthifolius* (EOST) and control (water), before being exposed to parasitism

Treatments	Parasitism of *T. pretiosum* [%]	Emergence of *T. pretiosum* [%]	Sex ratio of *T. pretiosum*
Control	81.25 ± 0.70 a	83.3 ± 0.69 a	0.7 ± 0.02a
EOEC 0.5%	66.5 ± 1.13 a	85.7 ± 1.08 a	0.7 ± 0.05a
EOEC 1%	72 ± 1.47 a	90.9 ± 1.42 a	0.7 ± 0.06a
EOST 0.5%	60.5 ± 1.52 a	88.4 ± 1.56 a	0.7 ± 0.07a
EOST 1%	50.25 ± 1.70 b	85.5 ± 1.52 a	0.8 ± 0.07a

Means followed by different letters in the column differ statistically using the Tukey test ($p < 0.05$)

The sex ratio did not differ between treatments, both in the application of EOs in pre-parasitism (H = 3.4098; df = 4; p = 0.4917) (Table 6) and post-parasitism (H = 10.358; df = 4; p = 0.3854) (Table 7). It always skewed towards females.

Table 7. Mean percentage (± SE) of emergence of *Trichogramma pretiosum* in *Plutella xylostella* eggs treated with essential oil of *Eucalyptus citriodora* (EOEC), *Schinus terebinthifolius* (EOST) and control (water), after being exposed to parasitism

Treatments	Emergence of *T. pretiosum* [%]	Sex ratio of *T. pretiosum*
Control	79.7 ± 3.08 a	0.8 ± 0.02 a
EOEC 0.5%	72.5 ± 2.30 a	0.8 ± 0.04 a
EOEC 1%	23.1 ± 3.08 c	0.9 ± 0.04 a
EOST 0.5%	23 ± 3.25 bc	0.8 ± 0.06 a
EOST 1%	16.4 ± 2.03 bc	0.9 ± 0.04 a

Means followed by different letters in the column differ statistically using the Dunn test ($p < 0.05$)

Effects of EOs on *Trichogramma pretiosum* adults

The average number of dead insects was lower in parasitoids exposed to water and 0.5% EOEC than in those exposed to the other oil treatments, which did not differ statistically (p = 0.9182) (Table 8).

Table 8. Average number (± SE) of *Trichogramma pretiosum* females dead in contact with essential oil of *Eucalyptus citriodora* (EOEC), *Schinus terebinthifolius* (EOST) and control (water), 3 h after treatment

Treatments	Average number of dead adults	Percentage of dead adults
Control	0 ± 0.00 a	–
EOEC 0.5%	0 ± 0.00 a	–
EOEC 1%	0.6 ± 0.26 b	6%
EOST 0.5%	0.3 ± 0.14 b	3%
EOST 1%	0.3 ± 0.12 b	3%

Means followed by the same letters in the column do not differ statistically using the Dunn test ($p > 0.05$)

Discussion

The ovicidal effect of essential oils is possibly related to the presence of active substances that render insect development unviable or impair it (Krinski *et al.* 2018). Among the major compounds present in EOEC (Table 2) are citronellal (79.92%) and isopulegol (6.14%), which have already been attributed with insecticidal activity (Bandeira *et al.* 2022). According to Rodrigues (2018), constituents of *E. citriodora* EO may have a synergistic effect, enhancing their lethality.

Even though increasing oil concentration does not affect *P. xylostella* egg viability, this factor has been shown to increase insect embryonic mortality (Krinski *et al.* 2018). This aspect was studied by Cai *et al.* (2020), who found 100% and 67.74% inviability of *P. xylostella* eggs when exposed to concentrations of 8.5 and 5.95 mg · l^{-1} of citronellal, respectively. Even though the presence of this aldehyde in EOEC was recorded, the concentration of each substance in the oils was not analyzed, so it is possible that even at a higher concentration, the amount of citronellal was not sufficient to kill 100% of the embryos or larvae.

In addition to chemical aspects, the viscosity of the oils may likely have also influenced egg viability. The oils form an insulating film, making gas exchange by the embryo or larva difficult. This effect has already been observed with the presence of mineral oils in the eggs of other lepidopterans such as *Grapholita funebrana* (Treitschke) (Lepidoptera: Tortricidae) (Rizzo *et al.* 2018).

The insecticidal and/or feeding deterrent action of *P. xylostella* exposed to leaf discs containing essential oils is possibly attributed to the neurotoxic action of the compounds present in the oils, which can cause larval death both by contact and ingestion (Mayanglambam *et al.* 2022). Studies conducted with *E. citriodora* EO on *P. xylostella* detected that the major constituents of this oil (isopulegol and citronellal) (Table 2) have a positive interaction, enhancing the toxic action on the larvae, resulting in up to 80% mortality (Filomeno *et al.* 2017). Santos *et al.* (2020) also observed mortality of *P. xylostella* larvae exposed to citronellal, citronellol, and isopulegol, major constituents of EOEC. Therefore, it is likely that the percentage of dead *P. xylostella* larvae recorded in this study (70.68%) is also associated with these substances. The essential oil extracted from *Acacia nilotica* (L. Willd. ex Delile) (Fabaceae) seeds exhibited the highest mortality rates of 60% and 96.66% in *P. xylostella* at 24 and 48 h after treatment, respectively. The authors attribute this mortality to a decrease in the activity of acetylcholinesterase (AchE) in the larvae (Vivekanandhan *et al.* 2024).

The essential oils used in this study had myrcene, β-phellandrene, and α-pinene (*S. terebinthifolius*) and citronellal and citronellol (*E. citriodora*) as major constituents. Citronellal and citronellol are repellent substances for some groups of insects, such as mosquitoes (Shasany *et al.* 2000; Wany *et al.* 2013). Additionally, α-pinene and myrcene identified in the composition of EOST are also associated with the repellence of aphids (Sales *et al.* 2017). However, this repellence was not observed in *T. pretiosum* females in study. Also, Silva

et al. (2023) observed that females of *Diaeretiella rapae* (McIntosh) (Hymenoptera: Braconidae) were attracted to kale plants sprayed with pink pepper oil (0.5%), while citronella oil (*Cymbopogom winterianus*) had a repellent action, and lemon-scented eucalyptus oil did not influence the chemotaxis of this parasitoid at the same concentration.

These volatile organic compounds are commonly found in green leaves (Green Leaf Volatiles) and can interfere in different ways with the orientation of herbivores and natural enemies (Schuman 2023). Therefore, the absence of perception by *T. pretiosum* to the compounds present in EOST and EOEC (1%) may be related to different mechanisms of perception and interpretation of chemical signals among organisms. This is because there are different olfactory sensory characteristics associated with changes in structures such as types of antennae, sensilla, and odorant proteins, which are usually linked to the ecological context and the evolutionary process of each species (Fleischer *et al*. 2018). On the other hand, the differential chemotactic response of the parasitoid females between the concentrations of the same oil (*E. citriodora*) is possibly related to the insect's ability to trigger a motor response to a certain stimulus intensity, which, when in excess or lacking, does not act as a semiochemical.

In this study, the chemotactic response of the parasitoid was evaluated only to the volatiles of the oils; however, some studies have already found that the interaction of oils, plant extracts, and phytohormones with the plant can interfere with the orientation of *T. pretiosum*. (Lopes and Sant'Ana 2019) previously reported the attraction of *T. pretiosum* females to rice plants treated with methyl jasmonate and salicylic acid, compared to those sprayed with water and ethanol. It was also found that the presence of methyl jasmonate associated with eggs of *Tuta absoluta* (Meyrick) (Lepidoptera: Gelechiidae) on tomato plants increased the foraging behavior and parasitism performance of *T. pretiosum* (Weber *et al*. 2024).

The low effect of the essential oils previously applied to the eggs on the parasitism rate of *T. pretiosum* in this study had already been observed in eggs of *E. kuehniella* treated with the EOs *Hyptis marrubioides* (Epling) (Lamiaceae) and *Ocimum basilicum* (Linnaeus) (Lamiaceae), which are considered harmless according to the IOBC criteria, indicating that they are safe for *T. pretiosum* (Bibiano *et al.* 2022). Our results showed that *P. xylostella* eggs treated with lemon-scented eucalyptus (0.5% and 1%) and pink pepper oil (0.5% and 1%) before the exposition to parasitism, also reduced the emergence of *T. pretiosum* to less than 30% suggesting a potential selectivity of these treatments for this species. Conversely, Parreira *et al.* (2019) found lower parasitism of *T. pretiosum* (9.4 to 30.7%) on *E. kuehniella* eggs exposed to the essential oils of *Carapa guianensis* (Aubl.) (Meliaceae), *Allium sativum* (Linnaeus) (Liliaceae), *Citrus sinensis* (Linnaeus) (Rutaceae), *Mentha piperita (Linnaeus)* (Lamiaceae), *Origanum vulgare* (Linnaeus) (Lamiaceae), *Piper nigrum* (Linnaeus) (Piperaceae), *Syzygium aromaticum* (Linnaeus) (Myrtaceae), and *Thymus vulgaris* (Linnaeus) (Lamiaceae).

In the present study, this result may be related to the presence of monoterpenes such as β-pinene, present in *S. terebinthifolius*. It has been reported that this component has a repellent effect on some insects, such as *Rhyzopertha dominica* (Fabricius) (Coleoptera: Bostrichidae) (Nascimento *et al*. 2018). Similar effects were observed for adults of *Bemisia tabaci* (Genn.) (Hemiptera: Aleyrodidae) and *Trialeurodes ricini* (Misra) (Hemiptera: Aleyrodidae), which were repelled with pink pepper essential oil at a concentration of 1% (Hussein *et al*. 2017) and for *Culex pipiens* (Linnaeus) (Diptera: Culicidae) exposed to 0.5 to 4 $\mu l \cdot cm^{-2}$ of this oil (Nenaah *et al*. 2022).

On the other hand, since there was no significant difference in the emergence percentages between the oils and the control at any concentration, it can be inferred that the oils, when applied to the eggs before parasitism, had no toxic effect on the parasitoid. However, the same was not observed when *P. xylostella* eggs parasitized by *T. pretiosum* were immersed in the oils after parasitism, which, except for EOEC (0.5%), had a lower emergence percentage than the control treatment. The difference in the effect of the oils on the eggs is possibly due to the development stage of the parasitoid (egg-pupa) that was affected by the product.

In pre-parasitism tests, the female came into contact with the eggs after they had dried and had the opportunity to oviposit. Considering that many toxic monoterpenes evaporate at room temperature within 24 to 48 h, it is possible that *T. pretiosum* larvae did not suffer the toxic effects of the oils upon hatching from the eggs. On the other hand, when the immature parasitoid was in the third instar or prepupa stage (Milonas *et al*. 2020) and the host egg was subjected to the oils, the immature parasitoid was affected by the toxic action of the oils, with a visible reduction in emergence.

Other essential oils, such as *Prangos ferulacea* (Lindl.) (Umbelliferae), when applied after parasitism by *Trichogramma embryophagum* (Hartig) (Hymenoptera: Trichogrammatidae), also showed toxic effects on the immature stages of the parasitoid, reducing the parasitoid emergence rate (Ercan *et al*. 2013). According to the authors, *P. ferulacea*, one of the major constituents responsible for toxicity, is α-pinene, a substance also found in the EOs tested in this work (Table 1–2).

The immature stages of *T. pretiosum* are sensitive and can be affected by chemical product applications on their host eggs. This was also evidenced in

applications of neem oil on *T. absoluta* eggs parasitized by *T. pretiosum* after parasitism, reducing parasitoid emergence at the egg stage (16.75%), larva (13.98%), and pupa (74.05%) (Rampelotti-Ferreira *et al*. 2017). The sex ratio of *T. pretiosum*, in turn, was not affected in any of the treatments. This is commonly skewed towards females, increasing the species' success, a characteristic desired in biological control programs as it contributes to the success of pest management (Sousa *et al*. 2017).

The results of these bioassays indicate that the combined use of the two management techniques for *P. xylostella* in cabbage can be done, however, with a safe interval of days to avoid applying the product to already parasitized eggs, which could compromise the development of the parasitoid.

Our results show few impacts on the tested parasitoids, differing from works such as that of Monsreal-Ceballos *et al*. (2018), who observed that Trichogrammatidae parasitoids have low tolerance to biopesticides formulated with EOs in laboratory tests. Similarly, Khan *et al*. (2015) tested 19 chemical-synthetic products under laboratory conditions, of which 10 were not selective for adult *T. pretiosum*, causing mortality of up to 100%. The oils of *Lippia origanoides* (Kunth) (Verbenaceae), *Cymbopogon winterianus* (Jowitt) (Poaceae), and *Cymbopogon citratus* (DC) Stapf.) (Poaceae), at the same concentrations as those evaluated in our work, caused a mortality of 50 to 57% in *T. pretiosum* adults, and were considered non-selective (Sombra *et al.* 2022). Conversely, the botanical species tested in the present study were selective for adult *T. pretiosum*, with low mortality rates.

Given that the essential oils of *E. citriodora* and *S. terebinthifolius* have toxic action on eggs and larvae and are feeding deterrents to *P. xylostella* larvae but have low impact on immature and adult parasitoids, it may be inferred that they have potential for use in integrated management programs in cabbage crops. However, before defining the associated use protocols of these techniques, semi-field and field tests should be conducted.

Conclusions

The essential oils of *E. citriodora* and *S. terebinthifolius* are toxic to eggs and larvae of *P. xylostella* act as feeding deterrents, and have minimal impact on parasitoids. Thus, they are promising for integrated pest management of *P. xylostella* in cabbage crops.

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