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Selectivity of essential oils to the egg parasitoid *Trichogramma pretiosum* Riley (Hymenoptera: Trichogrammatidae)[1]

Seletividade de óleos essenciais ao parasitoide de ovos *Trichogramma pretiosum* Riley (Hymenoptera: Trichogrammatidae)

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ABSTRACT - The diversity of arthropod pests has required the combined use of various control methods. The application of essential oils showing insecticidal, repellent or phage-inhibiting activity, together with the release of natural enemies, can improve integrated pest management provided the oils display selectivity. The aim of this study was to evaluate the selectivity of the oils of rosemary pepper [*Lippia origanoides* Kunth (Verbenaceae)], citronella [*Cymbopogon winterianus* Jowitt. (Poaceae)] and lemongrass [*C. citratus* (DC) Stapf.] for *Trichogramma pretiosum* Riley (Hymenoptera: Trichogrammatidae), comparing five concentrations (0.01, 0.05, 0.1, 0.5 and 1.0%), and a control (neutral detergent at 1.0%). Residual toxicity was evaluated using adult mortality, calculating lethal concentrations (LC_{50}) in addition to the reductions in parasitism in eggs of *Spodoptera frugiperda* (J. E. Smith) (Lepidoptera: Noctuidae) and selectivity for the parasitoids. The three oils showed selectivity for the parasitoid *T. pretiosum*, resulting in a mortality rate of between 17.2% (rosemary pepper) and 32.2% (lemongrass) at the lowest concentration. The essential oil of rosemary pepper stood out with an LC_{50} of 0.43%, a reduction of only 22% in parasitism (Class 1 - Innocuous) and 88.0% emergence, at a dose of 0.01%. The LC_{50} of the lemongrass oil was 0.15%, with a 34.0% reduction in parasitism (Class 2 - Slightly harmful) and 74.0% emerged adults. For the citronella oil, the LC_{50} was 0.12%, with a reduction of 46.0% (Class 2 - Slightly harmful) and emergence of 62.0%. The selectivity of the essential oils makes possible to release *T. pretiosum*, integrating biological control with botanical insecticides, as long as non-sprayed eggs are parasitised by *T. pretiosum*.

Key words: Biopesticides. Biological control. Botanical insecticides. Fall armyworm. Integrated Pest Management (IPM).

RESUMO - A diversidade de artrópodes-praga tem exigido a integração de diferentes métodos de controle. Assim, aplicações de óleos essenciais com atividade inseticida, repelente ou fagoinibidora, associadas às liberações de inimigos naturais, podem potencializar o manejo integrado de pragas, desde que apresentem seletividade. Nesse contexto, o objetivo foi avaliar a seletividade dos óleos de alecrim-pimenta *Lippia origanoides* Kunth (Verbenaceae), citronela *Cymbopogon winterianus* Jowitt. (Poaceae) e capim-santo *C. citratus* (DC) Stapf., sobre *Trichogramma pretiosum* Riley (Hymenoptera: Trichogrammatidae), comparando cinco concentrações (0,01; 0,05; 0,1; 0,5 e 1,0%), além da testemunha (detergente neutro à 1,0%). Avaliou-se a toxicidade residual por meio da mortalidade de adultos, calculando-se concentrações letais (CL_{50}), além das reduções no parasitismo em ovos de *Spodoptera frugiperda* (J. E. Smith) (Lepidoptera: Noctuidae) e seletividade aos parasitoides. Os três óleos exibiram seletividade ao parasitoide *T. pretiosum*, causando mortalidade entre 17,2 (alecrim-pimenta) e 32,2% (capim-santo), na menor concentração. Destacou-se o óleo essencial de alecrim-pimenta com CL_{50} de 0,43%, apenas 22% de redução do parasitismo (Classe 1 - Inócuo) e 88% de emergência quando à 0,01%. A CL_{50} do óleo de capim-santo foi 0,15%, com 34% de redução do parasitismo (Classe 2 - Levemente nocivo) e 74% de adultos emergidos. Já para o óleo de citronela, a CL_{50} foi de 0,12%, 46% de redução (Classe 2 - Levemente nocivo) e emergência de 62%. A seletividade dos óleos essenciais encontrada pode viabilizar liberações de *T. pretiosum,* integrando controle biológico e inseticidas botânicos, à medida que ovos não pulverizados, sejam parasitados por *T. pretiosum*.

Palavras-chave: Bioinseticidas. Controle biológico. Inseticidas botânicos. Lagarta-do-cartucho. Manejo Integrado de Pragas (MIP).

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INTRODUCTION

Trichogramma pretiosum Riley (Hymenoptera: Trichogrammatidae) stands out as the most commercialised of the parasitoids used in biological pest control programs around the world, parasitising the eggs of around 240 species of Lepidoptera (QUERINO *et al*., 2017). The adults measure from 0.5 to 1 mm in length, are yellowish-brown in colour, and have a dark abdomen, reddish eyes and short antennae, which are plumose in males and clavate in females (CÔNSOLI; PARRA; ZUCCHI, 2010). As soon as the larvae emerge, they begin to feed on the yolk and/or embryo of the host egg, causing its death (LAURENTIS *et al*., 2019). Parasitism efficiency in *T. pretiosum* is fundamental to the success of the biological control program for lepidopteran pests (LAURENTIS *et al*., 2019), such as *Spodoptera frugiperda* (JE Smith) (Lepidoptera: Noctuidae), *Chrysodeixis* (*Pseudoplusia*) *includens* (Walker) (Lepidoptera: Noctuidae), *Anticarsia gemmatalis* Hübner (Lepidoptera: Noctuidae) and *Neoleucinodes elegantalis* (Guenée) (Lepidoptera: Crambidae), where parasitism reached 95.0, 30.0, 40.2 and 19.0%, respectively (MAGALHÃES *et al*., 2012; OLIVEIRA *et al*., 2020). In maize (*Zea mays* L.) (Poaceae), the use of *T. pretiosum* improves productivity by up to 19.4%, which can result in increases of up to 700 Kg, increasing the gain per hectare by USD 96.50 (FIGUEIREDO *et al*., 2015). However, some biological control programs have demonstrated low efficiency due to such planning problems as an insufficient number of released parasitoids, a difficulty or lack of application technology, competition or predation by other natural enemies, and/or the absence of selective insecticides (FIGUEIREDO *et al*., 2015; PARRA; ZUCCHI, 1997).

The integration of biological and alternative controls by releasing *T. pretiosum* and spraying leaves with essential oils from aromatic plants can improve Integrated Pest Management (IPM) (ERCAN *et al*., 2013). Essential oils contain a variety of substances, such as alkaloids, phenols and terpenes, which can act on multiple action sites and express biocidal, repellent and/or antifeedant characteristics on arthropod pests, reducing the percentage of resistant individuals in a population and even showing a certain selectivity for non-target organisms, such as parasitoids (BASKAR; ANANTHI; IGNACIMUTHU, 2017; REGNAULT-ROGER; VINCENT; ARNASON, 2012; SOMBRA *et al*., 2020).

The essential oil of *Leptospermum petersonii* Bailey (Myrtaceae), comprised mainly of citronellal (3,7 - dimethyloct - 6 - en - 1 - al) and citral (3,7 - dimethyl - 2,6 - octadienal), showed selectivity for adults of *T. pretiosum*, in addition to moderate insecticidal and antifeedant activity, with post-ingestion effects (antibiosis) on the diamondback moth, *Plutella xylostella* (L.) (Lepidoptera: Plutellidae), reducing larval survival and growth, and oviposition by the moths (HEATHER; HASSAN, 2012). In contact and fumigation tests, the essential oils of *Origanum vulgare* L. (Lamiaceae) and *Thymus vulgaris* L. (Lamiaceae), were selective for females of *Trissolcus basalis* Wollaston (Hymenoptera: Scelionidae) (GONZÁLEZ *et al*., 2013), without affecting the behaviour of the parasitoid. However, some essential oils are unselective, or show lower selectivity, such as the essential oil of *Prangos ferulacea* L. (Umbelliferae), which is harmful to different life stages of *Trichogramma embryophagum* Htg. (Hymenoptera: Trichogrammatidae) (ERCAN *et al*., 2013).

A knowledge of the existence or non-existence of harmful effects (selectivity) of the different essential oils showing insecticidal, repellent or phago-inhibitory potential on the parasitism efficiency and adults of *T. pretiosum*, makes integration between biological and alternative controls possible, and helps in deciding when to release the parasitoid and employ systematic spraying, thereby improving the management of important lepidopteran pests (LAURENTIS *et al*., 2019; MAGALHÃES *et al*., 2012).

The aim of this study, therefore, was to evaluate the selectivity of the essential oils of rosemary pepper [*L. origanoides* Kunth (Verbenaceae)], citronella [*Cymbopogon winterianus* Jowitt (Poaceae)] and lemongrass [*Cymbopogon citratus* (DC) Stapf (Poaceae)] applied to eggs of *S. frugiperda* on *T. pretiosum*.

MATERIAL AND METHODS

The experiments were carried out in air-conditioned rooms (25 ± 3 °C, RH of 70 ± 10% and a photoperiod of 12 h) at the “Laboratório de Entomologia Aplicada (LEA)” of the Federal University of Ceará (UFC), at 3º44’32.474” S and 38º34’31.48” W, and an approximate altitude of 27.75 metres (Fortaleza, Ceará State, Brazil).

Breeding the *S. frugiperda*

Breeding began with caterpillars collected in areas of commercial maize cultivation in the districts of Quixeré and Limoeiro do Norte (Ceará State, Brazil), which were kept on an artificial diet proposed by Greene, Leppla and Dickerson (1976) until the formation of pupae. The pupae were removed from the diet residue and placed in glass tubes (100 x 25 mm) until the adults emerged. After emerging, the adults were transferred to cylindrical polyvinyl chloride (PVC) cages (10 x 25 cm) containing a 10.0% honey solution (food), lined with paper towels (oviposition) and closed at the ends with voile fabric. The paper towel and solution were replaced every two days, removing the egg masses and transferring them to Petri dishes (90 x 15 mm). After three days, the newly emerged caterpillars were transferred with the aid of a fine-haired brush to glass tubes (100 x 25 mm) containing an artificial diet, removing samples of the population to carry out the bioassays.

Breeding the *T. pretiosum*

The 'Ubajara' strain of *T. pretiosum* was obtained from the "LEA-UFC" (OLIVEIRA *et al*., 2020). The adults were kept in glass tubes (100 x 25 mm) and fed with honey painted on the inner wall of the tube with the aid of a fine-haired brush. The eggs of *Anagasta kuehniella* (Zeller) (Lepidoptera: Pyralidae) were used as an alternative host. The eggs were previously glued onto sky-blue cardboard sheets (4.0 x 2.0 cm) using a 20.0% dilution of gum arabic, and rendered inviable by exposure to ultraviolet germicidal light for 50 min. The cards containing the inviable eggs were offered to the females of *T. pretiosum*, closing the tubes with PVC® plastic film, and allowing contact for 24 hours. The cards were then removed and placed in individual glass tubes (100 x 25 mm) until the adults emerged. The *T. pretiosum* progeny were used in the bioassays.

Obtaining the essential oils

The essential oils were provided by "AGROPAULO Agroindustrial S/A", Jaguaruana, Ceará State, Brazil and extracted in the laboratory using the technique of steam distillation. The oils were stored under refrigeration in amber glass, and sent to the Natural Product Chemical Laboratory of "Embrapa Agroindústria Tropical" (Fortaleza, Ceará State, Brazil) (3°45'4.741"S and 38°34'33.906"W, at an altitude of around 38.41 m) for an analysis of the chemical composition by gas chromatography using a mass spectrometer (CG-MS), and determining the Kovats retention index (KI) and mass spectrum of each constituent using an Agilent CG-MS chromatograph. The resulting data were identified from the literature (ADAMS, 2007) (Table 1).

Experimental design

The experimental design of the bioassays was completely randomised (CRD) and arranged in a 3 x 5 factorial scheme to evaluate the selectivity of the essential oils of rosemary pepper (*L. origanoides*), citronella (*C. winterianus*) and lemongrass (*C. citratus*) on parasitism efficiency in eggs of *S. frugiperda* and on adults of *T. pretiosum* at concentrations of 0.01, 0.05, 0.1, 0.5 and 1.0% oil per litre of water, previously dissolved with neutral detergent (1:1). Five replications were set up, using 10 eggs of *S. frugiperda* as the working plot. The negative control consisted of a 1.0% neutral detergent solution.

Bioassay for adult selectivity

Adult selectivity was evaluated via residual toxicity on a treated surface, by spraying 100 ± 20 µL of the essential-oil solutions or the control internally on glass tubes (100 x 25 mm) using a manual sprayer (Figure 1A). Fifteen adults of *T. pretiosum* were released into the treated tubes after 24 hours and kept without food (Figure 1B). The tubes were closed with PVC® plastic film and placed in an upright position on shelves. The mortality rate was recorded 24 hours after each release; any insects that did not respond to mechanical stimulus carried out with a fine-haired brush were considered dead.

Table 1 - Characterisation of the major compounds of the essential oils of rosemary pepper [*Lippia origanoides* Kunth (Verbenaceae)], citronella [*Cymbopogon winterianus* Jowitt. (Poaceae)] and lemongrass [*C. citratus* (DC) Stapf.], obtained by gas chromatography using a mass spectrometer (CG-MS)

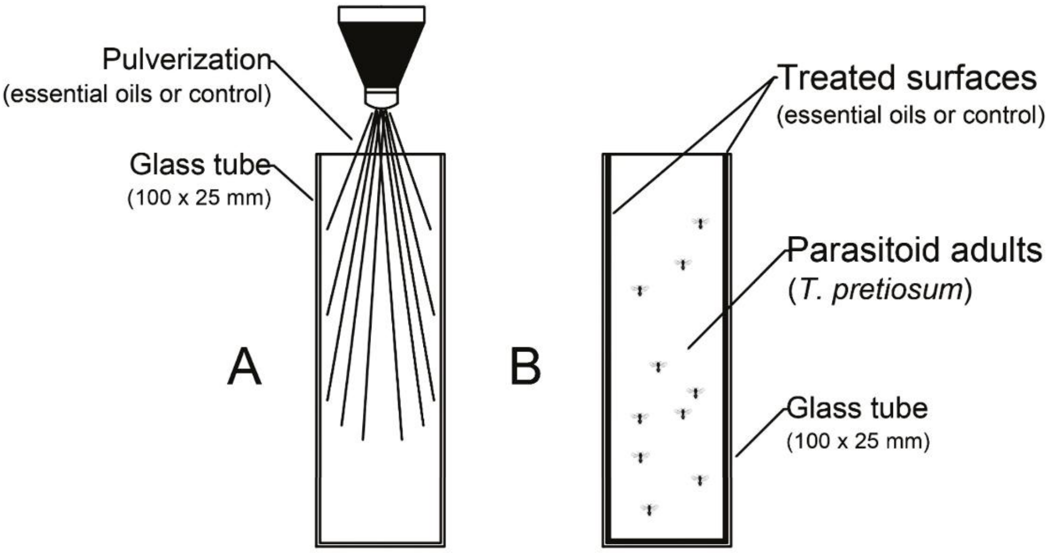
Compounds	KI[a]	Essential oils		
		L. origanoides	*C. winterianus*	*C. citratus*
		Content[b] (%)		
3-Carene	1014	-	-	29.01
2,4-Decadienal, (E,E)-	1314	-	-	25.95
Acetophenone	1058	32.75	-	-
α-Cadinene	1537	0.27	1.37	0.31
α-Citral	1266	-	0.44	16.22
α-Fenchene	946	1.96	-	-
α-Himachalene	1452	10.38	-	0.33
α-Pinene	940	5.08	0.23	-
α-Terpinene	1017	0.31	-	-
β-Caryophyllene	1409	-	3.44	-

Continuation Table 1

β-Myrcene	999	3.90	1.01	-
β-Pinene	979	0.39	0.16	-
Carvacrol	1333	30.37	-	-
D-Limonene	1040	-	-	0.94
Eugenol	1364	-	1.22	-
Isopulegol	1164	-	-	5.75
Geraniol	1249	-	3.44	-
Geranyl acetate	1396	-	1.67	0.45
Lavandulyl acetate	1283	-	5.90	-
Longifolene	1407	-	-	0.82
Menthone	1197	-	55.97	-
Methyl thymyl ether	1244	1.95	-	-
p-Cymenene	1091	0.62	-	-
p-Menth-8-en-2-one, trans-	1188	-	-	1.90
Perillen	1107	-	-	1.71
Rose oxide	1115	-	1.21	-
Terpinolene	1083	7.96	0.38	-
Trans-β-Ocimene	1047	-	0.09	0.83
Valencene	1496	-	1.12	-
γ-Terpinene	1049	0.42	18.35	0.57
(S)-cis-Verbenol	1280	-	-	10.42
Total		96.36	96.00	95.21

[a] Kovats Retention Index (KI) by GC-MS; [b] Component content of the essential oil

Figure 1 - Bioassay for contact toxicity. Spraying into glass tubes (A) and release of adults of *Trichogramma pretiosum* Riley (Hymenoptera: Trichogrammatidae) 'Ubajara' (B) after 24 hours



Mortality was corrected relative to the control, using formulae proposed by Abbott (1925):

$$\%M = \frac{NDIT}{TNIT} \times 100 \quad (1)$$

where: %M = Percentage of mortality; NDIT = Number of dead insects for the treatment; NTIT = Total number of insects for the treatment.

$$Mc(\%) = \frac{\%Mo - \%Mt}{100 - \%Mt} \times 100 \quad (2)$$

where: Mc = corrected mortality; Mo = observed mortality; Mt = mortality for the negative control.

Bioassay for parasitism selectivity

Eggs of *S. frugiperda* were collected and glued equidistantly onto sky-blue cardboard sheets (2.0 x 2.0 cm) using a 20.0% solution of gum arabic with the aid of a stereoscopic microscope and a fine-haired brush. The cards, containing 10 eggs, were placed on filter paper and sprayed (100 ± 20 μL) separately with one of the essential-oil solutions or the control using a manual sprayer (Figure 2A). After 30 minutes, each card was exposed for 48 hours to one mated female of *T. pretiosum* (Figure 2B) of up to 24 hours of age, previously placed in an individual glass tube (100 x 25 mm) which was closed with PVC® plastic film. Following this period, the females were removed using a fine-haired brush, and the cards evaluated after 12 days, noting the number of parasitised eggs (with and without an emergence hole) and adult emergence, calculating possible reductions in the rates of parasitism (RP), and correcting them relative to the control.

The reduction in the rate of parasitism (RP) was used to classify the essential oils according to the toxicity classes for phytosanitary products established by the International Organisation for Biological and Integrated Control of Noxious Animals and Plants/West Palearctic Regional Section (IOBC/WPRS): Class 1, toxicity less than 30.0% = harmless; Class 2, toxicity between 30.0% and 79.0% = slightly harmful; Class 3, toxicity between 80.0% and 99.0% = moderately harmful; and Class 4, toxicity greater than 99.0% = harmful (HASSAN; ABDELGADER, 2001).

Bioassay for susceptibility of the parasitised eggs

Cards containing 10 eggs of *S. frugiperda*, collected during breeding and glued to sky-blue cardboard sheets (2.0 x 2.0 cm) using a 20.0% solution of gum arabic, were exposed to parasitism for 24 hours in glass tubes (100 x 25 mm) containing mated females of *T. pretiosum* 'Ubajara' (Figure 3A). After exposure, the cards were removed and placed on filter paper where they were sprayed with 100 ± 20 μL of the essential-oil solutions or the control using a manual sprayer, and left to rest for 30 minutes (Figure 3B). The treated cards were placed into individual glass tubes (100 x 25 mm), closed with PVC® plastic film, and arranged in an upright position on shelves. The cards were evaluated after 12 days, recording the percentage of emerged adults and the sex ratio (PARREIRA *et al*., 2018).

Statistical analysis

The mean values for corrected mortality (Mc) were submitted to Probit analysis (FINNEY, 2009). From the dose-mortality curves, estimates of the lethal concentrations (LC_{50}) were generated for an adult *T. pretiosum* mortality rate of 50.0%. The data were subjected to analysis of variance (ANOVA) and the mean values compared by Tukey's test at a level of 5.0% ($p \leq 0.05$) in the case of a significant difference, using the SISVAR® statistical software (FERREIRA, 2014).

Figure 2 - Bioassay for selectivity. Spraying the cards containing eggs of *Spodoptera frugiperda* (J. E. Smith) (Lepidoptera: Noctuidae) (A), and exposure of the cards containing eggs to parasitism by females of *Trichogramma pretiosum* Riley (Hymenoptera: Trichogrammatidae) 'Ubajara' (B)

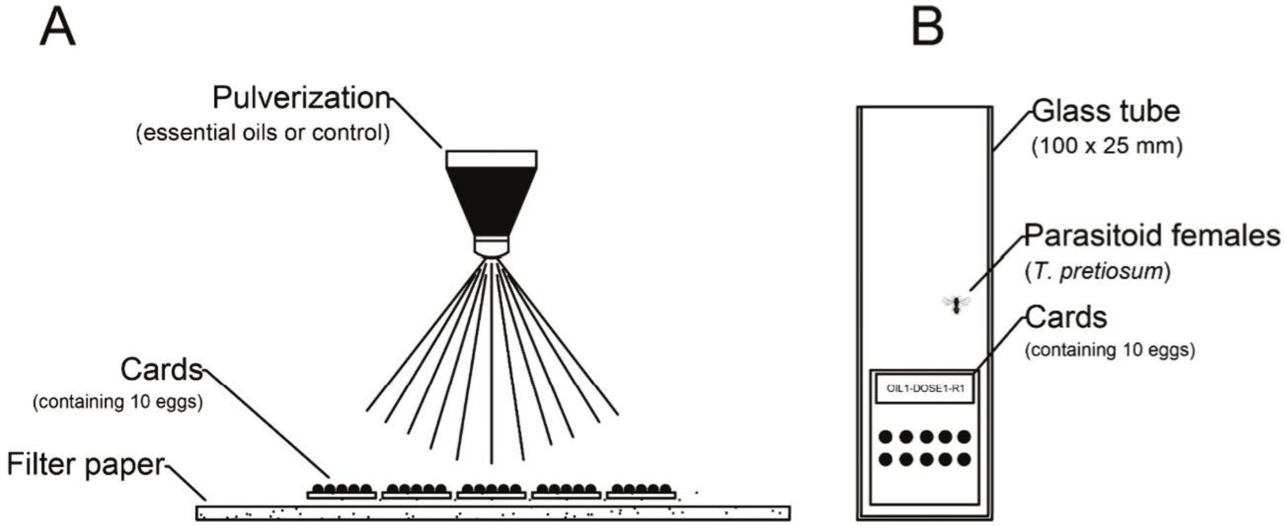
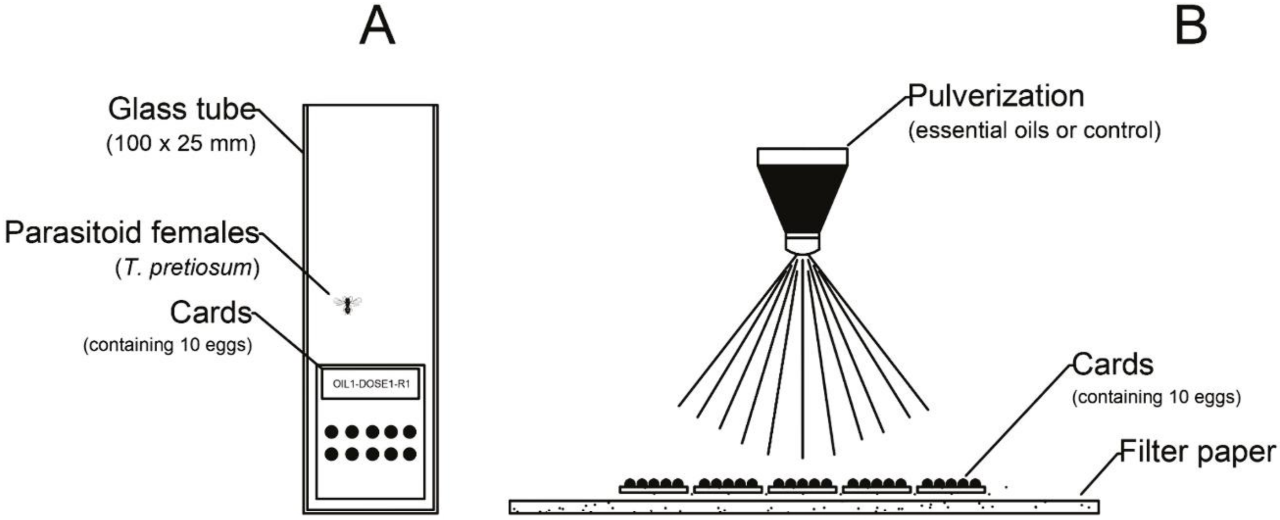


Figure 3 - Bioassay for susceptibility. Exposure of the cards containing eggs of *Spodoptera frugiperda* (JE Smith) (Lepidoptera: Noctuidae) to parasitism by females of *Trichogramma pretiosum* Riley (Hymenoptera: Trichogrammatidae) 'Ubajara' (A) and spraying the cards post-parasitism with the essential oils or the control (B)



RESULTS AND DISCUSSION

There were differences between the three essential oils regarding corrected mortality (Mc) ($p < 0.01$), reduction in parasitism (RP) ($p < 0.01$) and adult emergence (EM) ($p < 0.01$) in *T. pretiosum* 'Ubajara'. The concentrations also differed ($p < 0.01$) (Table 2), showing a direct relationship between the residual toxicity to adults, reduction in parasitism, or toxicity to eggs parasitized by *T. pretiosum*, and the increase in concentration of the essential oils ($p < 0.01$).

The three essential oils displayed some degree of selectivity to adults of *T. pretiosum* at the lowest concentrations, with mortality rates (Mc) of between 17.2% (rosemary pepper) and 32.2% (lemongrass). The essential oil of citronella differed by 0.1% and caused 26.8% Mc after 24 hours of exposure, followed by the essential oil of rosemary pepper, with an Mc of 36.2%, and the essential oil of lemongrass, with 47.2%. The oil from rosemary pepper apparently had no effect on the behaviour of the surviving parasitoids, while the essential oil of lemongrass stood out for causing lethargy and even paralysis in adults of *T. pretiosum*, resulting in up to 61.2% mortality at the highest concentration (1.0%) (Table 3).

The lethal concentrations (LC_{50}) made it possible to observe the selectivity of the three essential oils for the adults of *T. pretiosum* (Table 4), highlighting the oil of rosemary pepper, which obtained a calculated LC_{50} of 0.43%, while the oils of citronella and lemongrass obtained values of 0.12% and 0.15%, respectively. However, the overlapping confidence intervals (CI 95.0%) showed no differences between the lethal concentrations (LC_{50}).

Spraying the eggs of *S. frugiperda* at the higher concentrations reduced the rates of parasitism and adult emergence in *T. pretiosum* (Figure 4AB). In the case of eggs treated with the essential oil of rosemary pepper (0.01%), parasitism was 56.0% and adult emergence 92.9% (Figure 4AB). The difference between the essential oils was more marked at the concentration of 0.1%, where spraying the eggs with the essential oil of lemongrass resulted in only 8.0% parasitism, despite an adult emergence of 100.0%, while in eggs treated with the essential oil of citronella, parasitism was 28.8%, with 98.6% emergence; for the oil of rosemary pepper, parasitism was 36.0% and emergence was 77.8% (Figure 4AB). There was an inversely proportional relationship between the concentration (0.01% - 1.0%) and the rate of parasitism, as seen with the essential oil of rosemary pepper by the reduction in parasitism from 56.0% (0.01%) to 28.0% (1.0%) between the lowest and highest concentrations.

Only the concentration of 0.01% of the oil of rosemary pepper was considered selective for the females of *T. pretiosum*, reducing parasitism by 22.0% (Class 1 - Innocuous). The essential oil of lemongrass (0.1%) inhibited parasitism by 70.0% (Class 2 - Mildly harmful), as did the essential oil of citronella, which reduced parasitism by 53.0% (Class 2 - Mildly harmful) (Table 5).

When spraying took place following parasitism of the *S. frugiperda* eggs, the three essential oils displayed a certain degree of selectivity for *T. pretiosum*. An inverse relationship was found between the concentration and adult emergence, as with the 0.01% essential oil of rosemary pepper, which afforded 88.0% emergence, including 51.4% females, similar to the

Table 2 - Analysis of variance (ANOVA) for corrected mortality (Mc), reduction in parasitism (RP) and adult emergence (EM) in *Trichogramma pretiosum* Riley (Hymenoptera: Trichogrammatidae) treated with essential oils of rosemary pepper [*Lippia origanoides* Kunth (Verbenaceae)], citronella [*Cymbopogon winterianus* Jowitt. (Poaceae)] and lemongrass [*C. citratus* (DC) Stapf.]

Source of variation	DF	Mean squares		
		MC (%)	RP (%)	EM (%)
Essential oils	2	1910.01**	1576.64**	4837.00**
Doses	4	1765.81**	2161.10**	3190.33**
Oils x Doses	8	102.36^{ns}	593.90**	237.83^{ns}
Error	60	177.36	118.13	321.33
C. V. (%)		34.67	17.71	31.23

** significant at a level of 1%; ^{ns} not significant

Table 3 - Corrected mortality (Mc) as a percentage (%), in adults of *Trichogramma pretiosum* Riley (Hymenoptera: Trichogrammatidae) exposed for 24 hours to surfaces treated with the essential oils of rosemary pepper [*Lippia origanoides* Kunth (Verbenaceae)], citronella [*Cymbopogon winterianus* Jowitt. (Poaceae)] and lemongrass [*C. citratus* (DC) Stapf.]

Essential oils	Concentrations (%)				
	0.01	0.05	0.1	0.5	1.0
Rosemary pepper	17.20 aB	29.60 aAB	36.20 abAB	37.60 aAB	43.00 aA
Citronella	23.00 aB	26.60 aB	26.80 bB	38.00 aAB	55.40 aA
Lemongrass	32.20 aB	46.60 aAB	47.20 aAB	55.60 aAB	61.20 aA
C. V. (%)	42.85	35.44	32.64	27.61	26.08
Value F	1.613^{ns}	2.939^{ns}	3.279*	2.978^{ns}	2.437^{ns}
Value-p	0.2078	0.0606	0.0445	0.0584	0.0961

Mean values followed by the same lowercase letter in a column and uppercase letter in a row do not differ statistically by Tukey's test at 5.0% probability. * significant at a level of 5%; ^{ns} not significant

Table 4 - Lethal concentrations (LC_{50}) (%) causing 50% mortality in adults of *Trichogramma pretiosum* Riley (Hymenoptera: Trichogrammatidae) after 24 hours of exposure to the essential oils of rosemary pepper [*Lippia origanoides* Kunth (Verbenaceae)], citronella [*Cymbopogon winterianus* Jowitt. (Poaceae)] and lemongrass [*C. citratus* (DC) Stapf.]

Essential oils	LC_{50} [a]	$CI_{95\%}$ [b]
Rosemary pepper	0.4381	0.0716 - 1.7445
Citronella	0.1209	0.0111 - 1.3366
Lemongrass	0.1519	0.0691 - 0.3441

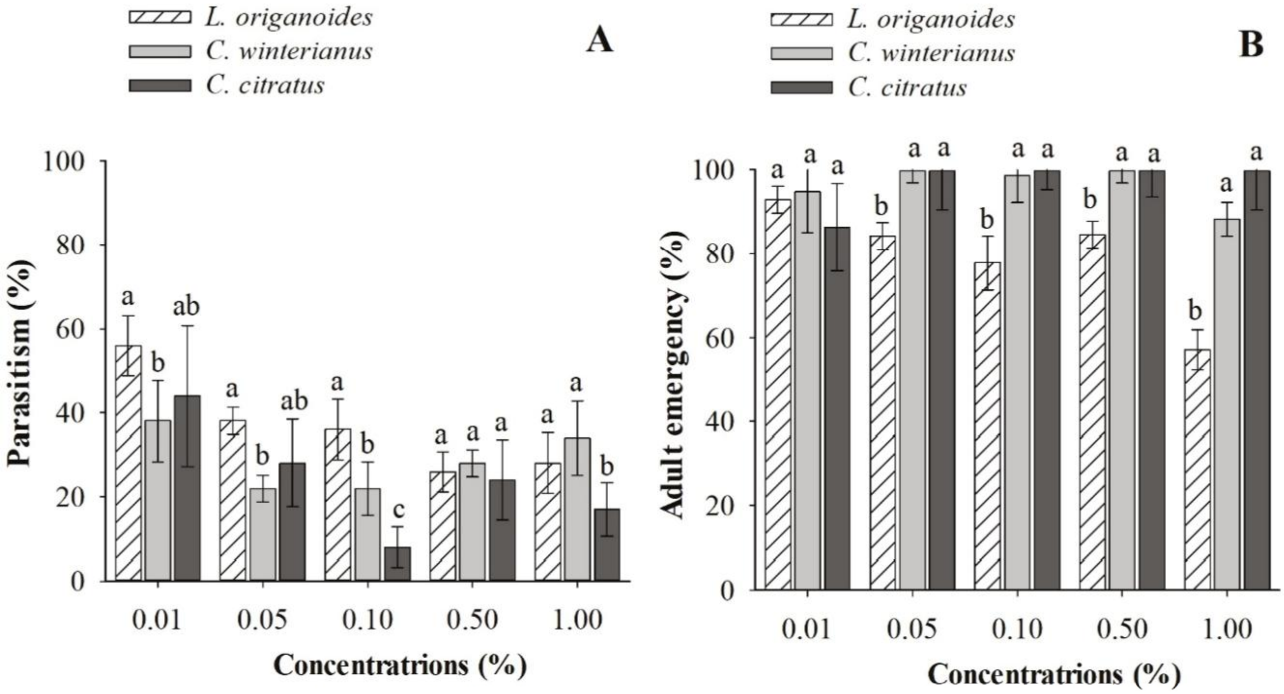
[a] Lethal concentration; [b] Confidence intervals (CI 95%)

results obtained with the essential oil of citronella (62.0% emergence and 50.5% females) and lemongrass (74.0% and 47.8%) (Figure 5).

The three essential oils had no effect on the sex ratio of *T. pretiosum* when the eggs were pulverised.

The essential oils of rosemary pepper (*L. origanoides*), citronella (*C. winterianus*) and lemongrass (*C. citratus*) showed a degree of selectivity for the parasitism of *T. pretiosum* 'Ubajara' in the eggs of *S. frugiperda* and in adults of the parasitoid, furnishing data on the possible interaction between the two methods of lepidopteran pest control (PARREIRA *et al*., 2019; VIANNA *et al*., 2009). The main differences between the three essential oils are largely due to the mechanisms of action of the major compounds, which induced low toxicity at lower concentrations, altering host selection and the emergence of *T. pretiosum*, after spraying the eggs of *S. frugiperda* (CAMPOS *et al*., 2019; EL-WAKEIL, 2013).

Figure 4 - Parasitism (%) and emergence (%) in adults of *Trichogramm pretiosum* Riley (Hymenoptera: Trichogrammatidae) in eggs of *Spodoptera frugiperda* (JE Smith) (Lepidoptera: Noctuidae) previously sprayed with the essential oils of rosemary pepper [*Lippia origanoides* Kunth (Verbenaceae)], citronella [*Cymbopogon winterianus* Jowitt. (Poaceae)] and lemongrass [*C. citratus* (DC) Stapf.]



Mean values followed by the same lowercase letter in a column and uppercase letter in a row do not differ statistically by Tukey's test at a level of 5%. ** significant at a level of 1%; ns not significant

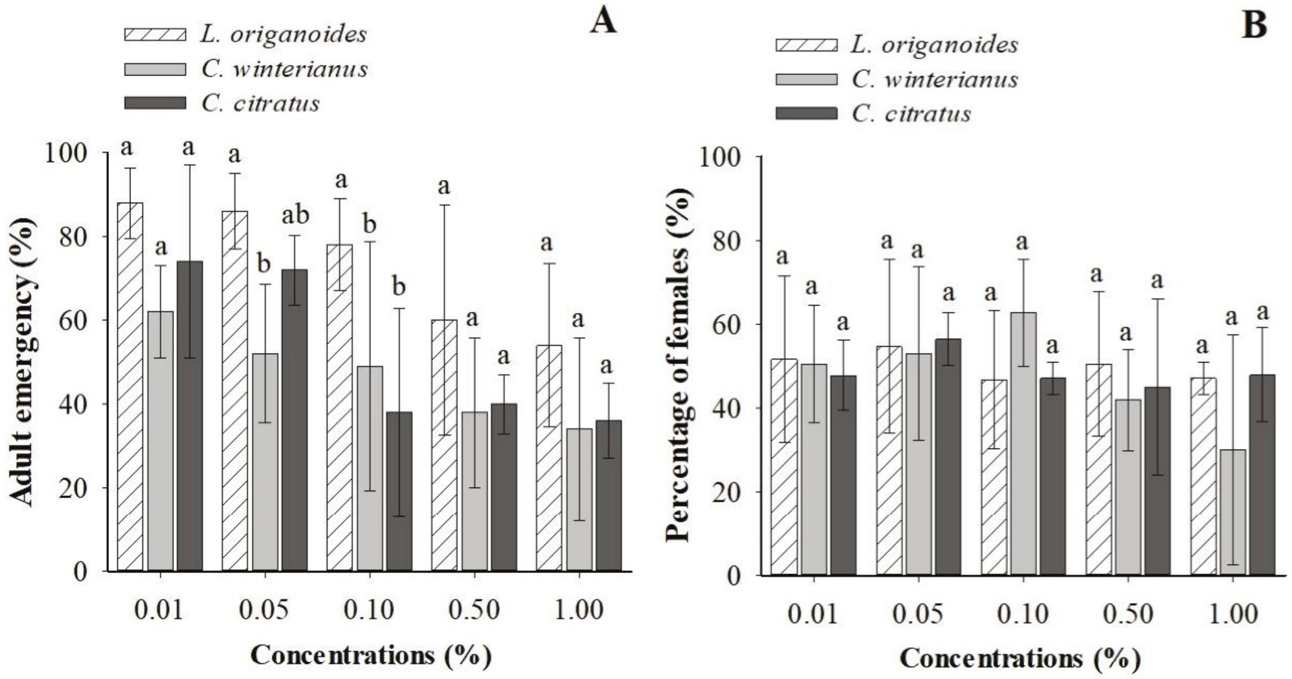
Table 5 - Estimated reductions in the rate of parasitism (RP) (%) by the essential oils of rosemary pepper [*Lippia origanoides* Kunth (Verbenaceae)], citronella [*Cymbopogon winterianus* Jowitt. (Poaceae)] and lemongrass [*C. citratus* (DC) Stapf.] in adults of *Trichogramma pretiosum* Riley (Hymenoptera: Trichogrammatidae)

Essential oils	Concentrations (%)				
	0.01	0.05	0.01	0.5	0.01
Rosemary pepper	22.0 cD	40.0 bC	42.0 cBC	51.2 aAB	53.2 aA
Citronella	46.0 aA	53.0 aA	56.0 bA	56.0 aA	57.4 aA
Lemongrass	34.0 bC	50.0 aB	70.0 aA	54.0 aB	50.0 aB
C. V. (%)	40.00	12.82	18.57	15.87	14.65
Value F	19.806**	6.373**	26.958**	0.277^{ns}	0.946^{ns}
Value-p	0.0000	0.0023	0.0000	0.7585	0.3907

Mean values followed by the same lowercase letter in a column and uppercase letter in a row do not differ statistically by Tukey's test at a level of 5%. ** significant at a level of 1%; ns not significant

The selectivity of the essential oils can be attributed to such factors as the development of detoxifying metabolic pathways and the excretion of toxic compounds by the parasitoids (KOUL; DHALIWAL, 2004), in addition to the low residual power of the essential oils, a result of the rapid degradation of compounds with insecticidal potential (SAXENA *et al.*, 2018). This low residual power can reduce the period of insecticidal activity of the essential oils in the field; however, the high capacity for causing mortality of some compounds can result in significant mortality a few hours after application, and allow temporal integration by releasing *T. pretiosum* before or after spraying (ISMAN; MIRESMAILLI; MACHIAL, 2011; MIRESMAILLI; ISMAN, 2014; MONSREAL-CEBALLOS *et al.*, 2017).

Figure 5 - Emergence (%) and sex ratio in adults of *Trichogramma pretiosum* Riley (Hymenoptera: Trichogrammatidae), after spraying the parasitised eggs of *Spodoptera frugiperda* (JE Smith) (Lepidoptera: Noctuidae) with the essential oils of rosemary pepper [*Lippia origanoides* Kunth (Verbenaceae)], citronella [*Cymbopogon winterianus* Jowitt. (Poaceae)] and lemongrass [*C. citratus* (DC) Stapf.]



The essential oil of rosemary pepper showed greater selectivity for *T. pretiosum*, with less toxicity to adults and a smaller reduction in parasitism, which is mainly due to the mechanisms of action of thymol (2 – isopropyl – 5 - methyl-phenol) and carvacrol (2 – methyl -5 - (1 - methylethyl) - phenol) (EL-WAKEIL, 2013), the two volatile monoterpenes that act to modulate the γ-aminobutyric acid (GABA) receptors found in the peripheral nervous system of insects, and to compete for the nicotinic acetylcholine receptors (nAChR), respectively (CAMPOS *et al*., 2019; EL-WAKEIL, 2013). However, due to volatilisation, the two compounds have low residual power, acting with less intensity 24 hours after spraying, and proving to be selective for adults of the parasitoid (CAMPOS *et al*., 2019; SAXENA *et al*., 2018).

The reduction in parasitism attributed to the essential oils of citronella and lemongrass is largely due to the major compounds, citral (3,7 – dimethyl - 2,6 - octadienal), citronellal (3,7 – dimethyloct – 6 – en – 1 - al) and geraniol [(E) 3,7-dimethyl – octa - 2,6 – diene – 1 - ol), respectively (SILVA *et al*., 2014). These compounds are volatile with repellent action, and were possibly detected by receptors on the antennae or tarsi of the *T. pretiosum* females, generating a non-preference for oviposition. The repellency of the compounds has already been noted in Hemiptera, such as *Brevicoryne brassicae* L. (Hemiptera: Aphididae) (RICCI *et al*., 2002) and *Bemisia tabaci* (Genn.) (Hemiptera: Aleyrodidae) (DELETRE *et al*., 2015); in Coleoptera, such as *Tribolium castaneum* (Herbst.) (Coleoptera: Tenebrionidae) (OLIVERO-VERBEL; NERIO; STASHENKO, 2010) and *Sitophilus zeamais* Motsch (Coleoptera: Curculionidae) (PEIXOTO *et al*., 2015); and in Diptera, *Anopheles gambiae* Giles (Diptera: Culicidae) (NERIO; OLIVERO-VERBEL; STASHENKO, 2010) and *Musca domestica* L. (Diptera: Muscidae) (CHAUHAN; MALKI; SHARMA, 2018).

The reduction in the emergence rate of *T. pretiosum* adults following spraying of the previously parasitized eggs (those containing the parasitoid) with the essential-oil solutions is possibly due to the ability of some compounds to diffuse through the chorion of the host and interrupt the embryonic development of the parasitoid (PARREIRA *et al*., 2018, 2019). The carvacrol present in the essential oil of rosemary pepper (*L. origanoides*) may have diffused through the chorion and acted on the nervous system, causing acetylcholinesterase inhibition, as seen when the essential oils of *O. vulgare* and *T. vulgaris* were used on immature stages of *Trissolcus basalis* Wollaston (Hymenoptera: Scelionidae) (EL-WAKEIL, 2013; GONZÁLEZ *et al*., 2013).

The lack of any difference in sex ratio showed that spraying (contact) the three essential oils did not change the nutritional quality of the *S. frugiperda* eggs as a food source (PARREIRA *et al*., 2019). Changes in the sex ratio are generally associated with a reduction in the quality of the nutritional resources of the host (VIANNA *et al*., 2009).

Selectivity can also manifest as ecological and physiological; when ecological, it is considered for selective use, i.e. less exposure of the parasitoids to essential oils, adapting and interspersing applications of essential oils with the release of *T. pretiosum* adults. Whereas physiological selectivity includes the use of insecticides of low toxicity, or those more toxic to the pest than to any natural enemies, as seen in the three essential oils, which presented low toxicity to the parasitoid, at lethal concentrations higher than those calculated for caterpillars of *S. frugiperda* (GONZÁLEZ *et al*., 2013; PARREIRA *et al*., 2018, 2019; SOMBRA *et al*., 2020). The results, after field validation, contribute to a possible integration of the two methods, and may increase efficiency, insofar as unsprayed eggs can be parasitised by the females of *T. pretiosum* (ERCAN *et al*., 2013; MIRESMAILLI; ISMAN, 2014).

CONCLUSIONS

1. The three essential oils, rosemary pepper (*L. origanoides*), citronella (*C. winterianus*) and lemongrass (*C. citratus*) showed selectivity for *T. pretiosum*;
2. The essential oil of rosemary pepper (*L. origanoides*) stood out as more selective, considered harmless or slightly harmful at concentrations below 0.01%. The essential oils of citronella and lemongrass were classified as slightly harmful.

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