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SHORT COMMUNICATION

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Sublethal effects of insecticides used in strawberry on *Trichogramma pretiosum* (Hymenoptera: Trichogrammatidae)

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Abstract

Aim of study: Assessment of toxicity and sublethal effects of registered insecticides currently used in strawberry cultivation in Brazil on *Trichogramma pretiosum* Riley adults.

Area of study: The study was conducted under laboratory conditions in Paraná (Brazil).

Material and methods: Previously non-parasitized *Duponchelia fovealis* Zeller (Lepidoptera: Cambridae) eggs were dipped into insecticide dilutions or control solution. Seven active ingredients were tested: thiamethoxam, abamectin, azadirachtin, spinetoram, chlorfenapyr, lambda-cyhalothrin and chlorpyriphos. Side-effects of pesticides were quantified by measuring mortality on *T. pretiosum* females in 24 h, longevity after exposure to the insecticides, parasitism and emergence rates, and offspring sex ratio. These traits were also measured on the second generation.

Main results: According to IOBC criteria, thiamethoxam was classified as harmless; abamectin, chlorfenapyr and spinetoram as slightly toxic; azadirachtin and lambda-cyhalothrin as moderately toxic and chlorpyriphos as toxic. The emergence rate of *T. pretiosum* second generation was not significantly affected by thiamethoxam, abamectin, azadirachtin, and chlorfenapyr. Sublethal effects caused by azadirachtin, abamectin and chlorfenapyr were verified in the second generation.

Research highlights: The information generated by this study is useful for designing future biological control strategies in integrated pest management programs against *D. fovealis.*

Additional key words: selectivity; biological control; egg parasitoid

Abbreviations used: AI (active ingredient); E (percentage of reduction of the capacity of a given biological feature); IOBC (International Organization for Biological Control); IPM (Integrated Pest Management); SE (standard error); RH (relative humidity).

Authors' contributions: ICP and MACZ obtained funding, supervised the work and coordinated the research project. DMA and MACZ designed the study. DMA performed the laboratory trials. JMMA, ESA and DMA analyzed and interpreted the data. JMMA and ESA wrote the manuscript. All authors read, critical revised the manuscript for important intellectual content and approved the final manuscript.

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Introduction

The members of the Trichogrammatidae family rank among the most important biotic agents employed around the world (Gallego *et al.*, 2019; Araujo *et al.*, 2020; Schäfer & Herz, 2020). The Trichogrammatidae family consists of parasitoids of insect eggs; they are amenable to mass production (Jalali, 2013) and highly efficient for controlling Lepidoptera pests (Feltrin-Campos *et al.*, 2019). Among these pests, the European pepper moth, *Duponchelia fovealis* Zeller (Lepidoptera: Crambidae), is widely distributed and causes crops losses in Europe, Asia, Africa, and North America (CABI, 2020). *Duponchelia fovealis* is considered a key-pest for strawberry in European countries (Bonsignore & Vacante, 2010) and was also detected in South America attacking strawberry fields in Brazil (Zawadneak *et al.*, 2017).

In Brazil, registered chemical pesticides to control European pepper moth on strawberry (Ministério da Agricultura, Pecuária e Abastecimento, 2020) are lacking, which has led farmers, agricultural technicians and researchers to seek for alternatives, including biological control agents such as parasitoids of the *Trichogrammatidae* family (Rodrigues *et al.*, 2017). Since the *Trichogramma* genus consists of cosmopolitan parasitoids, understanding host interactions is an essential step for developing a biological control strategy, as different species exhibit variations in host preference (Paes *et al.*, 2018; Tabebordbar *et al.*, 2020). *Trichogramma galoi* Zucchi and *Trichogramma pretiosum* Riley proved promissory to be used in the biological control of *D. fovealis* (Paes *et al.*, 2018). However, non-selective pesticides in agricultural production systems pose a limitation to the successful implementation of biological control programs employing Trichogrammatidae due to their sensitivity to insecticide active ingredients (AIs) (Gallego *et al.*, 2019). Furthermore, the effect of pesticides used in strawberry crops in Brazil on the fitness of *Trichogramma* species for *D. fovealis* eggs has been scarcely studied (Rodrigues *et al.*, 2017). In this context, the current work aimed to assess the toxicity and sublethal effects of insecticides registered and currently used in strawberry cultivation in Brazil on *T. pretiosum* by applying them on *D. fovealis* eggs in pre-parasitism non-choice tests.

Material and methods

Insects

Duponchelia fovealis were obtained from a laboratory colony (Federal University of Paraná, Curitiba, Paraná, Brazil) established from wild locally-collected insects and reared at 25 ± 2 °C, 70 ± 10 % RH (relative humidity), and a 14:10 h light:dark photoperiod as described by Zawadneak *et al.* (2017). Adults were kept in plastic cages (17 × 15 cm) and fed on a nutritional solution developed by Zawadneak *et al.* (2017). The walls of these cages were wrapped with a paper towel for egg deposition. Eggs adhered to the paper towel were transferred on the same paper towel to Petri dishes (90 × 15 mm; ten eggs per dish) until larval emergence, and then transferred to glass test tubes (2.8 × 8.5 cm) covered with cotton wrapped in a piece of voile. Adults of *D. fovealis* were fed using cotton moistened into a solution containing beer, honey and water.

A colony of *T. pretiosum* was purchased from Promip® (Engenheiro Coelho, São Paulo, Brazil) and reared in the laboratory into a climatic chamber (25 ± 2 °C, 70 ± 10 % RH, and a 14:10 h light:dark photoperiod). The wasps were reared on UVL (ultraviolet light)-sterilized eggs of Zeller (Lepidoptera: Pyralidae) from a laboratory colony. The eggs were glued with arabic gum (30 %) onto blue paper cards (4 × 1 cm) and exposed to adult *T. pretiosum* in glass vials (10 × 1.5 cm). After 24 h of exposure, the cards were transferred to new glass vials, where they were held until adult emergence. Adult *T. pretiosum* were provided with honey as droplets smeared on the inside wall of the glass vials.

Insecticides

Seven commercial formulations, with different insecticide AIs were used (IRAC, 2020), as listed in Table S1 [suppl]. These compounds were selected because of their current and main use in the chemical management of pests in strawberry crops in Brazil. The doses tested were the maximum authorized or recommended by the manufacturer. Application rates of the insecticide formulations were prepared by diluting the products in distilled water according to the manufacturer's instructions for the maximum field dose allowed.

Bioassays with the adult stage of *Trichogramma pretiosum*

Previously non-parasitized *D. fovealis* eggs were treated by dipping the cards (20 eggs each) into the insecticide dilutions or control solution for 5 seconds according to de Paiva *et al.* (2018). Then, the cards were air-dried for 1 hour and then transferred to glass Petri dishes (9 × 1 cm) with filter paper to remove moisture excess at ambient temperature. Each card was offered to a *T. pretiosum* female (24 h old) separately in a glass vial (5 × 1 cm). After 24 h of exposure, the cards were removed and transferred to a clean glass vial until parasitoid emergence. The female was fed on honey droplets. The side-effects of the insecticides were quantified by measuring the mortality of *T. pretiosum* females in 24 h, longevity of the females after the exposure to the insecticides, parasitism rate (by counting the number of dark eggs), emergence rate (eggs with exit holes/parasitized eggs) and offspring sex ratio (number of females/total number of insects).

After emergence, female offspring were isolated in glass vials and a card with 20 untreated *D. fovealis* eggs was offered to each female for parasitism during 24 h. The estimated parameters, observed on a daily basis, were progenitor mortality rate, number of parasitized eggs, emergence rate, sex ratio of the offspring and longevity (survival time until death).

In both bioassays, distilled water was used as a control. The bioassays were conducted under controlled conditions (25 ± 2 °C, 70 ± 10 % RH, and a 14:10 h light:dark photoperiod). In the case of the bioassay on the parental generation of parasitoids, every treatment was repeated four times and there were 10 replicates, corresponding to 10 host egg cards (20 eggs each), dipped into one of the seven insecticide solutions or water (n = 40 per treatment). In the case of the bioassay on the first generation, the number of cards varied between 9 and 46 depending on the parasitoid availability, which was affected by the previous bioassay.

Statistical analysis

The experiments followed a completely randomized design. Data did not follow the normality and homocedasticity assumptions and, consequently, were analyzed by the non-parametric Kruskal-Wallis test, and means were separated using the Dunn's test at 5% significance level. Data analyses were performed with R 3.6.2 environment (R Core Team, 2019).

Subsequently, the percentage reduction in emergence from parasitized eggs, adult longevity and percentage of parasitism relative to the control was evaluated using this equation: $E(\%) = [1 - (Q/q) \times 100)]$, where E is the percentage of reduction of the capacity of a given biological feature, Q is the average value of the analyzed parameter for a given insecticide, and q represents the mean value of the parameter obtained in the control. Based on the results, each insecticide was classified according to the International Organization for Biological Control (IOBC) criteria for laboratory tests: class 1 = harmless ($E < 30\%$ reduction of emergence, longevity, or fecundity), class 2 = slightly toxic ($30\% \leq E \leq 79\%$ reduction), class 3 = moderately toxic ($80\% \leq E \leq 99\%$ reduction), and class 4 = toxic ($E > 99\%$ reduction) (Amano & Haseeb, 2001).

Results and discussion

The percentage of survival for *T. pretiosum* females of the F_0 generation was significantly different between the control and abamectin, spinetoram, chlorpyriphos, chlorfenapyr and lambda-cyhalothrin treatments, 24 h after exposure to the insecticides (Fig. S1a [suppl]). In contrast, no significant differences were detected between the control and thiamethoxam and azadirachtin (Fig. S1a [suppl]). In the case of the F_1 generation, chlorfenapyr significantly reduced survival of *T. pretiosum* females 24 h after the treatment. No significant differences were found between the control and the thiamethoxam, abamectin and azadirachtin treatments (Fig. S1b [suppl]).

The longevity of *T. pretiosum* females from the F_0 generation decreased significantly for all AIs ($p< 0.001$) when compared to the control (Table 1). These reductions differed among AIs, thus according to IOBC criteria, thiamethoxam, azadirachtin, lambda-cyhalothrin were classified as slightly toxic, whereas abamectin, spinetoram, chlorfenapyr and chlorpyriphos were classified as moderately toxic (Fig. 1). This agrees with previous studies, involving the same methodological approach as the one reported in the current work, showing reductions in the longevity of *T. pretiosum* females that stayed in contact with host eggs treated with these AIs (Moura *et al.*, 2004; Khan *et al.*, 2015; de Paiva *et al.*, 2018).

The effect of insecticides on parasitism rate by *T. pretiosum* females from the F_0 generation is shown in Table 1. A significant decrease was detected for abamectin, chlorfenapyr, spinetoram, azadirachtin, lambda-cyhalothrin and chorpyriphos when compared to the control treatment ($p< 0.001$). This allowed for classifying thiamethoxam as harmless; abamectin, chlorfenapyr and spinetoram as slightly toxic; azadirachtin and lambda-cyhalothrin as

Table 1. Mean values (± SE) of longevity of adults and percentage of eggs parasitized of the F_0 generation, adult emergence rate and offspring sex ratio of the F_1 generation of *Trichogramma pretiosum* (Hymenoptera: Trichogrammatidae) after application of insecticide formulations to eggs of *Duponchelia fovealis* (Lepidoptera: Crambidae) under laboratory conditions.

Treatment	Longevity (days)	Parasitized egg (%)	Emergence rate (%)	Offspring sex ratio (%)
Control	7.7±0.4d	83.0±1.9d	86.4±1.1d	56.6±2.0
Thiamethoxam	4.0±0.3c	69.1±3.7d	66.4±3.0c	48.0±3.7
Abamectin	0.5±0.1a	48.8±4.4c	43.4±3.1b	46.1±4.3
Azadirachtin	4.5±0.3c	10.3±1.7a	56.1±5.4c	50.9±6.3
Spinetoram	0.4±0.1a	31.1±2.7b	7.5±1.4a	68.0±4.7
Chlorfenapyr	0.2±0.0a	49.1±4.2c	59.4±3.4c	52.9±4.9
Lambda-cyhalothrin	1.9±0.3b	2.0±0.4a	66.7±7.0cd	68.8±4.9
Chlorpyriphos	0.1±0.0a	0.0±0.0a	ND	ND
χ^2	209.83	197.03	93.19	5.38
Degrees of freedom	7	7	6	6
p-value	< 0.001	< 0.001	< 0.001	0.496

Different letters indicate significant differences among treatments verified by Kruskal-Wallis and Dunn tests. ND = No data.

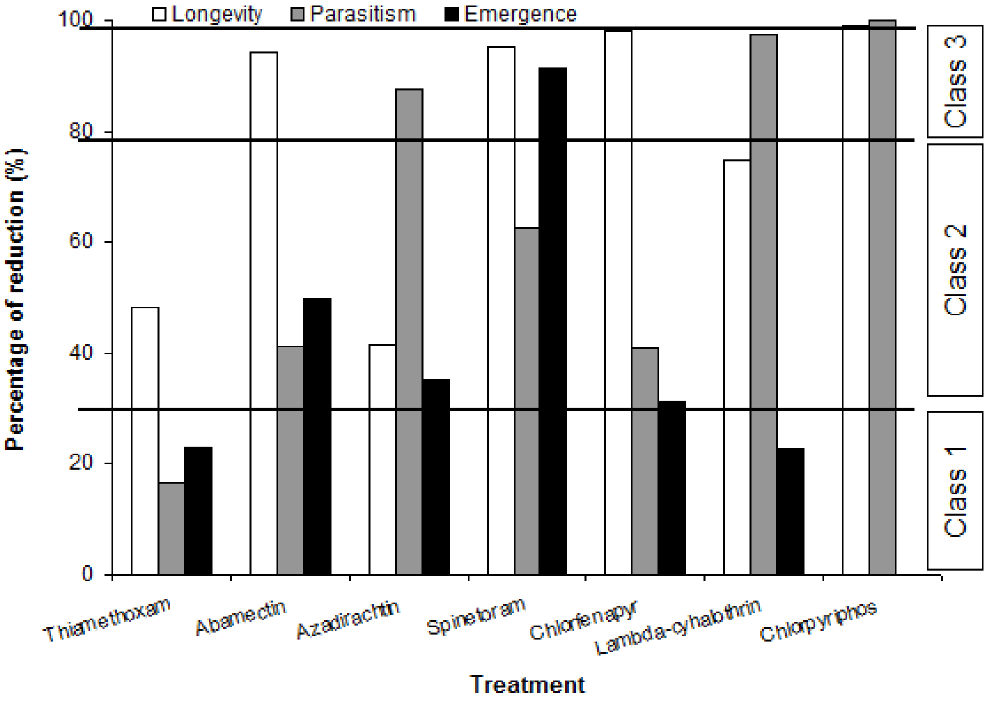

Figure 1. Reduction of adult parasitism rate and longevity of F_0 generation and emergence of F_1 generation of *Trichogramma pretiosum* (Trichogrammatidae) in eggs of *Duponchelia fovealis* (Lepidoptera: Crambidae) previously treated with insecticide formulations under laboratory conditions. Class toxicity according to the IOBC: class 1 = harmless, percentage of reduction (E) < 30%; class 2 = slightly harmful, $30 \leq E \leq 79\%$; class 3 = moderately harmful, $80 \leq E \leq 99\%$; and class 4 = harmful, E > 99%.

moderately toxic and chlorpyriphos as toxic (Fig. 1). The present results are in agreement with those by Moura *et al.* (2004) who observed reductions of 12.7% in the parasitism rate of *T. pretiosum* females when thiamethoxam was applied on the host eggs. Similarly, reduction in parasitism rate ranging from 46.0 (Moura *et al.*, 2004) to 77.4% (de Paiva *et al.*, 2018) was observed for chlorfenapyr, ranking this AI as slightly toxic. However, the effects of abamectin were different on the parasitism rate of two strains of *T. pretiosum*, one of them showing higher sensitivity to this AI than the other (Carvalho *et al.*, 2001). Reduction by 71.35% on parasitism was observed for spinetoram (Takahashi, 2016). Azadirachtin and lambda-cyhalothrin were classified as moderately toxic in the current study, since they reduced *T. pretiosum* parasitism rate likely due to the repellent effect of these AIs (Rodrigues *et al.*, 2017; de Paiva *et al.*, 2018), which can be detected by *Trichogramma* females when these AIs are on the host eggs (Potrich *et al.*, 2015). In addition, azadirachtin can affect embryo formation and development of host eggs (Correia *et al.*, 2013), leading to eggs with insufficient quality for being parasitized by *Trichogramma.* Lambda-cyhalothrin reduced the parasitism rate of females that had contacted the residues and, consequently, the offspring generated were also small, as previously reported (Carvalho *et al.*, 2001). The acute toxicity of chlorpyriphos on *T. pretiosum* was also observed by de Paiva *et al.* (2018) using the same methodological approach, indicating that this product is incompatible with this parasitoid species.

Moreover, treating eggs prior to parasitism with all AIs reduced significantly the emergence of the parasitoids from the F_0 generation (Table 1). This is in accordance with previous results on the effects of lambda-cyhalothrin and abamectin (Carvalho *et al.*, 2001), although the variability in emergence rate reduction can be due to genetic differences among the *Trichogramma* populations used in the tests, which lead to different tolerance to the insecticides. Chlofenapyr can cause reductions in the emergence of *T. pretiosum* (Moura *et al.*, 2004; de Paiva *et al.*, 2018); however, these previous studies reported lower effects of chlorfenapyr on emergence rate of *T. pretiosum* than the present study, likely due to differences on the populations of *T. pretiosum* used. The harmful effects of spinetoram and lambda-cyhalothrin on the parasitism rate by the F_0 generation and chlorpyriphos on the reduction of the emergence rate of the F_1 generation of *T. pretiosum* made not possible to perform observations of the biological parameters of the F_1 generation for these treatments.

No statistical differences were observed on the offspring sex ratio of the F_1 generation of *T. pretiosum* (p= 0.496) (Table 1); however, abamectin, azadirachtin and chlorfenapyr significantly affected this parameter on the F_2 generation of *T. pretiosum* by increasing the number of males (p< 0.001) (Table 2). Nevertheless,

Table 2. Mean values (± SE) of longevity of adults and percentage of eggs parasitized of the F_1 generation, adult emergence rate and offspring sex ratio of the F_2 generation of *Trichogramma pretiosum* (Hymenoptera: Trichogrammatidae) after application of insecticide formulations to eggs of *Duponchelia fovealis* (Lepidoptera: Crambidae) under laboratory conditions.

Treatment	n	Longevity (days)	Parasitized egg (%)	Emergence rate (%)	Offspring sex ratio (%)
Control	46	4.2±0.3c	78.4±3.0b	87.5±1.2	50.7±2.5b
Thiamethoxam	44	3.8±0.2bc	78.9±3.1b	77.9±2.1	45.5±3.9b
Abamectin	35	3.0±0.3b	5.3±1.6a	84.7±1.9	12.5±3.2a
Azadirachtin	9	3.8±0.4bc	63.9±9.5b	86.2±2.3	0.0±0.0a
Chlorfenapyr	10	1.2±0.5a	56.0±10.6b	82.0±2.7	7.8±4.2a
χ^2		17.63	64.05	7.46	25.25
Degrees of freedom		4	4	4	4
p-value		0.002	< 0.001	0.1113	< 0.001

Different letters indicate significant differences among treatments verified by Kruskal-Wallis and Dunn tests.

these results must be taken with caution because of the low number of *T. pretiosum* individuals that survived after insecticide treatments (Table 2). The sublethal effect on the F_2 generation of *T. pretiosum* could be a result of latent effects which are expressed in the subsequent life stage to the one in which the parasitoid was initially exposed to the insecticide, as observed by de Paiva *et al.* (2018). The sublethal effects of insecticides on parasitism by *T. pretiosum* females from the F_1 generation is shown in Table 2. A statistically significant decrease was found in relation to the control for abamectin, while no significant decrease was found for thiamethoxam, azadirachtin and chlorfenapyr. Moreover, the longevity of *T. pretiosum* females from the F_1 generation decreased significantly with respect to the control for abamectin, which was classified as harmless, and chlorfenapyr, classified as slightly toxic (Table 2). The emergence rate of the F_2 generation of *T. pretiosum* was not significantly affected by thiamethoxam, abamectin, azadirachtin and chlorfenapyr in relation to the control (p= 0.111) (Table 2). Therefore, these pesticides seemed to unleash physiological and behavioral changes that affect the development, sex ratio, longevity, among other characteristics, reducing the action of these natural enemies in the agro-systems (Desneux *et al.*, 2007). The results of this study indicate the necessity to assess the sublethal effects to evaluate the impact of insecticides on *T. pretiosum*, and not only for one generation as recommended by de Paiva *et al.* (2018).

In conclusion, under laboratory conditions, thiamethoxam can be considered compatible with *T. pretiosum*; however, the other AIs tested in this study presented side-effects on this parasitoid, reducing its beneficial effects for pest control. Abamectin, azadirachtin and chlorfenapyr caused sublethal effects on *T. pretiosum*, reducing parasitism in F_1 and F_2 generations and modifying the sex ratio of the F_2 generation. Chorpyriphos was incompatible with *T. pretiosum*. However, more experiments under field and semi-field conditions are needed in order to confirm the observations reported in the current work and establish whether thiametoxam is compatible with *T. pretiosum*.

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