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, critically revised the manuscript for important intellectual content and approved the final manuscript.

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**Supplementary material** (Table S1 and Fig. S1) accompanies the paper on SJAR’s website

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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; Tabebord var et al., 2018). Riley proved promissory to be used in the biological control of Du. 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 Du. 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 Du. fovealis eggs in pre-parasitism non-choice tests.

Material and methods

Insects

Du. 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 Du. 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 Du. 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 Du. 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
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 chlorpyriphos 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

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### Table 1. Mean values ($\pm$ 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                     |

| $\chi^2$           | 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.
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 *F₀* 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. Chlorfenapyr can cause reductions in the emergence of *T. pretiosum* (Moura et al., 2004; de Paiva et al., 2017); 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 *F₀* generation and chlorpyriphos on the reduction of the emergence rate of the *F₁* generation of *T. pretiosum* made not possible to perform observations of the biological parameters of the *F₁* generation for these treatments.

No statistical differences were observed on the offspring sex ratio of the *F₁* generation of *T. pretiosum* (*p* = 0.496) (Table 1); however, abamectin, azadirachtin and chlorfenapyr significantly affected this parameter on the *F₂* generation of *T. pretiosum* by increasing the number of males (*p* < 0.001) (Table 2). Nevertheless,
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, 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, 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, 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, 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, and F, generations and modifying the sex ratio of the F, generation. Chorpyrphos 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 thiamethoxam is compatible with T. pretiosum.

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