

# Insecticide toxicity to *Trichogramma pretiosum* (Hymenoptera: Trichogrammatidae) females and effect on descendant generation

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**Abstract** The effect of nine insecticides used in tomato production was evaluated on adults of two *Trichogramma pretiosum* Riley (Hymenoptera: Trichogrammatidae) populations from Rive and Afonso Cláudio, Espírito Santo State, Brazil. The experiment was developed in an acclimatized chamber at  $25 \pm 1^\circ\text{C}$ ,  $70 \pm 10\%$  relative humidity and 14 h photophase. Eggs of *Anagasta kuehniella* (Lepidoptera: Pyralidae), previously immersed in insecticides solutions were offered to females of both *T. pretiosum* populations. *Bacillus thuringiensis*, lufenuron and triflumuron had lowest negative effects on parasitism and viability of individuals of these populations; however, abamectin and pyrethroids (betacyflurin 50 and 125 g/l and esfenvalerate) insecticides reduced parasitism rates. *T. pretiosum* emerged from *A. kuehniella* eggs treated with esfenvalerate but were not able to parasitize non treated eggs of this host. *B. thuringiensis*, lufenuron and triflumuron may be used in integrated pest management programs to

control tomato pests, because they have moderated negative effect on parasitoid wasps.

**Keywords** *Trichogramma pretiosum* · *Lycopersicon esculentum* · Selectivity · Biological control

## Introduction

Parasitoids of the genus *Trichogramma* are important for the biological control in fruit (Pratissoli et al. 2004a, 2005) forest (Oliveira et al. 2000; Soares et al. 2007) and tomato (Miranda et al. 1998) plantations including *Tuta absoluta* (Meyrick) (Lepidoptera: Pyralidae) in Brazil (Pratissoli and Parra 2001). The chemical control of that tomato pest (Pratissoli et al. 2003) and plant resistance (Leite et al. 2001) are used. Natural enemies of the genus *Trichogramma* are an alternative for pest control and to reduce populations of *T. absoluta*, the most important pest of this culture in Brazil, and insecticide applications in the tomato culture (Pratissoli and Parra 2001). However, chemical control is, still, largely used if the biological control fails and therefore is necessary studies about the associated use of insecticides with parasitoids (Pratissoli et al. 2003) and predators (Zanuncio et al. 1998, 2003, 2005).

Methods to test the side effects of pesticides have been developed as a function of the beneficial arthropods and pesticides studied (Desneux et al. 2007). Negative effects of insecticides on populations of the parasitoid *Trichogramma* have been reported (Suh et al. 2000; Brunner et al. 2001; Takada et al. 2001; Vieira et al. 2001), whereas some studies show that insecticides may increase the performance of natural enemies (Komez et al. 2001; Rafalimanana et al. 2002; Delpuech et al. 2005).

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Pesticides may interfere with the feeding behavior by repellent, feeding inhibitor, or reducing olfactory capacity effects (Desneux et al. 2007). Mortality frequently is the only effect that insecticides are screened for, while more conspicuous sublethal effects in beneficial insects, such as altered behavior, reduced reproduction, and reduced longevity, are overlooked. However, studies showed that sublethal effects can severely reduce the performance of biological control agents (Stapel et al. 2000; Desneux et al. 2007).

Insects are affected in different ways when exposed to insecticides and it is important to assess the impact of these chemicals on parasitoid species for its use in integrate pest management programs (Elzen et al. 2000; Hill and Foster 2000; Zehnder et al. 2007). Many organisms react differently when in contact with certain compounds what makes necessary to understand the impact of insecticides on parasitoids to preserve these biological control agents (Elzen et al. 2000; Hill and Foster 2000). In this sense, some insecticides have residual effects on natural enemies affecting the reproductive potential of their offspring (Godoy et al. 2004; Jenkins and Isaacs 2007; Moscardini et al. 2008). Thus, the objective of this study was to determine the effect of nine insecticides, used in the control of *T. absoluta* in tomato plantations, on the emergence and parasitic efficiency of two populations of the parasitoid *Trichogramma pretiosum* Riley (Hymenoptera: Trichogrammatidae) and verify the effect on the second generation of parasitoids.

## Methods

The insecticides: abamectin (1.8%; Syngenta Proteção de Cultivos Ltda; São Paulo; Brazil), *Bacillus thuringiensis* (25,000 IU/mg; Syngenta Proteção de Cultivos Ltda; São Paulo; Brazil), betacyflurin (50 g/l and 125 g/l; Bayer S.A.; São Paulo; Brazil), esfenvalerate (25 g/l; Sumitomo Chemical do Brasil Repres. Ltda; São Paulo; Brazil), lufenuron (50 g/l; Syngenta Proteção de Cultivos Ltda; São Paulo; Brazil), methoxyfenozide (240 g/l; Dow AgroSciences Industrial Ltda; São Paulo; Brazil), tebufenozide (240 g/l; Dow AgroSciences Industrial Ltda; São Paulo; Brazil) and triflumuron (250 g/kg; Bayer S.A.; São Paulo; Brazil) (numbers in parentheses is concentration of active ingredient in commercial product) were evaluated on two *T. pretiosum* populations. Individuals of these two populations were collected in tomato crops in Rive and Afonso Cláudio, Espírito Santo State, Brazil. The populations were obtained from the different localities. Afonso Cláudio has extensive tomato plantations and its *T. pretiosum* population is exposed to more insecticides than the Rive's population. The bioassays were conducted with recently

emerged females from two *T. pretiosum* populations of the same generation. The control treatment had only distilled water. The insecticides evaluated were those used to control tomato crop pests. These are the most used products to control tomato crop pests in Espírito Santo State according to the "Research Institute and Technical Attendance and Rural Extension (INCAPER)".

## Insects rearing

### Host

*Anagasta kuehniella* (Zeller) (Lepidoptera: Pyralidae) was chosen as host because its eggs have been used for mass rearing and field release of parasitoid in Brazil. This host is easy to rear and to multiply the parasitoids for mass release in Brazil. *A. kuehniella* was reared with a diet of integral wheat (67%) and corn (30%) flours and beer yeast (3%). Eggs of *A. kuehniella* (0.4 g) were randomly distributed over the diet. Adults of *A. kuehniella* were daily collected during 5 days with a vacuum cleaner and transferred to PVC tubes (diameter: 200 mm, and length: 25 cm). This rearing system was maintained in an acclimatized chamber ( $25 \pm 1^\circ\text{C}$ ,  $70 \pm 10\%$  relative humidity (rh) and 14:10 L:D).

### Parasitoid

Both *T. pretiosum* populations were collected in commercial tomato plantations using  $8.0 \times 2.5$  cm cardboard strips with  $5 \text{ cm}^2$  central area containing sterile *A. kuehniella* eggs previously exposed to ultraviolet light for 50 min. Each population was multiplied and maintained with sterile *A. kuehniella* eggs glued onto blue rectangle cardboards ( $2.5 \times 10$  cm) with 10% Arabic gum in an acclimatized chamber ( $25 \pm 1^\circ\text{C}$ ,  $70 \pm 10\%$  rh and 14:10 L:D) in glass containers ( $14 \times 7$  cm) containing honey droplets (as food). These containers were covered with microperforated PVC film.

## Experimental procedure

### First generation

From 25 pairs of each *T. pretiosum* population up to 24 h after emergence, females were individualized in tubes ( $3.0 \times 0.5$  cm) with a honey droplet (as food) in its internal wall. Forty sterile *A. kuehniella* eggs (50 min exposed to germicide lamp) were glued on a blue cardboard strip ( $2.5 \times 0.5$  cm) and immersed for 5 s in solutions of each insecticide in the commercial concentrations indicate by the manufacturer for the control of *T. absoluta* in tomato plantations and maintained at room

temperature for 1 h to allow drying. Control eggs were immersed in distilled water. The treatment cardboard strips were then exposed to parasitism by *T. pretiosum* in tubes (3.0 × 0.5 cm) for 24 h in an acclimatized chamber (25 ± 1°C, 70 ± 10% rh and 14:10 L:D). After this period, the cardboards were transferred to glasses tubes (2.5 × 10 cm) and kept in an acclimatized chamber until emergence of adults.

### Second generation

Twenty-five females of *T. pretiosum* obtained from the eggs exposed to the different insecticides were individually placed into the tubes (3.0 × 0.5 cm) and fed with a honey droplet. One cardboard (2.5 × 0.5 cm) with forty sterilized *A. kuehniella* eggs was offered per female parasitoid (for a 24 h period).

### Statistical analysis

The parameters evaluated were percentage of parasitism and emergence in both generations of *T. pretiosum* populations. Data were transformed to  $\arcsin\sqrt{x+1}$ , submitted to variance analysis, and means were compared using a Scott–Knott test at 5% probability (Scott and Knott 1974).

## Results

### Impact on the first generation

The percentage of parasitism of *A. kuehniella* by *T. pretiosum* Rive population was similar in untreated control and with use of *B. thuringiensis*, lufenuron and triflumuron

insecticides. The methoxifenozone and tebufenozide significantly reduced the percentage of parasitism by about 30%, while abamectin, betacyflutin (50 and 125 g/l) and the esfenvalerate insecticides drastically reduced the parasitism rate of this population (Table 1). The parasitism percentage on Afonso Cláudio population was similar to the Rive population in the treatments using esfenvalerate, lufenuron, methoxifenozone and triflumuron and different to the abamectin, *B. thuringiensis*, betacyflutrin and tebufenozide insecticides. The Rive population showed higher parasitism than Afonso Cláudio population in eggs without treatment with insecticide (Table 1).

The percentage of emergence of *T. pretiosum* from Afonso Cláudio population was lower with esfenvalerate, whereas Rive population presented lower values with abamectin. The results of Rive population using *B. thuringiensis* were similar to those of the control. The percentage of emergence of adults from Rive population was lower with abamectin, beacyflutrin (125 g/l), methoxifenozone and triflumuron than those of Afonso Cláudio population. However, this population had lower emergence of adults than that of Rive when betacyflutrin (50 g/l) was used (Table 2).

### Impact on the second generation

The population from Afonso Cláudio had lower parasitism with the insecticides abamectin, betacyflutrin, methoxifenozone, tebufenozide and triflumuron in the second generation when compared with control group. Similar results were found for the Rive's population exposed to abamectin and betacyflutrin (125 g/l) such as found with the abamectin and betacyflutrin (125 g/l) for the population of Rive. The parasitism rate of this population was lower

**Table 1** Percentage of parasitism (mean ± standard error) of *Anagasta kuehniella* eggs treated with insecticides by two populations of *Trichogramma pretiosum*, first generation 25 ± 1°C, 70 ± 10% relative humidity and 14 h photo phase. *F* and *P* values

Insecticide	Rive population	Afonso Cláudio population
Abamectin (1.8%)	2.76 ± 0.77 D, b	11.90 ± 1.35 F, a
<i>Bacillus thuringiensis</i>	69.54 ± 2.43 A, b	79.00 ± 2.46 A, a
Betacyflutrin (50 g/l)	13.70 ± 1.49 C, a	6.50 ± 1.27 F, b
Betacyflutrin (125 g/l)	8.10 ± 1.49 C, b	22.70 ± 2.50 E, a
Esfenvalerate (25 g/l)	0.40 ± 0.19 D, a	0.40 ± 0.19 G, a
Lufenuron (50 g/l)	70.28 ± 2.98 A, a	70.00 ± 1.54 B, a
Methoxifenozone (240 g/l)	45.30 ± 3.69 B, a	53.76 ± 3.22 D, a
Tebufenozide (240 g/l)	47.26 ± 3.13 B, b	61.00 ± 2.54 C, a
Triflumuron (250 g/l)	66.00 ± 2.90 A, a	65.70 ± 3.72 C, a
Control (distilled water)	70.00 ± 2.19 A, a	62.48 ± 3.37 C, b
	<i>F</i>	<i>P</i>
Insecticide	289.895	0.00001
Population	13.367	0.00034
Insecticide × population	5.489	0.00007

Means followed by the same capital letter, per column, or small letter, per row, are not different by the Scott–Knott at  $P > 0.05$

**Table 2** Emergence percentage (mean  $\pm$  standard error) from *Anagasta kuehniella* eggs treated with insecticides by two populations of *Trichogramma pretiosum*, first generation 25  $\pm$  1°C, 70  $\pm$  10% relative humidity and 14 h photo phase. *F* and *P* values

Insecticide	Rive population	Afonso Cláudio population
Abamectin (1.8%)	9.04 $\pm$ 2.69 D, b	57.62 $\pm$ 5.78 B, a
<i>Bacillus thuringiensis</i>	94.25 $\pm$ 1.19 A, a	96.88 $\pm$ 0.89 A, a
Betacyflutrin (50 g/l)	81.63 $\pm$ 6.74 B, a	59.96 $\pm$ 9.43 B, b
Betacyflutrin (125 g/l)	61.03 $\pm$ 9.45 C, b	88.60 $\pm$ 7.27 A, a
Esfenvalerate (25 g/l)	1.44 $\pm$ 0.80 D, a	0.00 $\pm$ 0.00 C, a
Lufenuron (50 g/l)	55.50 $\pm$ 2.41 C, a	59.33 $\pm$ 2.70 B, a
Methoxifenozone (240 g/l)	66.21 $\pm$ 4.75 C, b	89.71 $\pm$ 1.87 A, a
Tebufenozide (240 g/l)	78.11 $\pm$ 3.31 B, a	88.70 $\pm$ 2.01 A, a
Triflumuron (250 g/l)	63.07 $\pm$ 4.91 C, b	86.94 $\pm$ 2.36 A, a
Control (distilled water)	90.46 $\pm$ 2.53 A, a	90.83 $\pm$ 1.86 A, a
	<i>F</i>	<i>P</i>
Insecticide	80.068	0.00001
Population	28.683	0.00005
Insecticide $\times$ population	8.728	0.00007

Means followed by the same capital letter, per column, or small letter, per row, are not different by the Scott–Knott at  $P > 0.05$

**Table 3** Parasitism percentage (means  $\pm$  standard error) in *Anagasta kuehniella* eggs from the first generation, treated with insecticides by two populations of *Trichogramma pretiosum*, second generation 25  $\pm$  1°C, 70  $\pm$  10% relative humidity and 14 h photo phase. *F* and *P* values

Insecticide	Rive population	Afonso Cláudio population
Abamectin (1.8%)	57.12 $\pm$ 5.16 B, a	64.72 $\pm$ 4.10 C, a
<i>Bacillus thuringiensis</i>	85.52 $\pm$ 2.51 A, a	91.40 $\pm$ 2.01 A, a
Betacyflutrin (50 g/l)	81.70 $\pm$ 4.62 A, a	89.50 $\pm$ 1.86 A, a
Betacyflutrin (125 g/l)	63.00 $\pm$ 3.01 B, a	66.90 $\pm$ 2.38 C, a
Esfenvalerate (25 g/l)	0.00 $\pm$ 0.00 C	nd
Lufenuron (50 g/l)	83.08 $\pm$ 2.56 A, a	84.68 $\pm$ 2.14 A, a
Methoxifenozone (240 g/l)	80.90 $\pm$ 2.19 A, a	51.48 $\pm$ 2.48 D, b
Tebufenozide (240 g/l)	83.40 $\pm$ 2.05 A, a	47.66 $\pm$ 3.12 D, b
Triflumuron (250 g/l)	74.70 $\pm$ 2.08 A, a	55.92 $\pm$ 3.35 D, b
Control (distilled water)	85.24 $\pm$ 2.60 A, a	75.20 $\pm$ 1.65 B, b
	<i>F</i>	<i>P</i>
Insecticide	25.13	<0.0001
Population	40.82	<0.0001
Insecticide $\times$ population	15.30	<0.0001

Means followed by the same capital letter, per column, or small letter, per row, are not different by the Scott–Knott at  $P > 0.05$ . nd: not determined

with the methoxifenozone, tebufenozone, triflumuron and the control than those of the Rive population in the second generation (Table 3).

The emergence of individuals of both *T. pretiosum* populations was not affected by the insecticides in the first generation, except for the esfenvalerate that prevented parasitism in the first generation and for that reason had no emergence of adults in the second generation (Table 4).

## Discussion

The results showed that the parasitic efficiency and adult emergence of *T. pretiosum* were affected for the insecticide used and for the population origin of the parasitoid. Within the insecticides studied, pyrethroids and abamectin decrease

the parasitism and adult emergence in both generations of the parasitoid. The insecticides acting as growth regulator and *B. thuringiensis* can be used in integrated pest management programs, since they have lowest effect on the natural enemy *T. pretiosum*.

The reduced parasitism of individuals of both *T. pretiosum* populations with the pyrethroids (betacyflutrin (50 and 125 g/l) and esfenvalerate) and abamectin might have been caused by the high toxicity of these products and their repellent action on *T. pretiosum* females such as reported for other parasitoids (Jacobs et al. 1984; Singh and Varma 1986), since parasitoids can be olfactory orientation affected by insecticides resulting in a low capacity to found their hosts (Desneux et al. 2004a).

A higher mortality of *T. pretiosum* adults of populations from Rive and Afonso Cláudio with the pyrethroids and

**Table 4** Percentage of emergence (mean  $\pm$  standard error) from *Anagasta kuehniella* from individuals of the first generation, treated with insecticides by two populations of *Trichogramma pretiosum*, second generation 25  $\pm$  1°C, 70  $\pm$  10% relative humidity and 14 h photo phase. *F* and *P* values

Insecticide	Rive population	Afonso Cláudio population
Abamectin (1.8%)	82.54 $\pm$ 5.38 C, a	81.48 $\pm$ 3.92 B, a
<i>Bacillus thuringiensis</i>	92.43 $\pm$ 1.60 A, a	96.35 $\pm$ 0.52 A, a
Betacyflutrin (50 g/l)	98.75 $\pm$ 0.32 A, a	97.18 $\pm$ 0.62 A, a
Betacyflutrin (125 g/l)	83.19 $\pm$ 2.20 C, b	93.42 $\pm$ 1.32 A, a
Esfenvalerate (25 g/l)	–	–
Lufenuron (50 g/l)	87.58 $\pm$ 1.26 B, b	94.45 $\pm$ 1.13 A, a
Methoxifenozone (240 g/l)	88.78 $\pm$ 1.84 B, a	93.45 $\pm$ 2.14 A, a
Tebufenozide (240 g/l)	87.75 $\pm$ 1.96 B, a	90.48 $\pm$ 2.10 A, a
Triflumuron (250 g/l)	96.62 $\pm$ 0.83 A, a	89.67 $\pm$ 2.63 A, b
Control (distilled water)	83.56 $\pm$ 2.76 C, a	78.81 $\pm$ 1.56 B, a
	<i>F</i>	<i>P</i>
Insecticide	20.92	<0.0001
Population	8.13	0.0045
Insecticide $\times$ population	5.32	<0.0001

Means followed by the same capital letter, in the column, or small letter, in the row, are not different by the Scott–Knott at  $P > 0.05$

abamectin can also explain the lower parasitism by individuals of these populations. The high mortality of *Trichogramma cacoeciae* Marchal and *T. pretiosum* was also reported with insecticides of these chemical groups (Youssef et al. 2004; Rocha and Carvalho 2004), and pyrethroids are more toxic in case of aphid parasitoids when compared with carbamate and carbamyltriazole (Desneux et al. 2004b). The parasitism rate of individuals (*T. pretiosum*) from Rive's population was similar with the growth regulators lufenuron and triflumuron, while it was higher with lufenuron in case of Afonso Cláudio population. It shows that these growth regulators can be used in integrated management programs of Lepidoptera pests aiming to conserve *Trichogramma* species (Suh et al. 2000; Brunner et al. 2001; Hewa-Kapuge et al. 2003; Pratisoli et al. 2004b, c; Moura et al. 2005) The moderate reduction on parasitism rate for Rive's population of this parasitoid with the methoxifenozone and tebufenozone shows that these insecticides could be used, in integrated pest management programs, but pending restrictions rules.

The higher impact of some insecticides on the emergence of *T. pretiosum* from Rive than those of Afonso Cláudio population corroborates reports of biological differences among *Trichogramma* species populations, which can be related to biotic (adaptability and intrinsic capacity of the population in a specific environment) and abiotic (environmental conditions) factors of their origin place (Pratisoli et al. 2003; Moura et al. 2005). Thus, the population from Afonso Cláudio may be resistant or tolerant to insecticides (Croft 1990; Li et al. 2007), since this region has large tomato plantations resulting in a higher use of insecticides than Rive region. In addition difference between the two populations can be also due to stimulus-independent behavioristic resistance as reported in some insects by Georgioui (1972).

Both populations of *T. pretiosum* showed different responses to the insecticides. The Rive's population had the parasitism more affected negatively, although the Afonso Cláudio population had presented lower parasitism in the control group. These findings corroborates that different populations can be affected in different ways (Pratisoli and Parra 2001) suggesting that many parasitoid's populations should be used in test of insecticide toxicity.

The two pyrethroid insecticides betacyflutrin and esfenvalerate (beside abamectin) presented great reduction on the parasitism rate and the percentage of emergence of individuals of both *T. pretiosum* populations, especially those from Rive. *B. thuringiensis* and the growth regulators (lufenuron and triflumuron) presented the lowest reduction on parasitism and percentage of emergence. It demonstrates that these insecticides can be used in integrated programs of pest management in the tomato crops. However these findings should be tested in field conditions to assess the selectivity of these chemicals into more realistic conditions (in field conditions).

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