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Selectivity of Insecticides Against Adult *Trichogramma pretiosum* Riley (Hymenoptera: Trichogrammatidae) on Cassava

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Abstract

In this study, the effects of various chemical and biological insecticides on adults of the parasitoid species *Trichogramma pretiosum* (Riley) were examined in cassava. The chemical insecticides zeta-cypermethrin, lufenuron + profenofós, imidacloprid, thiamethoxam, and teflubenzuron and the biological agents *Bacillus thuringiensis* and *Baculovirus erinnyis* were evaluated. For each treatment group, the effects were evaluated using *T. pretiosum* mortality rates, longevity of females, the number of eggs parasitized by the F_0 generation, and number of emergent F_1 parasitoids. The insecticides were classified using the International Organization for Biological Control categories. The tested insecticides had little effect on longevity and survival of adults, but all affected parasitism (F_0) and were moderately harmful to the parasitoid. In relation to the emergent (F_1) generation, the pesticides were either harmless or slightly harmful, except for zeta-cypermethrin which was classified as harmful with a 100% reduction in parasitoid emergence. The results indicate that the tested insecticides were moderately harmful to *T. pretiosum* in the laboratory; future studies will examine the effects of the insecticides under semi-field and field conditions to confirm their toxicity.

Keywords: chemical and biological control, Parasitoid, *Erinnyis ello*, pesticide, integrated pest management

1. Introduction

Cassava is an important food plant that is widely cultivated in Africa, Asia, Oceania and Latin Americat (CONAB, 2017). In Brazil, the largest producer in Latin America (CONAB, 2017), with crop showed an increase of 3.7% between 2016 and 2017. An estimated 21 million tons of cassava roots have been planted, corresponding to more than 1.4 million hectares of cultivated land (IBGE, 2017). Unfortunately, crop productivity can be reduced by the action of pest insects, such as the moth *Erinnyis ello*, which is a major pest that can cause defoliation of cassava plants (Pratissoli, Zanuncio, Barros, & Oliveira, 2002). In general, attempts to control pests generally make use of chemical or biological insecticides.

Biological control of caterpillars is carried out by application of *Baculovirus erinnyis* or *Bacillus thuringiensis*, which are selective for some beneficial species of entomofauna on cassava plants. Chemical control mainly involves application of pyrethroid and organophosphate insecticides (MAPA, 2016). However, the use of broad-spectrum chemical insecticides can also directly or indirectly affect beneficial insects such as parasitoids and predators. These organisms are of great importance in agroecosystems for maintenance of populations of insect pests below the level of economic damage (Carvalho, Parra, & Baptista, 2001).

Among the natural enemies of pest species present on cassava plants, more than 30 natural control agents have been described for *E. ello*, such as parasitoids, predators, and pathogens (bacteria, fungi, and viruses) that act on eggs, caterpillars, and pupae. Parasitoids of the genus *Trichogramma* attack eggs and therefore have the advantage of controlling the pest before the occurrence of damage to the crop (Botelho, 1997; Parra, Botelho, Côrrea, & Bento, 2002). Oliveira, Gomez, Rohden, Arce, and Duarte (2010) recorded natural parasitism of *E.*

ello eggs by *Trichogramma marandobai*, *Trichogramma manicobai* and *Trichogramma pretiosum* in cassava cultivars in Mato Grosso do Sul.

Integrated pest management using chemical and biological methods is feasible in cassava as in other crop species; one of the important aspects of an integrated management program is that the different control methods employed do not adversely interact to reduce their individual effectiveness (Oliveira, Antigo, Carvalho, & Glaeser, 2013).

With regard to cassava, few studies have investigated the selectivity of chemical insecticides on *T. pretiosum* parasitoids. Therefore, the objective of this study was to evaluate the effects of various insecticides on *T. pretiosum* in cassava crops.

2. Material and Methods

2.1 Insecticides

The following chemical insecticides (active ingredient, commercial brand, and dosage) were evaluated: imidacloprid, Confidor, 110 mL/ha; lufenuron + profenofós, Curyom 550 EC, 300 mL/ha; teflubenzuron, Nomolt 150 CS, 165 mL/ha; thiamethoxam, Actara 250 WG, 150 g/ha; zeta-cypermethrin, Mustang 350 EC, 150 mL/ha. The biological agents *Bacillus thuringiensis* (Dipel, 500 g/ha) and *Baculovirus erinnyis* (50 mL/ha) were also tested. The treatments lufenuron + profenofós and zeta-cypermethrin were evaluated at the manufacturer's maximum recommended dose. The compounds imidacloprid, teflubenzuron, and thiamethoxan are still in the registration phase by the Instrução Normativa Conjunta (Joint Normative Instruction) nº1, of June 16, 2014 (MAPA, 2016) and they were used at doses suggested by the manufacturers. For *B. thuringiensis* and *B. erinnyis*, the maximum recommended doses for cassava were used (Agrofit, 2018). Distilled water was used as a control.

2.2 Side-effects on the Maternal Generation (= F_0) Parasitoids

20 *T. pretiosum* females, up to 24 hours old, obtained from the creation of laboratory were placed into individual glass tubes (8.5 cm high × 2.5 cm diameter) and fed with a honey droplet deposited on the inner wall of the tube. Tubes were sealed with PVC plastic film perforated by an entomological pin for aeration. 20 blue cards (1.0 × 1.0 cm), each containing 30 eggs of the alternate host *Anagasta kuehniella* (Zeller) (Lepidoptera: Pyralidae) adhered using 10% diluted arabic gum, were immersed for five seconds in the various insecticides described in section 2.1. The cards were then placed on a paper towel at room temperature for one hour to remove excess solution and placed into each tube containing a single *T. pretiosum* female for 24 hours; this protocol is based on those proposed by Brugger et al. (2010), and Vianna et al. (2009).

The culture tubes were maintained in an air-conditioned room at 25±2 °C, 60±10% RH, and 12-hour photophase. Each treatment consisted of 20 replicates, each replicate consisting of a card carrying 30 *A. kuehniella* eggs that had potentially been parasitized. A randomized experimental design was used. Mortality of the adults from potentially parasitized eggs was evaluated; the longevity of females after parasitism, and the parasitism rate of the F_0 generation (number of eggs parasitized by females over 24 hours) were also evaluated. In order to verify possible effects on individuals of the F_1 generation, the rate of emergence [(Number of eggs with parasitoid exit orifice/Total number of parasitized eggs) × 100] of parasitoids from the treated eggs was evaluated.

2.3 Statistical Analysis and Classification of Insecticides

Datasets were first tested for a normal distribution (Kolmogorov test) and homoscedasticity (Bartlett test) and transformed if required; the data were then subjected to ANOVA, and significant differences between means were identified using the Tukey test ($\alpha < 0.05$). Statistical analyses were performed using Statgraphics® Centurion version XVI (Statistical Graphics Corp. 1994-2000).

In accord with IOBC recommendations, the tested insecticides were classified by the percentage reduction (PR) of parasitoid beneficial abilities (survival, parasitism, and emergence) in relation to the control treatment: class 1 = innocuous, reduction less than 30%; class 2 = slightly harmful, 30-79%; class 3 = moderately harmful, 80-99%; and class 4 = harmful, > 99% (Sterk et al., 1999). PR = 100 – (% General average of insecticide treatment/Overall mean of control treatment) × 100.

3. Results and Discussion

3.1 Effect of Insecticides on Mortality of Parasitoids in the Maternal (F_0) Generation

The mortality rates of *T. pretiosum* (F_0) females that came into contact with *A. kuehniella* eggs treated with all products were less than 20% and thus were considered innocuous according to IOBC toxicity classes (Table 1).

Table 1. Mortality (±SD), percentage of reduction (PR) and toxicological class for adults of *Trichogramma pretiosum* after contact in *Anagasta kuehniella* eggs treated with insecticides used in cassava crops. Means followed by the same lowercase letter in the column do not differ by test Tukey ($p > 0.05$)

Treatment	**Commercial Product Dosage**	**Mortality (%)**	**PR[1] (%)**	**Class[2]**	**Longevity of females (days)**
Control	-	0.0±0.00 b	-	-	11.00 a
Teflubenzuron	165 mL/ha *	0.0±0.00 b	-	1	9.05 a
Thiamethoxam	150 g/ha *	20.0±0.09 a	20.0	1	10.93 a
Imidacloprid	110 mL/ha *	20.0±0.09 a	20.0	1	11.00 a
Zeta-Cypermethrin	150 mL/ha	20.0±0.09 a	20.0	1	8.25 a
Lufenuron+Profenofos	300 mL/ha	15.0±0.08 a	15.0	1	9.11 a
Baculovirus erinnyis	50 mL/ha	10.0±0.06 a	10.0	1	8.05 a
Bacillus thuringiensis	500 g/ha	0.0±0.00 b	-	1	10.10 a

Note. * Products in phase of registration by Joint Normative Instruction nº1, of June 16, 2014, dose used supplied by the manufacturer. [1] Average percentage reduction in survival of *T. pretiosum*. [2] Toxicity classes recommended by Sterk et al. (1999).

The effect of an insecticide on beneficial organisms varies according to the mode of action, the dose, the crop treated, and the natural enemy species studied. Neurotoxic insecticides generally have poor selectivity with regard to parasitoids and other natural enemies (Parra et al., 2002). However, the insecticides used in the present study were largely innocuous in terms of adult mortality.

The neonicotinoid thiamethoxam was reported to cause 100% mortality in adult *Trichogramma galloi* (Zucchi 1988) and was therefore assessed as harmful (Oliveira et al., 2013). The difference in rates of mortality found here and by Oliveira et al. (2013) may be related to the dose used, as the latter study used the maximum dose in a sugar cane crop (1000 g/ha), while the present study used the maximum dose suggested by the manufacturer (150 g/ha).

Imidacloprid, another neonicotinoid, has been reported to be harmful to survival of adult *Trichogramma brassicae* (Bezdenko) after 3 hours of exposure (Hewa-kapuge, McDougall, & Hoffmann, 2003) and to adult *Trichogramma chilonis* (Ishii) (Preetha, Stanley, Suresh, Kuttalam, & Samiyappan, 2009).

Zeta-cypermethrin is a broad spectrum pyrethroid and, in the present study, induced 20% mortality in adult *T. pretiosum* under laboratory conditions (Table 1). Therefore, this insecticide was classified as innocuous. This finding is in agreement with Souza (2011), who did not observe negative effects using a compound of the same chemical group on *T. pretiosum*.

Lufenuron (benzoylurea) + profenophos (organophosphate) was classed as innocuous to survival of female *T. pretiosum* parasitizing treated *A. kuehniella* eggs. Oliveira et al. (2013) reported similar results for *T. galloi* after treatment of *Diatraea saccharalis* (Fabricius, 1794) (Lepidoptera: Crambidae) eggs with triflumuron, an insecticide belonging to the same chemical group (benzoylurea). These authors noted that adult phase parasitoids did not undergo ecdysis.

The effect of *B. erinnyis* on the survival of adult *T. pretiosum* has not previously been studied. This insecticide was classified as innocuous to adults of this parasitoid (Table 1). *Baculovirus anticarsia* has been previously been used on adult *T. pretiosum* and found to be innocuous (Amaro, Bueno, Pomari-Fernandes, & Neves, 2015).

Exposure of *T. pretiosum* to *B. thuringiensis*-treated eggs did not affect survival. Similar results have been obtained for *Trichogramma dendrolimi* Matsumura (Takada, Kawamura, & Tanaka, 2001) and *Trichogramma pratissolii* (Querino & Zucchi) (Pratissoli, Polanczyk, Vianna, Andrade, & Oliveira, 2006).

T. pretiosum females that came in contact with insecticides through 24 hour exposure to treated eggs showed an average longevity of approximately nine days (Table 1); no significant differences were present among the treatments ($p > 0.05$).

3.2 Effect of Insecticide Treatment on the Number of Parasitized Eggs (F_0)

The number of eggs parasitized by *T. pretiosum* (F_0) females was evaluated for each treatment. The results indicated that all of the tested insecticides reduced the rate of parasitism. Therefore, the treatments were classified as moderately harmful to the parasitoid (Table 2).

Table 2. Number (±SD) of parasitized eggs and percent reduction (PR) of *Trichogramma pretiosum* in *Anagasta kuehniella* eggs treated with insecticides used in cassava crops. Means followed by the same lowercase letter in the column do not differ by test Tukey ($p > 0.05$)

Treatment	**Commercial Product Dosage**	**Eggs parasitized (%)**	**PR[1] (%)**	**Class[2]**
Control	-	35.3±1.61 a	-	-
Teflubenzuron	165 mL/ha *	2.3±0.25 b	93.40	3
Thiamethoxam	150 g/ha *	6.0±0.52 b	82.93	3
Imidacloprid	110 mL/ha *	2.7±0.36 b	92.35	3
Zeta-Cypermethrin	150 mL/ha	1.0±0.14 b	97.05	3
Lufenuron+Profenofos	300 mL/ha	2.1±0.34 b	93.91	3
Baculovirus erinnyis	50 mL/ha	6.1±0.62 b	82.73	3
Bacillus thuringiensis	500 g/ha	5.5±0.57 b	84.46	3

Note. * Products in phase of registration by Joint Normative Instruction nº1, of June 16, 2014, dose used supplied by the manufacturer. [1] Average percentage reduction in survival of *T. pretiosum*. [2] Toxicity index recommended by Sterk et al. (1999).

Carvalho, Moura, Bueno, (2006) classified teflubenzuron as innocuous with respect to parasitism by *T. pretiosum*. This difference in classification may be related to the use of a higher insecticide dose in the present study.

Thiamethoxam was classified as moderately harmful in a study of parasitism by *T. galloi* on eggs of *Diatraea saccharalis* (Lepidoptera: Crambidae) (Oliveira et al., 2013). Conversely, Pratissoli et al. (2009), *Sitotroga cerealella* (Lepidoptera: Gelechiidae), and *A. kuehniella*, found that the insecticide selectivity of *T. pretiosum* in the hosts *Anticarsia gemmatalis* (Lepidoptera: Noctuidae), *Sitotroga cerealella* evaluated thiamethoxam on the host *Anticarsia gemmatalis* Lepidoptera: Pyralidae).

Similar results to those here have been reported in studies of imidacloprid treatment for *Trichogramma platneri* (Nagarkatti) (Brunner, Dunley, Doer, & Beers, 2001), *T. atopovirilia* (Maia, Carvalho, Leite, Oliveira, & Makyama, 2010), and *T. pretiosum* (Carvalho et al., 2010).

Zeta-cypermethrin has previously been reported to have a moderately harmful effects on parasitism of *T. pretiosum* on eggs of *Ephestia kuehniella* (Lepidoptera: Pyralidae) and *Sitotroga cerealella* (Lepidoptera: Gelechiidae) (Bastos, Almeida, & Suinaga, 2006).

Lufenuron reduced parasitism by *T. pretiosum* on eggs of *Spodoptera frugiperda* (Lepidoptera: Noctuidae), *A. kuehniella*, *S. cerealella* (Bastos et al., 2006) and *A. gemmatalis* (Lepidoptera: Noctuidae), similar to the results obtained here.

Baculovirus erinnyis was moderately harmful to parasitism by *T. pretiosum* (F_0). One possible mechanism for these could be related to a repellent activity of the virus. In a test of free choice in the laboratory, it was found that females avoided contact with virus-treated eggs (unpublished data).

In contrast to other published results that classified *B. thuringiensis* as innocuous (Amaro et al., 2015; Vianna et al., 2009; Filho, Botton, Grutzmacher, Giolo, & Manzoni, 2006), our findings show a moderately harmful effect of *B. thuringiensis* (Table 2). These differences may be related to the source of the biological agent, which may vary with regard to the number of viable spores.

3.3 Effect of Insecticides on Emergence of the First (F_1) Generation

Zeta-cypermethrin reduced the rate of emergence of *T. pretiosum* (F_1) from treated *A. kuehniella* eggs and was classified as harmful. Teflubenzuron and lufenuron + profenophos were classified according table the IOBC as slightly harmful (Table 3). The remaining insecticides were all classified as innocuous (Table 3).

Table 3. Emergence (± SD) and percentage reduction (PR) the *Trichogramma pretiosum* (F_1) (Hymenoptera: Trichogrammatidae) in *Anagasta kuehniella* (Lepidoptera: Pyralidae) eggs treated with insecticides used in cassava crops. Means followed by the same lowercase letter in the column do not differ by test Tukey ($p > 0.05$)

Treatment	**Commercial Product Dosage**	**Emergency (%)**	**PR[1] (%)**	**Class[2]**
Control	-	75.0±1.43 a	-	-
Teflubenzuron	165 mL/ha *	42.7±0.25 a	43.03	2
Thiamethoxam	150 g/ha *	80.0±0.70 a	-	1
Imidacloprid	110 mL/ha *	73.4±0.80 a	2.19	1
Zeta-Cypermethrin	150 mL/ha	0.0±0.00 b	100.0	4
Lufenuron+Profenofos	300 mL/ha	52.5±0.62 a	30.04	2
Baculovirus erinnyis	50 mL/ha	87.1±0.89 a	-	1
Bacillus thuringiensis	500 g/ha	87.14±0.89 a	-	1

Note. *Product in the stage of registration by Joint Normative Instruction nº1, of June 16, 2014, dose used supplied by the manufacturer. [1] Average percentage reduction in survival of *T. pretiosum*. [2] Toxicity index recommended by Sterk et al. (1999).

The reduced rate of emergence of parasitoids (F_1) from eggs treated with teflubenzuron or lufenuron + profenophos may be related to the mode of action of benzoylurea compounds, which act as growth regulators and inhibit synthesis of chitin. Thus, these compounds may affect formation of the parasitoid since the larvae hatch within their host and initiate feeding on a substrate contaminated with insecticide (Cônsoli, Botelho, & Parra, 2001; Oliveira et al., 2013). Oliveira et al. (2013) reported a 98.38% reduction in *T. galloi* emergence from *D. saccharalis* eggs treated with triflumuron, another benzoylurea compound, and classified the insecticide as moderately harmful. However, Carvalho et al. (2003) did not find a significant effect of lufenuron (benzoylurea insecticide) on emergence of *T. pretiosum* in treated *A. kuehniella* eggs and classified the insecticide as innocuous.

The results obtained for thiamethoxan were consistent with those of Moura, Carvalho, and Rigitano (2005), who classified this insecticide as innocuous for the emergence of *T. pretiosum* from treated eggs at different immature phases of the parasitoid. However, our results differ from those of Oliveira et al. (2013) for *T. galloi*, who classified the insecticide as slightly harmful. These differences may be related to the doses used: Oliveira et al. (2013) used the maximum recommended dose for sugar cane, which was approximately seven-fold higher than the dose used in the present research. The two studies also investigated different *Trichogramma* and host species.

In the present study, imidacloprid was classified as innocuous in contrast to the studies by Brunner et al. (2001) and Carvalho et al. (2003), who found this insecticide to be toxic to *T. platneri* and *T. pretiosum* under laboratory conditions. The different outcomes might be related to the use of different species of parasitoids.

Zeta-cypermethrin was classified as harmful as it caused a 100% reduction in the emergence of *T. pretiosum* from *A. kuehniella* eggs. Similar results were obtained by Bastos et al. (2006), who observed a reduction in the emergence of *T. pretiosum* in the hosts *A. kuehniella* and *S. cerealella*. This reduction demonstrates the toxicity the insecticide that would be acts directly on the central nervous system of the insects, resulting in high mortality rates.

No previous investigations have been reported on the effect of *B. erinnyis* on emergence of egg parasitoids. In the present study, we classified this biological insecticide as innocuous.

The biological insecticide *B. thuringiensis* was found innocuous for the emergence of parasitoids. Similarly, Amaro et al. (2015) reported that this insecticide was harmless for the emergence of *T. pretiosum* pupae, while Pratissoli et al. (2006) classified the insecticide as innocuous to *T. pratissolii* after feeding the parasitoids with honey containing *B. thuringiensis*.

In general, the different insecticides evaluated were innocuous to the survival of *T. pretiosum*. However, many showed some effect on parasitism. There is a possibility of parasitoid repellency by the tested products or by transovarian action.

If *T. pretiosum* is included in integrated pest management programs for the biological control of *E. ello* cassava crops, our results suggest that the releases be carried out 24 hours before spraying with insecticides so that use of insecticides does not affect the development of *T. pretiosum* in the field.

4. Conclusion

The tested compounds were classified as innocuous for survival and moderately injurious to parasitism (maternal generation). With regard to parasitoid emergence (F_1), the insecticides were innocuous or slightly harmful, with the exception of zeta-cypermethrin which was harmful.

As the evaluated insecticides were moderately harmful to parasitism by *T. pretiosum* in the laboratory, new semi-field and field tests are required in order to confirm their toxicities on the biological characteristics studied.

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