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Selectivity of insecticides used in corn crops to adult *Trichogramma atopovirilia* (Hymenoptera: Trichogrammatidae)

Selectividad de insecticidas usados en maíz a los adultos *Trichogramma atopovirilia* (Hymenoptera: Trichogrammatidae)

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Abstract: The aim of this work was to evaluate the toxicity of the insecticides imidacloprid/ß-cyfluthrin (Connect 100/12.5 SC), chlorfenapyr (Pirate 240 SC), chlorpyrifos (Astro 450 EW), novaluron (Rimon 100 CE), spinosad (Tracer 480 SC) and triflumuron (Certero 480 SC), as used in corn crops *(Zea mays*), to parent generation females and to F_1 and F_2 generation specimens of *Trichogramma atopovirilia*. Eggs of *Anagasta kuehniella* were glued to cardstock strips and placed under a germicidal lamp to kill embryos. These were then sprayed with the chemical products using a Potter tower and exposed to parasitism 24, 48, and 96 hours after application of the compounds for a span of 24 hours. We evaluated the number of dead specimens and the number of eggs parasitized by parent generation females, as well as the percent emergence and parasitic capacity of the F_1 and F_2 generations. Chlorfenapyr, spinosad, chlorpyrifos, and imidacloprid/ß-cyfluthrin were moderately harmful to adult *T. atopovirilia*, while novaluron was slightly harmful. Triflumuron was harmless and could be used in integrated pest management programs intended to preserve adult *T. atopovirilia* in corn crops.

Key words: Pesticides. Parasitoids. *Zea mays*. Toxicity. *Anagasta kuehniella.*

Resumen: Se evaluó la toxicidad de los insecticidas imidacloprid/ ß-ciflutrina (Connect 100/12,5 SC), clorfenapir (Pirate 240 SC), clorpirifos (Astro 450 EW), novalurom (Rimon 100 CE), spinosade (Tracer 480 SC) y triflumurom (Certero 480 SC), utilizados en el cultivo de maíz *(Zea mays*), para adultos de la generación maternal de *Trichogramma atopovirila* y para especímenes de las generaciones F_1 y F_2. Huevos de *Anagasta kuehniella* fueron adheridos a cartulinas e irradiados con una lámpara antigermicida para matar a los embriones. Luego, se les aplicó mediante una torre de Potter los productos químicos y se expusieron durante 24 h a parasitismo a las 24, 48 y 96 h después de la aplicación de los compuestos. Se evaluó el número de especímenes muertos y el número de huevos parasitados por hembra de la generación maternal, también el porcentaje de emergencia y la capacidad de parasitismo de las generaciones F_1 y F_2. Clorfenapis, espinosade, clorpirifós e imidacloprid/ß-ciflutrina fueron moderadamente perjudiciales para los adultos de *T. atopovirila*, mientras novalurom fue levemente perjudicial. Triflumurom fue inocuo y puede ser utilizado en programas de manejo integrado de plagas donde se busque preservar adultos de *T. atopovirila* en el cultivo de maíz.

Palabras clave: Pesticidas. Parasitoides. *Zea mays*. Toxicidad. *Anagasta kuehniella.*

Introduction

Fall armyworm *Spodoptera frugiperda* (J.E. Smith, 1797) (Lepidoptera: Noctuidae) is the most common pest to affect corn crops, being present every crop year and causing increasingly extensive damage to plantations, potentially affecting as much as 37% of the crop (Mendes *et al*. 2008). That and other pests of corn crops are mainly controlled by chemical products, which in turn have been causing emergence of secondary pests, resurgence and selection of resistant populations, in addition to causing a negative impact on the environment (Economic and Social Research Council 2009). Bearing that in mind, the use of alternative pest control methods to protect corn crops is a matter of the greatest importance as an effort to minimize these damaging effects, including biological control using parasitoids and predators.

Among natural enemies that help control fall armyworm in corn crops are parasitoids of the genus *Trichogramma*, drawing worldwide attention for being egg parasites and killing their hosts before emergence and attack to the plant (Lundgren *et al*. 2002).

Some authors noted the presence of *Trichogramma atopovirilia* Oatman & Platner,1983 (Hymenoptera: Trichogrammatidae) parasitizing eggs of *S. frugiperda* in field conditions, demonstrating their potential as pest control agents in corn crops (Alvarez and Roa 1995; Zucchi and Monteiro 1997). Beserra and Parra (2004) evaluated the parasitic capacity and number of adults emerging per egg of *S. frugiperda* in a laboratory and observed that mean values were higher for *T. atopovirilia* than for *Trichogramma pretiosum* Riley, 1879 (Hymenoptera: Trichogrammatidae). They reported that *T. atopovirilia* has stronger chances of increasing its population in a shorter time than *T. pretiosum*, thus being apparently more suitable for biological control of *S. frugiperda*.

The effectiveness of these parasitoids in integrated pest control management programs, however, is conditional on the use of chemical products that will not affect the parasitism and development of parasite populations, in other words, it is conditional to the application of selective compounds (Carvalho *et al*. 2002; Degrande *et al*. 2002; Foerster 2002).

In studying the effect of 40 agrochemicals in various commercial formulations on adult individuals of *T. atopo-*

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virilia, Manzoni *et al*. (2007) verified that 45% (18) were found to be harmless, 15% (6) were slightly harmful, 12.5% (5) were moderately harmful and 27.50% (11) were harmful. The variations in how this parasitoid species responded to pesticides demonstrate the need for studies in search of new molecules to find compatibility between chemical and biological methods for this particular parasitoid species.

Given the above, the objective of this work is to evaluate the effect of new insecticides recommended for corn crops on *T. atopovirilia* in order to gather information that will help decision making as to which pesticides to use in pest management programs involving corn crops, ultimately seeking to preserve this parasitoid species.

Material and Methods

The following insecticides were used in this study (technical and commercial name, formulation, dosage and chemical group are listed for each one): imidacloprid/ß-cyfluthrin (Connect 100/12.5 SC - 0.33/0.04 g a.i. L^{-1}, Neonicotinoid/Pyrethroid), chlorfenapyr (Pirate 240 SC - 0.6 g a.i. L^{-1}, Pyrazole Derivative), chlorpyrifos (Astro 450 EW - 0.75 g a.i. L^{-1}, Organophosphate), novaluron (Rimon 100 CE - 0.05 g a.i. L^{-1}, Benzoylurea), spinosad (Tracer 480 SC - 0.16 g a.i. L^{-1}, Spinosyn) and triflumuron (Certero 480 SC - 0.048 g a.i. L^{-1}, Benzoylurea). Water was used as control treatment.

Twenty females up to 24 hours old per treatment were collected from a laboratory nursery and were placed separately in 8 cm x 2.5 cm glass tubes, fed with honey droplets smeared on the inside of the tubes which were then sealed with PVC film. Approximately 125 eggs of *Anagasta kuehniella* (Zeller, 1879) (Lepidoptera: Pyralidae) up to 24 hours old were glued to 5 cm x 0.5 cm strips of blue cardstock and placed under a germicidal lamp to kill embryos according to Parra (1997), then pulverized with chemical compounds using a Potter spray tower regulated at a pressure of 15 lb/in^2 and applied volume of 1.5 ± 0.5 µL/cm^2.

The treated eggs were placed in an environmental chamber at 24±2ºC, RH 70±10%, with 14 hours of photophase, being then offered to female individuals of *T. atopovirilia* 24, 48, and 96 hours after application of the insecticides, for 24 hours. The female individuals were then kept in the same tubes in order to assess their mortality throughout, while the cards containing the supposedly parasitized eggs were transferred into new tubes and stored in an environmental chamber until emergence of F_1 generation parasitoids. Items being assessed included daily mortality over eight days, parasitic capacity, and percentage of insect emergence.

To evaluate the effects of pesticides on the newly emerged F_1 generation adults originating from the eggs of *A. kuehniella* previously treated and then exposed to parasitism 24, 48, and 96 hours after application of the products, twenty female individuals of *T. atopovirilia* per treatment were placed in separate glass tubes, each receiving 125 untreated eggs of *A. kuehniella* up to 24 hours old, glued to card strips and placed under a germicidal lamp to kill embryos, as mentioned previously. The parasitic period was 24 hours, at the end of which the females were discarded and the card strips containing the supposedly parasitized eggs were kept in an environmental chamber under the above mentioned conditions, until full development and emergence of F_2 generation parasitoids was reached. Items assessed included parasitic rate of F_1 generation females and emergence percentage of F_2 generation specimens.

Each treatment consisted of five replicates and each experimental portion comprised four card strips with eggs previously offered to the wasps for parazitation. The bioassays used a completely randomized design in a three exposure times x seven compounds factorial arrangement, totaling 21 treatments.

The data were submitted to analysis of variance and the mean values were compared using the Scott-Knott cluster analysis at the 5% significance level (Scott and Knott 1974). The evaluated pesticides were further grouped into the following toxicity categories as a function of the reduction in parasitoid survival rate according to IOBC recommendations: class 1 = harmless (<30% reduction), class 2 = slightly harmful (30% to 79% reduction), class 3 = moderately harmful (80% to 99% reduction) and class 4 = harmful (>99% reduction) (Sterk *et al*. 1999, Van de Veire *et al*. 2002). The control treatment was used as reference parameter. The mean percentage of reduction in parasitoid survival was obtained using the following equation: % of reduction = 100 – [(% general mean of treatment with pesticide / % general mean of control treatment) x 100].

Results and Discussion

The parasitic capacity of female individuals of *T. atopovirilia* that had come into contact with host eggs 24, 48, and 96

Table 1. Number (±SE) of eggs parasitized by parent generation females of *Trichogramma atopovirilia* after coming into contact the eggs of *Anagasta kuehniella*, 24h, 48h and 96h after their contamination with the compounds.

Treatment	24h	48h	96h	Reduction (%)[2]	Classes[3]
Control	16.30±1.17bB[1]	28.42±0.70aB	12.20±1.80bA	–	–
Chlorfenapyr	3.61±0.57aC	4.00±0.37aC	1.42±0.24aB	84.1	3
Spinosad	4.40±0.34aC	4.80±0.26aC	1.51±0.28aB	81.0	3
Triflumuron	16.90±1.20bB	36.41±0.93aA	5.50±0.78cB	0.0	1
Chlorpyrifos	1.71±0.22aC	1.21±0.10aC	1.31±0.14aB	92.4	3
Imidacloprid/ ß-cyfluthrin	0.92±0.08aC	3.52±0.41aC	0.43±0.04aB	91.3	3
Novaluron	21.20±0.80bA	37.50±0.96aA	9.20±1.14cB	0.0	1
CV (%) = 37.75					

[1] Means followed by the same lower-case letter in a row and same upper-case letter in a column do not differ by the Scott-Knott test (P>0.05); [2]Mean percentage of reduction in parasitism; [3]Toxicity class according to Sterk *et al*. (1999).

Table 2. Emergence (%) (±SE) of F_1 generation *Trichogramma atopovirilia* originating from females that had come into contact with eggs of *Anagasta kuehniella,* 24h, 48h and 96h after their contamination with the compounds.

Treatment	24h	48h	96h	Reduction (%)[2]	Classes[3]
Control	99.22±0.24aA[1]	98.24±2.39aA	96.88±0.70aA	–	–
Chlorfenapyr	39.92±4.69aB	8.53±2.36bC	26.44±5.88aC	74.55	2
Spinosad	85.57±1.01aA	70.44±0.20aB	65.75±6.72aB	24.65	1
Triflumuron	94.83±0.74aA	97.49±0.00aA	68.64±7.78bB	11.33	1
Chlorpyrifos	96.67±0.00aA	100.00±0.00aA	90.84±2.91aA	2.31	1
Imidacloprid/ ß-cyfluthrin	100.00±1.22aA	95.96±1.15aA	73.33±8.70bB	8.50	1
Novaluron	85.66±0.52aA	67.42±6.50aB	31.35±3.86bC	37.33	2
CV (%) = 25.2					

[1] Means followed by the same lower-case letter in a row and same upper-case letter in a column do not differ by the Scott-Knott test (P>0.05); [2]Mean percentage of reduction in emergence; [3]Toxicity class according to Sterk *et al*. (1999).

hours after their contamination was reduced by virtually every compound, although triflumuron and novaluron allowed 100% parasitism and thus these were classified as harmless (class 1); the remaining products were considered moderately harmful (class 3) (Table 1). These results agree with Carvalho *et al*. (2001) and Parreira (2007), who failed to identify negative effects of triflumuron and novaluron respectively on the parasitic capacity of *T. pretiosum* when exposed to eggs of *A. kuehniella* 24 and 48 hours after contamination with the product.

A substantial decrease in the number of eggs parasitized by parent generation females of *T. atopovirilia* when exposed to eggs treated with chlorpyrifos 24, 48 and 96 hours after contamination was also verified by Moscardini *et al*. (2008). These authors evaluated fenitrothion and methidathion, organophosphate products, and concluded that the reduced parasitism probably resulted from the mortality inflicted on female individuals, whether by direct contact with residues or by ingestion of such products during the parasitic process.

As for emergence of F_1 generation parasitoids, chlorfenapyr was noted to be slightly harmful to females exposed to host eggs 24, 48 and 96 hours after application of the compound. Insecticide novaluron was slightly harmful, noting that 96 hours after application it presented one of the lowest mean values of emergence, approximately 31.3%. The remaining compounds were rated as harmless (class 1) (Table 2).

Parreira (2007) verified that novaluron caused a reduction in the emergence percentage of F_1 generation individuals of *T. pretiosum* when exposed to treated eggs of alternative host *A. kuehniella*, similarly to the result found in this work, where novaluron was rated as slightly harmful (class 2) (Table 2).

The parasitic capacity of F_1 generation females of *T. atopovirilia* could not be evaluated in chlorfenapyr and chlorpyrifos treatments when the parent generation females were exposed to contaminated eggs 24, 48 and 96 hours after contamination due the high mortality inflicted by these products soon after insect emergence (Table 3).

None of the insecticides were noted to affect the number of eggs parasitized by the F_1 generation of *T. atopovirilia* 24 hours after application of the compounds, with novaluron causing a decreasing reduction in the number of parasitized eggs throughout the evaluations. When parasitoids came into contact with host eggs 48 and 96 hours after contamination with triflumuron and novaluron, no reduction occurred in the parasitic rate, with mean values 46,9 and 43,9, and 21,1 and 22,4 for parasitized eggs / female respectively (Table 3).

Similar results for triflumuron and novaluron were found by Carvalho *et al*. (2001) and Stefanello Jr *et al*. (2008), who, in evaluating the effects of these products on the parasitic capacity of *T. pretiosum* on treated eggs of *A. kuehniella*, observed that they were slightly harmful.

Table 3. Number (±SE) of eggs parasitized by F_1 generation of *Trichogramma atopovirilia,* after parent females had come into contact with eggs of *Anagasta kuehniella*, 24h, 48h and 96h after their contamination with the compounds.

Treatment	24h	48h	96h	Reduction (%)[2]	Classes[3]
Control	48.80±1.03aA[1]	31.60±1.38bB	34.60±0.66bA	–	–
Chlorfenapyr	*	*	*	*	*
Spinosad	42.50±1.24aA	34.23±1.10aB	21.90±3.01bA	14.2	1
Triflumuron	48.10±1.51aA	46.95±0.89aA	21.10±0.07bA	0.0	1
Chlorpyrifos	*	*	*	*	*
Imidacloprid/ ß-cyfluthrin	44.68±1.09aA	27.45±1.29bB	24.80±4.59bA	15.8	1
Novaluron	55.95±0.69aA	43.96±1.36bA	22.45±1.52cA	0	1
CV(%) = 24.5					

[1] Means followed by the same lower-case letter in a row and same upper-case letter in a column do not differ by the Scott-Knott test (P>0.05); [2]Mean percentage of reduction in number of parasitized eggs; [3]Toxicity class according to Sterk *et al*. (1999). *The number of insects was insufficient to assess this characteristic.

Table 4. Emergence (%) (±SE) of F_2 generation of *Trichogramma atopovirilia* originating from F_1 generation females that had come into contact with eggs of *Anagasta kuehniella*, 24h, 48h and 96h after their contamination with the compounds.

Treatment	24h	48h	96h	Reduction (%)[2]	Classes[3]
Control	96.80±0.25aA[1]	99.15±0.10aA	97.20±0.46aA	–	–
Chlorfenapyr	*	*	*	*	*
Spinosad	99.20±0.22aA	99.42±0.11aA	76.68±8.59aB	6.0	1
Triflumuron	96.80±0.37aA	99.77±0.06aA	97.66±0.34aA	0.0	1
Chlorpyrifos	*	*	*	*	*
Imidacloprid/ ß-cyfluthrin	98.80±0.26aA	99.44±0.25aA	58.98±10.77bB	12.3	1
Novaluron	97.03±0.29aA	99.76±0.10aA	95.49±0.97aA	0.3	1
CV(%) = 19.2					

[1] Means followed by the same lower-case letter in a row and same upper-case letter in a column do not differ by the Scott-Knott test (P>0.05); [2]Mean percentage of reduction in emergence; [3]Toxicity class according to Sterk *et al*. (1999). *The number of insects was insufficient to assess this characteristic.

As for the emergence of F_2 generation of *T. atopovirilia* originating from F_1 generation females that had come into contact with eggs of *A. kuehniella* 24 hours after their contamination, it was noted that parasitoid emergence was not affected, with mean values ranging from 96.8% to 99.2% (Table 4).

Chlorfenapyr and chlorpyrifos caused 100% of mortality in F_1 generation insects immediately after their emergence (Tables 3 and 4), preventing evaluation of the emergence percentage of F_2 generation specimens. Similar results were found by Moscardini *et al*. (2008), who, in evaluating the effect of fenitrothion and methidathion on *T. pretiosum*, failed to obtain the emergence percentage of F_2 generation specimens due to 100% of mortality in F_1 generation parasitoids.

Spinosad, triflumuron and novaluron did not affect the emergence of F_2 generation females throughout the evaluation period, being rated as harmless (class 1). The evaluation at 96 hours revealed that spinosad and imidacloprid reduced the percentage of parasitoid emergence, with mean values 76.7% and 58.9% respectively (Table 4).

Parreira (2007) evaluated the action of triflumuron, imidacloprid and novaluron on the emergence of F_2 generation specimens of *T. pretiosum* originating from F_1 generation females that had come into contact with eggs of *A. kuehniella* 24 and 48 hours after contamination and noted that these compounds did not cause reduction in the emergence percentage, being thus rated as harmless. Carvalho *et al*. (2003) studied the effect of triflumuron on *T. pretiosum* and also observed innocuousness when this parasitoid was exposed to eggs of alternative host *A. kuehniella* 24 and 48 hours after application of the product, causing no reduction in the emergence of F_2 generation insects.

As a function of the reduction in emergence percentage of F_2 generation females as caused by spinosad, triflumuron, imidacloprid/ß-cyfluthrin and novaluron, these products were rated as class 1 = harmless (Table 4).

Spinosad and chlorpyrifos caused 95% and 100% of mortality in the fourth evaluation (4 days after application) and on the first evaluation (24 hours after application) respectively, while triflumuron caused 65.0% of mortality eight days after application. Imidacloprid/ß-cyfluthrin only caused 10.0% of mortality 24 hours after application, increasing throughout the evaluation period to 95.0% of mortality on day eight. Novaluron revealed a similar pattern to the control treatment, presenting a peak mortality of 70,0% (Fig. 1A).

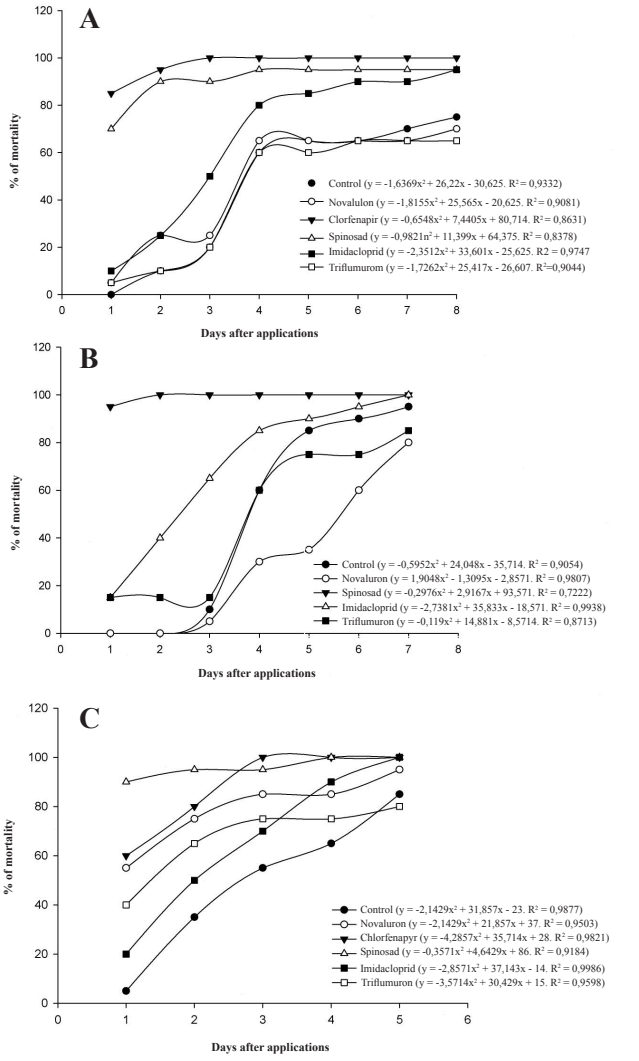

Figure 1. Mortality curves over time of parent generation females of *Trichogramma atopovirilia* after they had come into contact with eggs of *Anagasta kuehniella* treated and exposed to parasitism, 24h **A**, 48h **B** and 96h **C** after their contamination.

Chlorfenapyr and chlorpyrifos caused 100% of mortality in insects right in the first evaluation, while spinosad caused 100% of mortality in the second evaluation. Triflumuron and novaluron revealed a similar pattern to the control treatment, with mean values of mortality at around 95.0% and 80.0% on the last evaluation day respectively (Fig. 1B).

Chlorpyrifos caused 100% of mortality 24 hours after being applied, while chlorfenapyr caused 60.0% of mortality in the first evaluation, reaching 100% on day three. Spinosad presented 90.0% of mortality right on day one and 100% on day four. Triflumuron, imidacloprid/ß-cyfluthrin and novaluron caused 40.0%, 20.0% and 55.0% of mortality respectively in the first evaluation, increasing to around 95.0% on the last day. The control treatment presented 5.0% of mortality in the first evaluation, increasing to around 85.0% on the last day (Fig. 1C).

The results found in this work can contribute to a better understanding of the potentialities of combined use of parasitoids *T. atopovirilia* and selective compounds, ultimately seeking to control fall armyworm infestation in corn crops. However, additional studies are required using natural hosts, preferably under field conditions, to finally validate or refute the toxicity of compounds to this beneficial species.

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