

## Effects of insecticides used in corn on immature stages of *Trichogramma atopovirilia* (Hymenoptera: Trichogrammatidae)

Efectos de insecticidas usados en el maíz sobre los estados inmaduros de *Trichogramma atopovirilia* (Hymenoptera: Trichogrammatidae)

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**Abstract:** The chemical control of *Spodoptera frugiperda* (Lepidoptera: Noctuidae) is difficult because caterpillars live inside the corn (*Zea mays*) cob and they do not come into contact with insecticides. Research on the use of parasitoids of the genus *Trichogramma* (Hymenoptera: Trichogrammatidae) has contributed to advances in alternatives for the control of this pest. However, there is little information on the effects of pesticides used in corn on these parasitoids. Therefore, the objective of this study was to evaluate the physiological selectivity of insecticides for *Trichogramma atopovirilia* in the egg-larval, pre-pupal and pupal phases. The bioassays were carried out under controlled conditions, exposing eggs of *Anagasta* (= *Ephestia*) *kuehniella* (Lepidoptera: Pyralidae) to parasitism for a 24-h period. The insecticides were sprayed using a Potter tower on host eggs containing the parasitoids in the egg-larval period, pre-pupal and pupal phases. The insecticides evaluated in g a. i. L<sup>-1</sup> were: imidacloprid/β-cyfluthrin (Connect 100<sup>TM</sup> SC - 0.033), chlorfenapyr (Pirate 240<sup>TM</sup> SC - 0.6), chlorpyrifos (Astro 450<sup>TM</sup> EW - 0.75), novaluron (Rimon 100<sup>TM</sup> CE - 0.05), spinosad (Tracer 480<sup>TM</sup> SC - 0.16) and triflumuron (Certo 480<sup>TM</sup> SC - 0.048). Water was used as a control. According to the results, the products were classified in the following toxicological classes for *T. atopovirilia*: imidacloprid/β-cyfluthrin, novaluron and triflumuron as harmless (class 1); spinosad and chlorfenapyr as slightly harmful (class 2); and Chlorpyrifos was harmful (class 4) to the parasitoid. Therefore, new studies under greenhouse and field conditions are necessary to confirm these toxicity results.

**Key words:** *Zea mays*. Parasitoids. Pesticides. Biological control. Physiological selectivity.

**Resumen:** El control químico de *Spodoptera frugiperda* (Lepidoptera: Noctuidae) es difícil debido a que las larvas quedan dentro de los cartuchos de las plantas de maíz (*Zea mays*), dificultando el contacto con los insecticidas. Las investigaciones sobre parasitoides del género *Trichogramma* (Hymenoptera: Trichogrammatidae) han contribuido a los avances en la búsqueda de alternativas de control. Sin embargo, existe poca información sobre los efectos de los plaguicidas usados en el maíz sobre estos parasitoides. Así, el objetivo de este estudio fue evaluar la selectividad fisiológica de insecticidas para *Trichogramma atopovirilia* durante las fases de huevo-larva, pre-pupal y pupal. El bioensayo se llevó a cabo en laboratorio bajo condiciones controladas, donde los huevos de *Anagasta* (= *Ephestia*) *kuehniella* (Lepidoptera: Pyralidae) fueron expuestos al parasitismo por 24 horas empleando el método de la torre de Potter, que contiene los parasitoides en sus diferentes fases. Los insecticidas evaluados, en g.L<sup>-1</sup> de i.a., fueron: imidacloprida/β-ciflutrina (Connect 100<sup>®</sup> SC - 0,033), clorfenapir (Pirate 240<sup>®</sup> SC - 0,6), clorpirifos (Astro 450<sup>®</sup> EW - 0,75), novalurum (Rimon 100<sup>®</sup> CE - 0,05), spinosad (Tracer 480<sup>®</sup> SC - 0,16) y triflumuron (Certo 480<sup>®</sup> SC - 0,048). Se usó agua como tratamiento control. Los productos se agruparon en clases toxicológicas de acuerdo a las recomendaciones de la OILB. Imidacloprida/β-ciflutrina, triflumuron y novalurum fueron inofensivos (clase 1) para *T. atopovirilia*; spinosad y clorfenapir fueron levemente perjudiciales (clase 2); y clorpirifos fue perjudicial (clase 4) al parasitoide. Se requieren más estudios en invernadero y de campo para determinar estos resultados de toxicidad.

**Palabras clave:** *Zea mays*. Parasitoides. Plaguicidas. Control biológico. Selectividad fisiológica.

### Introduction

In 2012, Brazil expects to produce 68 million and five hundred and ten thousand tons of corn, considering the two seasons of the year, with planted area of approximately 14 million and 888 thousand hectares (IBGE 2012). However, among the factors that may affect the yield and quality of production is the incidence of pests, which can cause damage to crops and production, with a significant economic impact (Fernandes 2003).

Among these pests, the fall armyworm, *Spodoptera frugiperda* (Smith & Abbot, 1797) (Lepidoptera: Noctuidae) is considered the main one. Its attack occurs from

the plant emergence until bolting and silking. The damage caused by this pest can reach 60% depending on the cultivar, degree of infestation, stage of plant development and time of the attack (Cruz *et al.* 1999, 2002, 2006; Lourenção and Santos 2005). The chemical method is the most widely used against this pest, using several insecticide groups. Such control is difficult due to the behavior of the pest, which remains inside the plant cartridges, thus reducing the contact with insecticides. The failures in the application of the pesticides usually result in recurrence and selection of resistant populations of *S. frugiperda*, and may often cause biological imbalances (Gassen 1996).

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Among the natural enemies of the fall armyworm, egg parasitoids stand out. These insects are known to be highly effective against a large number of agricultural pests. Those belonging to the genus *Trichogramma* (Hymenoptera: Trichogrammatidae) are widely distributed geographically and play an important role as natural enemies of lepidopteran pests in various agroecosystems (Hassan and Abdelgader 2001).

Beserra and Parra (2004) observed that the average parasitism rate and the number of adults emerged per egg of *S. frugiperda* were higher for *Trichogramma atopovirilia* Oatman & Platner, 1983, compared to *Trichogramma pretiosum* Riley, 1879, and inferred that the first species is more likely to increase their population in a shorter time than *T. pretiosum*, therefore, being more suitable for the biological control of the pest. However, there is little information on the effects of pesticides used in corn crops on these parasitoids. Thus, this study aims to evaluate the effect of new insecticides used in corn on *T. atopovirilia* during its immature stages.

### Material and methods

The insecticides used with their technical names, trademarks, formulations, dosages and chemical groups were: imidacloprid/β-cyfluthrin (Connect 100™ SC - 0.033 g a.i. L<sup>-1</sup>, neonicotinoid/pyrethroid), chlorfenapyr (Pirate 240™ SC - 0.6 g a.i. L<sup>-1</sup>, pyrazole derivative), chlorpyrifos (Astro 450™ EW - 0.75 g a.i. L<sup>-1</sup>, organophosphate), novaluron (Rimon 100™ CE - 0.05 g a.i. L<sup>-1</sup>, benzoylurea), spinosad (Tracer 480™ SC - 0.16 g a.i. L<sup>-1</sup>, spinosyn) and triflumuron (Certero 480™ SC - 0.048 g a.i. L<sup>-1</sup>, benzoylurea). Water was used as control.

**Assessment of the effects of insecticides on *T. atopovirilia* during its immature stages.** Twenty *T. atopovirilia* females per treatment were individually placed in glass tubes (8 x 2.5 cm) and fed honey droplets deposited on the inner walls of the tubes and closed with PVC film. Approximately 125 eggs of *Anagasta kuehniella* Zeller, 1879 (Lepidoptera: Pyralidae), with up to 24 hours of age, were glued with arabic gum (50% diluted) onto a piece of blue cardboard (5 cm x 0.5 cm). Parasitism was allowed for 24 hours. The cards with parasitized eggs were kept in a climatic chamber at 24 ± 2 °C, RH 70 ± 10% and 14-h photophase until the parasitoids reached each stage of development desired for the bioassay.

Twenty cards with eggs of *A. kuehniella* per treatment, containing the parasitoid in the egg-larval period or in the pre-pupal or pupal phases, in a total of 140 cards for each developmental stage, were sprayed with the products using a Potter tower adjusted to the pressure of 15 lb./pol<sup>2</sup> with a volume application of 1.5 ± 0.5 μL/cm<sup>2</sup> (Vogt 2001). Then, they were distributed into glass tubes, which were kept in a climatic chamber under the same conditions described above. Each treatment consisted of five replications, where each one was constituted of four cards with eggs of *A. kuehniella* containing the parasitoid in its different immature phases.

The effects of pesticides on parasitoids of the F<sub>1</sub> generation were assessed for emergence rate [(number of eggs with an outlet orifice of the parasitoid /total number of parasitized eggs) x 100].

The effects of the compounds on the emerged insects from the F<sub>1</sub> generation were evaluated. Twenty females per treatment were individually placed in glass tubes and fed honey, deposited on the inner wall of the tubes, which were closed with PVC film. Around 125 eggs of *A. kuehniella*, untreated and fixed in a piece of blue paper, were offered to these females for a period of 24 hours.

The effects of the compounds tested on the parasitoids were measured by evaluating the parasitism rate (number of eggs/female of generation F<sub>1</sub>/24 hours), and according to the emergence rate.

A completely randomized experimental design was used in a 3 x 7 factorial arrangement (3 periods of development x 7 compounds, totaling 21 treatments), the data collected were submitted to analysis of variance, and means were compared by the Scott-Knott test at 5% significance level (Scott and Knott 1974).

According to the reduction in the survival rate and beneficial features of the parasitoids, the insecticides evaluated were grouped into toxicological categories, according to the 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 average reduction in the beneficial capacity of parasitoids (parasitism and emergence) was calculated with the following equation: % reduction = 100 - [(% general mean of treatment with pesticide/% general mean of the control treatment) x 100]. The average obtained in the control treatment was used as a reference to calculate the reduction in the beneficial capacity of parasitoids.

**Table 1.** Emergence rate (%) (±SE) of *Trichogramma atopovirilia* (F<sub>1</sub>) from treated eggs of *Anagasta kuehniella* containing the parasitoids in the egg-larval period, pre-pupal and pupal phases<sup>1</sup>.

Treatment	Egg-larval <sup>1</sup>	Pre-pupal <sup>1</sup>	Pupal <sup>1</sup>
Control	94.93 ± 2.46aA	95.14 ± 2.0aA	97.25 ± 1.45aA
Imidacloprid/β-cifluthrin	91.81 ± 3.98aA	91.07 ± 2.7aA	86.64 ± 3.89aB
Spinosad	33.25 ± 3.46bB	51.06 ± 3.0aC	51.79 ± 6.06aD
Chlorfenapyr	15.32 ± 3.46cC	81.68 ± 1.4bB	93.80 ± 1.19aA
Novaluron	93.11 ± 2.29aA	95.52 ± 1.9aA	94.02 ± 1.39aA
Triflumuron	92.84 ± 6.01aA	95.08 ± 1.0aA	87.24 ± 4.25aB
Chlorpyrifos	89.34 ± 1.72aA	91.19 ± 1.3aA	76.32 ± 5.79bC
CV(%) = 9.2			

<sup>1</sup> Means followed by the same letter, lowercase on the line and uppercase in the column, do not differ by the Scott-Knott test (P < 0.05).

**Table 2.** Effect of insecticides on emergence (%) of *Trichogramma atopovirilia* (F<sub>1</sub>) from treated eggs of *Anagasta kuehniella* containing the parasitoids in the egg-larval period, pre-pupal and pupal phases<sup>1</sup>.

Treatment	Egg-larval		Pre-pupal		Pupal	
	E(%) <sup>1</sup>	Class <sup>2</sup>	E(%) <sup>1</sup>	Class <sup>2</sup>	E(%) <sup>1</sup>	Class <sup>2</sup>
Control	----	----	----	----	----	----
Imidacloprid/β-ciflutrin	3.2	1	4.3	1	11.0	1
Spinosad	65.0	2	46.3	2	46.7	2
Chlorfenapyr	84.0	3	14.1	1	3.5	1
Novaluron	2.0	1	0.0	1	3.3	1
Triflumuron	2.2	1	0.0	1	10.3	1
Chlorpyrifos	6.0	1	4.1	1	21.5	1

<sup>1</sup> Mean percentage of reduction in emergence; <sup>2</sup> Toxicity class according to Sterk *et al.* (1999).

## Results and discussion

**Parasitoid emergence (F<sub>1</sub> generation).** Imidacloprid/β-cyfluthrin, novaluron and triflumuron did not affect the emergence rate of the F<sub>1</sub> - generation parasitoids from the maternal generation treated in the egg-larval period, and can be considered harmless (class 1) (Tables 1 and 2). Our results are similar to those of Matos (2007) who evaluated the selectivity of products used in citrus to *T. atopovirilia* and found that imidacloprid/β-cyfluthrin and triflumuron were slightly toxic.

Spinosad reduced the emergence of parasitoids independent of the developmental stage, being framed in class 2 (slightly harmful) (Table 2). Bueno *et al.* (2008) studied the effect of insecticides on immature stages of *T. pretiosum* and classified spinosad as toxic (class 4). Comparing the compound spinosad with other products tested within the egg-larval period, it was found that it had the second lowest emergence rate, e.g. 33.0% and, for the pre-pupal and pupal stages, spinosad caused the greatest reduction in adult emergence, of about 51.0% and 51.8%, respectively (Table 1). The major toxicity observed for the product spinosad may be related to the high value of octanol-water (Log K<sub>ow</sub>), which was 4.91. High values of Log K<sub>ow</sub> provide greater lipophilicity and a further penetration of the product through the corium (Guedes *et al.* 1992).

Chlorfenapyr was more toxic than the other compounds tested when sprayed on the host eggs containing parasitoids

in the egg-larval stage, causing a lower emergence rate, approximately 15.0%, being classified as moderately harmful (class 3); for the remaining developmental stages of the parasitoid, chlorfenapyr was considered harmless (class 1) (Tables 1 and 2). This effect might be explained in terms of the longer exposure period of the product through the host egg chorion, increasing contamination of the embryo due to their increased activity and contact with the product that penetrated the egg (Carvalho *et al.* 2001; Nörnberg *et al.* 2009).

Moura *et al.* (2005) found that chlorfenapyr caused a reduction in the emergence of the F<sub>1</sub>-generation *T. pretiosum* from eggs of *A. kuehniella* treated in the egg larval period and in the pre-pupal and pupal phases, averaging 19.4%, 2.4% and 37.3%, respectively. The greater toxicity of this product may be related to the high Log K<sub>ow</sub> value (4.83) and to the amount of active ingredient used by these authors (0.12 g a.i. L<sup>-1</sup>), since it is twice the amount used in this study.

Insects treated with chlorpyrifos showed an average of 85.61% regardless the developmental stage of the insect, so it was placed in class 1 (harmless) (Tables 1 and 2), however it caused the immediate death of all insects after emergence. It is likely that some residue of the product remained on the surface of the egg chorion of *A. kuehniella*, leading the death of the parasitoids that touch it. This is a clear clue of the high toxicity of chlorpyrifos for the parasitoid species. These data are similar to those found by Nörnberg *et al.* (2009), when assessing the effect of Lorsban™ 480 Br on immature stages of *T. pretiosum*.

**Table 3.** Number (±SE) of parasitized eggs per female of *Trichogramma atopovirilia* from the F<sub>1</sub> generation, from treated eggs of *Anagasta kuehniella* containing the parasitoids in the egg-larval period, pre-pupal and pupal phases<sup>1</sup>.

Treatment	Egg-larval <sup>1</sup>	Pre-pupal <sup>1</sup>	Pupal <sup>1</sup>
Control	41.05 ± 4.89aA	46.70 ± 2.58aA	39.12 ± 2.73aA
Imidacloprid/β-ciflutrin	45.45 ± 1.43aA	46.95 ± 1.27aA	40.85 ± 3.67aA
Spinosad	28.90 ± 5.24aB	30.10 ± 3.17aB	20.80 ± 9.38aB
Chlorfenapyr	*	37.70 ± 4.27aB	10.50 ± 3.45bB
Novaluron	41.55 ± 2.67aA	44.95 ± 2.50aA	47.05 ± 1.11aA
Triflumuron	46.55 ± 4.89aA	47.10 ± 2.61aA	43.75 ± 3.53aA
Chlorpyrifos	*	*	*
CV(%) = 22.5%			

<sup>1</sup> Means followed by the same letter, lowercase on the line and uppercase in the column, do not differ by the Scott-Knott test (P < 0.05).

\* Insufficient number of insects to evaluate this biological characteristic.

**Table 4.** Reduction in the number (E%) of parasitized eggs by *Trichogramma atopovirilia* (F<sub>1</sub>) from treated eggs of *Anagasta kuehniella* containing the parasitoids in the egg-larval period, pre-pupal and pupal phases<sup>1</sup>.

Treatment	Egg-larval		Pre-pupal		Pupal	
	E(%) <sup>1</sup>	Class <sup>2</sup>	E(%) <sup>1</sup>	Class <sup>2</sup>	E(%) <sup>1</sup>	Class <sup>2</sup>
Control	----	----	----	----	----	----
Imidacloprid/β-cifluthrin	0.0	1	0.0	1	0.0	1
Spinosad	29.6	1	35.5	2	46.8	2
Chlorfenapyr	*	*	19.3	1	73.1	2
Novaluron	0.0	1	4.0	1	0.0	1
Triflumuron	0.0	1	0.0	1	0.0	1
Chlorpyrifos	*	*	*	*	*	*

<sup>1</sup> Mean percentage of reduction in emergence; <sup>2</sup> Toxicity class according to Sterk *et al.* (1999).

\* Insufficient number of insects to evaluate this biological characteristic.

Giolo *et al.* (2006) studied the effects of organophosphate (phosmet, malathion and fenthion) products on immature stages of *T. pretiosum* and classified them as harmless (class 1). The selectivity of these compounds probably occurred in the concentrations used by these authors, which were 0.1, 0.2 e 0.05 g a.i. 100 L<sup>-1</sup>, respectively, less than the dose of chlorpyrifos used in this study.

Manzoni *et al.* (2007) evaluated the effects of carbaryl (0.00173 g a.i. L<sup>-1</sup>) and of the organophosphates fenitrothion (0.001 g a.i. L<sup>-1</sup>) and trichlorfon (0.0015 g a.i. L<sup>-1</sup>) on *T. atopovirilia* in its immature stage, and found that all compounds were harmful when sprayed at the egg-larval period and, as well as in the pre-pupal and pupal phases.

**Egg-larval/parasitized eggs.** At the egg-larval period, the lowest number of parasitized eggs was recorded for spinosad, with an average of 28.9 parasitized eggs per *T. atopovirilia* females. In the pre-pupal and pupal phases, the lowest parasitism averages were found for spinosad and chlorfenapyr (Table 3).

Imidacloprid/β-cyfluthrin, novaluron and triflumuron did not reduce the number of parasitized eggs per female of the F<sub>1</sub> generation, and these insecticides were considered harmless (class 1) (Tables 3 and 4). Novaluron and triflumuron are selective to *T. pretiosum*. However, imidacloprid/β-cyfluthrin is slightly harmful (class 2) when the parasitoid was treated at the egg-larval period (Carvalho *et al.* 2010). The results

found in this study are consistent with those of Matos (2007). This author found that triflumuron is harmless or slightly harmful in three immature stages of *T. atopovirilia*.

The selectivity of novaluron and triflumuron in the parasitism of *T. atopovirilia* may be related to the mode of action of these insect growth regulator insecticides.

**Pre-pupal and pupal stage/parasitized eggs.** Chlorfenapyr was harmless (class 1) to the parasitoid in the pre-pupal phase, but slightly harmful (class 2) to the pupa. It was not possible to analyze the ability of parasitism for the egg-larval period, due to the low number of insects that emerged from the F<sub>1</sub> generation and the high mortality of this generation after hatching (Tables 3 and 4). Moura *et al.* (2005) evaluated the effect of chlorfenapyr (0.12 g a.i. L<sup>-1</sup>) on the parasitism capacity of *T. pretiosum* of the F<sub>1</sub> generation from eggs of *A. kuehniella* treated in the egg-larval period and in the pre-pupal and pupal phases. Those authors found that this insecticide caused a reduction in parasitism capacity, being classified as slightly harmful (class 2).

Chlorpyrifos allowed the emergence of insects of the F<sub>1</sub> generation. However, it caused immediate mortality to parasitoids when they left the host egg, making it impossible to evaluate the parasitism rate; therefore, it was classified as harmful (class 4) (Tables 2 and 4). This result is similar to that found by Cañete (2005) which noted that this insecticide at a dosage of 120 g a.i. 100 L<sup>-1</sup> affected the parasitism

**Table 5.** Emergence rate (%) (±SE) of *Trichogramma atopovirilia* (F<sub>2</sub>) from treated eggs of *Anagasta kuehniella* containing the parasitoids in the egg-larval period, pre-pupal and pupal phases<sup>1</sup>.

Treatment	Egg-larval <sup>1</sup>	Pre-pupal <sup>1</sup>	Pupal <sup>1</sup>
Control	92.71 ± 1.72aA	97.59 ± 1.61aA	99.17 ± 0.34aA
Imidacloprid/β-cifluthrin	91.37 ± 1.37aA	88.34 ± 3.52aA	96.76 ± 1.53aA
Spinosad	93.43 ± 2.42aA	92.32 ± 2.24aA	76.50 ± 19.20bB
Chlorfenapyr	*	93.49 ± 1.55aA	98.81 ± 0.76aA
Novaluron	89.67 ± 1.84aA	94.23 ± 0.94aA	95.40 ± 1.59aA
Triflumuron	85.00 ± 1.29aA	93.28 ± 1.84aA	89.73 ± 1.87aA
Chlorpyrifos	*	*	*
CV(%) = 11.9			

<sup>1</sup> Means followed by the same letter, lowercase on the line and uppercase in the column, do not differ by the Scott-Knott test (P < 0.05).

\* Insufficient number of insects to evaluate this biological characteristic.

**Table 6.** Effect of insecticides on emergence (%) of *Trichogramma atopovirilia* (F<sub>2</sub>) from treated eggs of *Anagasta kuehniella* containing the parasitoids in the egg-larval period, pre-pupal and pupal phases<sup>1</sup>.

Treatment	Egg-larval		Pre-pupal		Pupal	
	E(%) <sup>1</sup>	Class <sup>2</sup>	E(%) <sup>1</sup>	Class <sup>2</sup>	E(%) <sup>1</sup>	Class <sup>2</sup>
Control	----	----	----	----	----	----
Imidacloprid/β-cyfluthrin	1.4	1	9.4	1	2.4	1
Spinosad	0.0	1	5.4	1	22.8	1
Chlorfenapyr	*	*	4.3	1	0.3	1
Novaluron	3.2	1	3.4	1	3.8	1
Triflumuron	8.3	1	4.4	1	9.5	1
Chlorpyrifos	*	*	*	*	*	*

<sup>1</sup> Mean percentage of reduction in emergence; <sup>2</sup> Toxicity class according to Sterk *et al.* (1999).

\* Insufficient number of insects to evaluate this biological characteristic.

capacity of *T. atopovirilia* from treated eggs of *Anticarsia gemmatalis* Hübner, 1818 (Lepidoptera: Noctuidae), since it caused 100% mortality of the parasitoids soon after hatching.

Spinosad was slightly harmful (class 2) to *T. atopovirilia* in the pre-pupal and pupal phases, causing a reduction of 35.5 and 46.8% in the parasitism rate, respectively, and harmless (class 1) for the egg-larval period of the parasitoid, with an average of 29.6% (Table 4). However, Carmo *et al.* (2010), when evaluating this same compound in the pupal stage of *T. pretiosum*, rated it as moderately harmful (class 3).

**Parasitoid emergence (F<sub>2</sub> generation).** All the products sprayed on the host eggs containing the parasitoid in the egg-larval period and in the pre-pupal phase did not reduce the emergence rate of *T. atopovirilia* from the F<sub>2</sub> generation and were considered harmless (class 1). However, spinosad decreased this biological characteristic in the pupal stage, with an average of 76.0% emergence (Tables 5 and 6). This result is similar to that found by Cañete (2005) who noted a reduction in the emergence of the F<sub>2</sub> generation of *T. atopovirilia* caused by spinosad.

Chlorfenapyr was classified as harmless (class 1) to the parasitoid in the pre-pupal and pupal phases. However, in the egg-larval period, it was not possible to calculate the emergence rate of the insects from the F<sub>2</sub> generation due to the high mortality of the insects of the F<sub>1</sub> generation caused by the product (Tables 1 and 6). Except for the egg-larval period,

these results are similar to those of Moura *et al.* (2005) which found that chlorfenapyr did not affect the emergence of *T. pretiosum* from the F<sub>2</sub> generation.

Imidacloprid/β-cyfluthrin, novaluron and triflumuron did not reduce the emergence of parasitoids of the F<sub>2</sub> generation when treated in the egg-larval, pre-pupal and pupal stages, and were considered harmless (class 1) (Tables 5 and 6). These results are similar to those found by Carvalho *et al.* (2010) and Matos (2007), when they evaluated the effects of imidacloprid/β-cyfluthrin, novaluron and triflumuron on the emergence rate of *T. pretiosum* and *T. atopovirilia* from eggs of *A. kuehniella* containing the parasitoids in the pupal stage. The effect of chlorpyrifos in the emergence of *T. atopovirilia* from the F<sub>2</sub> generation could not be calculated, due to the fact that the product caused 100.0% mortality of the F<sub>1</sub> generation (Table 1).

**Sex ratio.** The products did not affect the sex ratio of parasitoids when treated in the egg-larval period and in the pre-pupal and pupal phases. However, when the effects of products on *T. atopovirilia* were evaluated in each immature stage, there were significant differences between treatments (Table 7). The sex ratio of the parasitoids treated in the egg-larval period (F<sub>1</sub> generation) was not affected by any of the evaluated compounds (Table 7). These results were similar to those of Moura *et al.* (2005) when sprayed imidacloprid and chlorfenapyr/β-cyfluthrin on *T. pretiosum* and did not find any effect on this biological characteristic.

**Table 7.** Sex ratio (±SE) of the F<sub>1</sub>-generation specimens of *Trichogramma atopovirilia* from treated eggs of *Anagasta kuehniella*, containing the parasitoids in the egg-larval period, pre-pupal and pupal phases<sup>1</sup>.

Treatment	Egg-larval I	Pre-pupal I	Pupal I
Control	0.71 ± 0.03 aA	0.67 ± 0.14 aB	0.73 ± 0.07 aB
Imidacloprid/β-cyfluthrin	0.79 ± 0.02 aA	0.81 ± 0.01 aA	0.84 ± 0.02 aA
Spinosad	0.75 ± 0.03 aA	0.85 ± 0.02 aA	0.66 ± 0.05 aB
Chlorfenapyr	0.75 ± 0.03 aA	0.87 ± 0.03 aA	0.87 ± 0.02 aA
Novaluron	0.85 ± 0.02 aA	0.83 ± 0.02 aA	0.74 ± 0.02 aB
Triflumuron	0.74 ± 0.02 aA	0.80 ± 0.02 aA	0.77 ± 0.05 aB
Chlorpyrifos	0.79 ± 0.02 aA	0.73 ± 0.04 aB	0.67 ± 0.06 aB
CV (%) = 13.2			

<sup>1</sup> Means followed by the same letter, lowercase on the line and uppercase in the column, do not differ by the Scott-Knott test (P < 0.05).

### Conclusions

In accordance with the toxicity classification of the IOBC, imidacloprid/ $\beta$ -cyfluthrin, novaluron and triflumuron are harmless to *T. atopovirilia*, and spinosad and chlorfenapyr are slightly harmful. In contrast, chlorpyrifos is highly harmful to the parasitoid *T. atopovirilia*, requiring further studies under greenhouse and field conditions to prove its toxicity.

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