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Effect of chemical insecticides used in tomato crops on immature *Trichogramma pretiosum* (Hymenoptera: Trichogrammatidae)

Efecto de insecticidas químicos usados en el cultivo de tomate sobre inmaduros de *Trichogramma pretiosum* (Hymenoptera: Trichogrammatidae)

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Abstract: The effects of the chemical insecticides acetamiprid, lufenuron, imidacloprid, novaluron, triflumuron, and pyriproxifen on the developmental stages of *Trichogramma pretiosum* were evaluated. Eggs of *Anagasta kuehniella* glued on blue paper cards were offered to *T. pretiosum* females for 24 h. After parasitization, the eggs were treated by dipping the cards into the insecticide solutions or in water for 5 s after they had reached the egg-larval, pre-pupal, or pupal stages. The application of pyriproxifen to *T. pretiosum* during its egg-larval period was slightly harmful to the parasitization capacity of F_1 generation females and the emergence rate of F_1 and F_2 generation adults. When applied to *T. pretiosum* in the egg-larval period, acetamiprid was slightly harmful to the parasitization capacity of the F_1 generation females of *T. pretiosum*. When applied to *T. pretiosum* in the pupal stage, acetamiprid was slightly harmful to the parasitization capacity of F_2 generation females. When applied during the egg-larval period, imidacloprid was slightly harmful to the parasitization capacity of *T. pretiosum* females of the F_1 generation. Lufenuron applied on *T. pretiosum* in the pupal stage was slightly harmful to the parasitization capacity of the F_2 generation females. The insecticides novaluron and triflumuron, when applied during the immature stages to *T. pretiosum*, were harmless to its F_1 and F_2 generations.

Key words: Solanaceae. Insecticide selectivity. Parasitoids. Pesticides.

Resumen: Se evaluaron los efectos de los insecticidas químicos acetamiprid, lufenuron, imidacloprid, novaluron, triflumuron, y piriproxifen en las etapas de desarrollo de *Trichogramma pretiosum*. Se ofrecieron huevos de *Anagasta kuehniella* adheridos a cartón azul a las hembras de *T. pretiosum* durante 24 h. Después de parasitismo, los huevos fueron tratados por inmersión en las soluciones químicas o en agua durante cinco segundos, después de alcanzadas las etapas de huevo-larva, pre-pupa o pupa. La aplicación de piriproxifen a *T. pretiosum* durante su período de huevo-larva fue ligeramente perjudicial a la capacidad de parasitismo de las hembras de la generación F_1 y la tasa de emergencia de adultos de la generación F_1 y F_2. Cuando se aplicó a *T. pretiosum* en el período de huevo-larva, acetamiprid fue ligeramente perjudicial a la capacidad de parasitismo de las hembras de la generación F_1 de *T. pretiosum*. Cuando se aplicó a *T. pretiosum* en la fase de pupa, acetamiprid fue ligeramente perjudicial para la capacidad de parasitismo de las hembras de la generación F_2. Cuando se aplicó durante el período de huevo-larva, imidacloprid fue ligeramente perjudicial a la capacidad de parasitismo de las hembras de *T. pretiosum* de la generación F_1. Lufenuron aplicado en *T. pretiosum* en la fase de pupa fue ligeramente perjudicial para la capacidad de parasitismo de las hembras de la generación F_2. Los insecticidas novaluron y triflumuron cuando se aplicaron en los estados inmaduros de *T. pretiosum* fueron inofensivos para las generaciones F_1 y F_2.

Palabras clave: Solanaceae. Selectividad insecticidas. Parasitoides. Plaguicidas.

Introduction

Tomato crops are affected by a large number of pests and diseases (Santini 2001). In Brazil, tomato pests are controlled mainly by the indiscriminate use of chemical pesticides, which generally lead to biological imbalances and environmental pollution. A safer alternative for controlling some of the tomato pests is biological control utilizing parasitoids belonging to the genus *Trichogramma* (Hymenoptera: Trichogrammatidae). According to Pratissoli and Parra (2001) and Haji *et al*. (2002), trichogrammatids have significantly contributed to reducing the populations of lepidopteran pests and the number of insecticide applications on tomato crops.

According to Haji *et al*. (1998), *Trichogramma pretiosum* (Riley, 1879) (Hymenoptera: Trichogrammatidae) has been used in the control of the tomato leaf caterpillar *Manduca diffissa* (Butler, 1871) (Lepidoptera: Sphingidae), tomato moth *Tuta absoluta* (Meyrick, 1917) (*Scrobipalpuloides*) (Lepidoptera: Gelechiidae), and tomato fruit borer *Neoleucinodes elegantalis* Guenée, 1854 (Lepidoptera: Crambidae), which demonstrates the potential of this species in pest control. However, one of the major obstacles to the use of this and other parasitoids in tomato crops is the continued use of large quantities of chemical pesticides. Another limiting factor is that there are few studies about the toxic effects of the new molecules on natural enemies of pests in tomato crops

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(Degrande *et al*. 2002; Moura *et al.* 2005; Moura and Rocha 2006). Thus, the aim of the present work was to evaluate the toxicity of some new insecticides used in tomato crops applied during the immature developmental stages of *T. pretiosum* on F_1 generation adults, and also to evaluate the sublethal effects on the F_2 generation adults.

Material and Methods

The studies were carried out with parasitoids of the species *T. pretiosum* obtained from parasitized *Spodoptera frugiperda* (J. E. Smith, 1797) (Lepidoptera: Noctuidae) eggs, collected from a maize crop in the municipality of Piracicaba, São Paulo State, Brazil. The parasitoids were reared in the laboratory under controlled conditions (24±2 °C, 70 ± 10% RH, and 12h photoperiod) on eggs of the factitious host *Anagasta kuehniella* (Zeller, 1879) (Lepidoptera: Pyralidae). The insecticides used in the tests with *T. pretiosum*, together with their technical and trade names, dosages, formulations, and chemical groups, are presented in Table 1. They are the latest products registered for control of tomato crop pests in Brazil. Distilled water was used as a control.

To evaluate the effects of the insecticides on immature stages of *T. pretiosum*, twenty fertile *T. pretiosum* females, aged about 24 h, were placed in individual glass tubes (8 cm x 2.5 cm) and fed with honey. The tubes were closed with polyvinyl chloride (PVC) film. About 125 UV-killed eggs of *A. kuehniella* were glued onto 5 x 0.5-cm paper cards and exposed to the females for parasitization for 24 h (Parra 1997). The supposedly parasitized eggs were kept in an acclimatized chamber at 24±2 °C, 70±10% RH and exposed to a 12-h photoperiod until the parasitoids reached the desired developmental stage for the application of the insecticides.

A total of 20 paper cards per treatment with parasitized *A. kuehniella* eggs (parasitoids in the egg-larval or pre-pupal or pupal stages; 0-24 h, 72-96 h, and 168-192 h after parasitism, respectively) were dipped into the insecticide solutions or into water for 5 s, dried in the shade for about 30 min and then placed in glass tubes. The tubes were kept in the acclimatized chamber as previously described. The effects of the insecticides applied during the immature stages on *T. pretiosum* adults emerging from the treated host eggs were also studied. The same number of females, card size and number of host eggs as previously described were employed; however, untreated 0-24-h old *A. kuehniella* eggs were presented to *T. pretiosum* F_1 and F_2 generation females, and biological parameters like adult emergence, sex ratio, and parasitization capacity were studied.

The effects of the insecticides on each immature stage of the parasitoid were measured by evaluating percent emergence [(number of eggs with parasitoid emergence holes/total number of parasitized eggs) x 100], sex ratio (number of females/number of females + males) according to the equation proposed by Pereira *et al.* (2004), and parasitism ratio (number of parasitized eggs/female/24 h) of F_1 and F_2 generations females. Each treatment was replicated five times, with the experimental plot represented by four paper cards with parasitized host eggs. A completely randomized three x seven factorial experimental design with three periods of parasitoid development vs. seven substances, totaling 21 treatments were used. The obtained data were submitted to analysis of variance and the means were compared by the Scott-Knott grouping test at 5% significance (Scott and Knott 1974).

The insecticides were also classified using the International Organization for Biological Control (IOBC) protocols (Sterk *et al.*1999), considering the percent reduction of emergence and parasitism capacity of F_1 and F_2 generation females due to insecticide application at each immature stage in relation to the control treatment, as follows: class 1 = harmless (<30% reduction), 2 = slightly harmful (30-79% reduction), 3 = moderately harmful (80-99% reduction), and 4 = harmful (>99% reduction). The mean percent reduction of emergence capacity and number of eggs of the parasitoid were calculated with the equation: % reduction = 100 – [(% general mean of emergence or number of eggs in the treatment with the insecticide/% general mean of emergence or number of eggs of control) x 100].

Results and Discussion

Emergence and sex ratio of F_1 generation parasitoids. Only pyriproxifen affected the emergence of *T. pretiosum* (F_1 generation) negatively, 28.4% emergence, when applied to host eggs with the parasitoid in the egg-larval stage (Table 2). This compound also produced a 24-h delay in the emergence of F_1 adults. The observed effects are thought to be related to the mechanism of action of the compound, since some insect growth regulators are agonists or antagonists of the juvenile hormone, which is responsible for the growth and/or the development of the immature forms of the insects. Pyriproxifen mimics the action of the juvenile hormones in a number of physiological processes and is a potent inhibitor of embryogenesis, metamorphosis, and adult formation (Glancey *et al.* 1990; Ishaaya and Horowitz 1992; Miyamoto *et al.* 1993).

None of the evaluated insecticides reduced adult emergence in the F_1 generation when applied on the host eggs containing parasitoids in the pre-pupal stage. However, imida-

Table 1. Insecticides evaluated for selectivity to *Trichogramma pretiosum*.

Trade Name	Concentration/ Formulation	Technical Name	Dosages (g a.i. L^{-1} water)	Chemical Group
Mospilan	200 SP	Acetamiprid	0.05	Chloronicotinyl
Match	50 EC	Lufenuron	0.04	Benzoylphenylurea
Provado	200 CS	Imidacloprid	0.14	Chloronicotinyl
Rimon	100 EC	Novaluron	0.02	Benzoylphenylurea
Certero	480 CS	Triflumuron	0.14	Benzoylphenylurea
Cordial	100 EC	Pyriproxifen	0.1	Pyridyl ether

The control treatment consisted of only distilled water.

Table 2. Percent emergence (±SEM) of F_1 generation of *Trichogramma pretiosum* from treated *Anagasta kuehniella* eggs with parasitoids in different developmental stages.

Treatment	Egg-larval[1]	Class[2]	Pre-pupal[1]	Class[2]	Pupal[1]	Class[2]
Control	79.07±4.84aA	-	73.7±6.52aB	-	80.9±3.11aA	-
Acetamiprid	82.3±3.96aA	1	83.8±5.65aA	1	85.7±5.17aA	1
Imidacloprid	87.1±2.47aA	1	70.9±4.24bB	1	71.5±6.69bB	1
Lufenuron	89.5±3.66aA	1	86.8±2.02aA	1	71.3±10.27bB	1
Triflumuron	90.2±2.36aA	1	71.5±7.22bB	1	89.9±2.94aA	1
Novaluron	91.5±2.06aA	1	87.8±2.17aA	1	87.7±4.90aA	1
Pyriproxifen	28.4±3.88bB	2	80.4±4.43aA	1	89.6±2.4aA	1
CV (%) = 13.2						

[1] Means followed by the same lower case letter within lines and upper case letter within columns do not differ by the Scott-Knott test ($P < 0.05$). [2]Toxicity index recommended by Sterk *et al.* (1999).

cloprid and triflumuron treatments during the parasitoid pre-pupal stage reduced adult emergence in the F_1 generation by 16.2 and 18.7%, respectively, as compared to the emergence recorded when applied during the egg-larval period. The applications of imidacloprid and lufenuron on host eggs with parasitoids in the pupal stage led to a significant reduction of emergence of F_1 generation adults of *T. pretiosum* in comparison to the control and other treatments (Table 2). Carvalho *et al.* (2003) presented similar results for imidacloprid and lufenuron products, with only 70.3% and 72.8% adult emergence, respectively, when pupae in eggs of *A. kuehniella* were contaminated. Similar results were reported by Pratissoli *et al*. (2004), who observed that the number of *T. pretiosum* adults that emerged from lufenuron-treated *S. frugiperda* host eggs fell sharply.

Acetamiprid and novaluron were harmless, as they did not cause any reduction in adult emergence in the F_1 generation, regardless of the developmental stage of the parasitoid subjected to insecticide treatment. Different results were reported by Moura *et al.* (2005, 2006), who found that acetamiprid significantly decreased percent adult emergence in all stages tested. These differences in biological responses may be associated with distinct populations of *T. pretiosum*, as well as their geographical origins, as also discussed by Bleicher and Parra (1990) and Brunner *et al.* (2001).

When pyriproxifen was applied in the egg-larval stage, it was slightly harmful (class 2) due to 30-70% reduction in adult emergence in the F_1 generation. Acetamiprid, imidacloprid, lufenuron, triflumuron, and novaluron were classified as harmless (class 1) when they were applied during the pre-pupal and pupal stages of *T. pretiosum,* giving an adult emergence of >70% (Table 2). Similar results were obtained by Moura *et al.* (2005) for acetamiprid, which was also categorized as class 1. However, imidacloprid has been classified as slightly harmful (class 2), differing from the result obtained in this work. This difference is possibly associated with the dose used by other authors, which was eight times higher than that of the present study.

The sex ratio of males and females of the F_1 generation of *T. pretiosum* that emerged from treated host eggs during the egg-larval period and the pre-pupal and pupal stages was not affected by any of the evaluated insecticides ($P > 0.05$), and there was no statistical difference between treatments, which presented average ratios of 0.5-0.6.

Parasitism capacity of F_1 generation parasitoids. Only lufenuron did not significantly reduce the parasitism capacity of F_1 females of *T. pretiosum* treated during the egg-larval period. However, this insecticide significantly reduced (14.7 and 13.2% parasitized eggs, respectively) the parasitism capacity of F_1 females treated during the pre-pupal and pupal stages, when compared with those treated in the egg-larval stage (Table 3). Carvalho *et al*. (2003) also observed negative effects for 0.28 g a.i.L^{-1} and 0.4 g a.i.L^{-1} imidacloprid and lufenuron, respectively, pulverized onto eggs of *A. kuehniella* with *T. pretiosum* parasitoids in the pupal stage.

Table 3. Number (±SEM) of eggs parasitized by *Trichogramma pretiosum* F_1 females from treated *Anagasta kuehniella* eggs with parasitoids in different developmental stages.

Treatment	Egg-larval[1]	Class[2]	Pre-pupal[1]	Class[2]	Pupal[1]	Class[2]
Control	27.5±3.70aA	-	30.2±2.23aA	-	25.7±3.54aA	-
Acetamiprid	17.8±2.24bB	2	25.9±4.27aA	1	29.3±3.51aA	1
Imidacloprid	19.1±2.14aB	2	27.6±5.01aA	1	26.1±4.16aA	1
Lufenuron	33.3±1.96aA	1	18.6±4.65bA	1	20.1±1.92bA	1
Triflumuron	22.8±1.92aB	1	24.3±3.55aA	1	27.0±2.02aA	1
Novaluron	22.4±2.03aB	1	23.3±3.93aA	1	22.3±4.07aA	1
Pyriproxifen	11.7±1.64bB	2	25.9±4.35aA	1	19.3±2.79aA	1
CV (%) = 31.02						

[1]Means followed by the same lower case letter within rows and upper case letter within columns do not differ by the Scott-Knott test ($P < 0.05$). [2]Toxicity index recommended by Sterk *et al.* (1999).

Table 4. Percent of emergence (±SEM) of F_2 generation *Trichogramma pretiosum* wasps from treated *Anagasta kuehniella* eggs with parasitoids in different developmental stages.

Treatment	Egg-larval[1]	Class[2]	Pre-pupal[1]	Class[2]	Pupal[1]	Class[2]
Control	80.6±6.53aA	-	73.7±5.76aA	-	76.4±4.79aA	-
Acetamiprid	59.0±6.26aB	1	84.7±6.88aA	1	75.2±9.06aA	1
Imidacloprid	58.5±6.30aB	1	84.1±15.36aA	1	68.0±9.19aA	1
Lufenuron	87.8±5.99aA	1	64.1±12.61bA	1	48.7±7.83bA	2
Triflumuron	66.7±7.30aB	1	88.6±8.23aA	1	77.2±4.0aA	1
Novaluron	69.7±4.65aB	1	66.2±5.23aA	1	70.7±9.65aA	1
Pyriproxifen	56.1±5.07aB	2	72.5±7.86aA	1	58.3±5.75aA	1
CV (%) = 13.7						

[1]Means followed by the same lower case letter within rows and upper case letter within columns do not differ by the Scott-Knott test ($P < 0.05$). [2]Toxicity index recommended by Sterk *et al.* (1999).

Treatments with acetamiprid and pyriproxifen reduced the parasitism capacity of females from treated host eggs with parasitoids in the egg-larval period, as compared to other developmental stages of treatment with the insecticides. The parasitism capacity of F_1 generation females that emerged from parasitized host eggs treated during the pre-pupal and pupal stages was not negatively affected by the insecticides (Table 3). In a study of acetamiprid sprayed through a Potter tower, Moura and Rocha (2006) observed significant reduction in the capacity of parasitism (57.3%) of F_1 generation females when eggs of *A. kuehniella* with *T. pretiosum* were treated in the pupal stage. These conflicting results obtained in the treatment of the pupal stage are possibly associated with the different techniques used to treat host eggs.

IOBC classification (F_1 generation). According to the IOBC toxicity indices, acetamiprid, imidacloprid, and pyriproxifen are slightly harmful (class 2), reducing parasitism by 30-79%, but only when applied to host eggs with parasitoids in the egg-larval stage. Lufenuron, triflumuron, and novaluron were harmless (class 1) when applied to all immature stages of *T. pretiosum*, which reduced parasitism by less than 30% in comparison to the control (Table 3). Rocha and Carvalho (2004) reported that lufenuron and triflumuron were classified as slightly harmful (class 2) and imidacloprid as moderately harmful (class 3), differing from the present study. The possible discrepancy of toxicological classification is associated with the different methodology used, since the studies were carried out by spraying insecticides directly onto adults and not onto immature forms of the parasitoid.

Emergence and Sex ratio (F_2 generation). The percent emergence of F_2 generation parasitoids was reduced by all insecticides, except lufenuron, when *A. kuehniella* eggs with parasitoids in the egg-larval stage were treated. However, treatment with lufenuron in the pre-pupal and pupal stages of the parasitoid reduced the percent emergence of F_2 generation parasitoids, as compared to treatment during the egg-larval stage (Table 4). Carvalho *et al.* (2003) reported different results, in which lufenuron showed significant reduction in relation to the control only when eggs of *A. kuehniella* containing parasitoids in the pupal stage were infected, which did not happen in egg-larval or pre-pupal stages. These differences in biological responses may be associated with distinct populations of *T. pretiosum* studied, as well as their geographical origins, as previously mentioned.

Considering the percent reduction in the emergence of F_2 generation specimens, pyriproxifen was slightly harmful (class 2) to parasitoids in the egg-larval period, and lufenuron was slightly harmful only to parasitoids in the pupal stage. All the other compounds were harmless (class 1) to all immature stages (Table 4).

The sex ratio of F_2 generation parasitoids submitted to acetamiprid, imidacloprid, and triflumuron treatments was not adversely affected. Lufenuron treatment in the egg-larval and the pre-pupal stages reduced the proportion of females in the F_2 generation by from 0.2 to 0.4 on average, respectively. Novaluron application in the pre-pupal stage adversely affected the sex ratio of F_2 generation adults. Pyriproxifen treatment in pre-pupal stage was less harmful with regard to

Table 5. Sex ratio (± SEM) of F_2 generation *Trichogramma pretiosum* wasps from treated *Anagasta kuehniella* eggs with parasitoids in different developmental stages.

Treatment	Egg-larval[1]	Pre-pupal[1]	Pupal[1]
Control	0.7±0.08aA	0.6±0.11aA	0.6±0.06aA
Acetamiprid	0.4±0.06aA	0.6±0.12aA	0.7±0.08aA
Imidacloprid	0.5±0.05aA	0.7±0.12aA	0.6±0.07aA
Lufenuron	0.2±0.10aB	0.4±0.13aB	0.4±0.07aA
Triflumuron	0.6±0.04aA	0.7±0.08aA	0.6±0.02aA
Novaluron	0.6±1.64aA	0.2±0.10bB	0.5±0.05aA
Pyriproxifen	0.4±0.06bA	0.7±0.09aA	0.4±0.09bA
CV (%) = 6.98	-	-	

[1]Means followed by the same lower case letter within rows and upper case letter within columns do not differ by the Scott-Knott test ($P < 0.05$).

Table 6. Number (±SEM) of eggs parasitized by *Trichogramma pretiosum* F_2 females from treated *Anagasta kuehniella* eggs with parasitoids in different developmental stages.

Treatment	Egg-larval[1]	Class[2]	Pre-pupal[1]	Class[2]	Pupal[1]	Class[2]
Control	33.1±2.87aB		38.8±3.87aA		32.3±2.27aB	
Acetamiprid	39.5±5.50aA	1	35.6±3.25aB	1	22.3±2.53bC	2
Imidacloprid	49.5±1.61aA	1	36.1±4.85bB	1	40.9±2.80bA	1
Lufenuron	42.7±1.44aA	1	48.3±1.68aA	1	47.7±2.49aA	1
Triflumuron	29.4±3.51aB	1	29.0±5.77aB	1	24.5±2.15aC	1
Novaluron	37.5±1.64aA	1	32.4±2.42aB	1	40.1±1.11aA	1
Pyriproxifen	40.1±1.92aA	1	41.6±1.67aA	1	31.2±3.99bB	1
CV (%) = 13.7						

[1]Means followed by the same lower case letter within rows and upper case letter in columns do not differ by the Scott-Knott test ($P < 0.05$). [2]Toxicity index recommended by Sterk *et al.* (1999).

sex ratio of F_2 generation adults as compared to its treatment during the other developmental stages (Table 5).

Parasitism capacity of F_2 generation parasitoids. Insecticide application during the egg-larval stage of *T. pretiosum* did not significantly reduce the parasitism capacity of F_2 generation females. Treatments with acetamiprid, imidacloprid, triflumuron, and novaluron applied in the pre-pupal stage and acetamiprid and triflumuron applied in the pupal stage of *T. pretiosum* reduced the parasitism capacity of F_2 generation females (Table 6). The capacity of parasitism of the different stages of the F_2 generation of parasitoids exposed to lufenuron, triflumuron, and novaluron did not present any negative effect from the pesticides. Imidacloprid affected parasitism capacity, except in the egg-larval stage. Acetamiprid and pyriproxifen applied in the pupal stage of the parasitoid adversely affected the parasitism capacity of F_2 generation females.

IOBC classification (F_2 generation). Acetamiprid, when used during the pupal stage of the parasitoid, was slightly harmful (class 2) with respect to parasitism capacity of F_2 generation females, while the other insecticides were harmless (class 1) when applied during all immature stages (Table 6). The compounds that were harmful to *T. pretiosum* in the present study may be more or less toxic in field conditions due to the effect of various abiotic factors. Thus, there is a need for field validation of the laboratory results. Furthermore, the experiments must also be carried out using the natural host to evaluate the impact of the pesticides on this parasitoid species so that safer pesticides can be associated with the release of parasitoids in integrated pest control programs.

Conclusions

When applied to *T. pretiosum* in the egg-larval period, acetamiprid, imidacloprid, and pyriproxifen were slightly harmful to the parasitism capacity of F_1 generation females, and so was pyriproxifen to the emergence rates of F_1 and F_2 generation adults. When applied to *T. pretiosum* in the pupal stage, acetamiprid was slightly harmful to the parasitism capacity of F_2 generation females, as well as lufenuron to the percent emergence of F_2 generation adults. In general, acetamiprid, imidacloprid, lufenuron, and pyriproxifen were toxic to *T. pretiosum* when applied in different immature stages. Greenhouse and field studies are required to validate the laboratory trial results. Regardless of the immature stage of *T. pretiosum* at the time of pesticide application, triflumuron and novaluron were harmless to F_1 and F_2 generation parasitoids, thus being recommended as safe insecticides for integration with parasitoid use in pest control programs.

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