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Lethal and Sublethal Effects of Various Pesticides on *Trichogramma achaeae* (Hymenoptera: Trichogrammatidae)

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Abstract

Little information is available regarding the lethal and sublethal effects of pesticides on *Trichogramma achaeae* (Nagaraja and Nagarkatti; Hymenoptera: Tricogrammatidae) during integrated management of *Tuta absoluta* (Meyrick; Lepidoptera: Gelechiidae), an important pest for tomato production. Twenty-two pesticides sprayed on *Ephestia kuehniella* (Zeller; Lepidoptera: Pyralidae) eggs were evaluated on the mortality of adult parasitoids upon contact with the hosts 24 h after the treatments and their sublethal effects on the parasitoids were assessed in laboratory conditions. Tests were carried out with fresh solutions at the recommended concentration. According to the International Organisation for Biological and Integrated Control (IOBC) standards, chlorpyrifos is harmful to the parasitoid; merthiocarb, methomyl, spinosad lambda-cyhalothrin, and acrinatrin are moderately harmful; and chlorantraniliprole, lufenuron, hexythiazox, cyromazine and *Bacillus thuringiensis* have no effect on the parasitoid. Sulfur is slightly harmful, and azoxystrobin is harmless. Chlorpyrifos was the most lethal among these pesticides and killed all females in less than 24 h. All other pesticides affected the biological parameters of *T. achaeae* to varying degrees. Regarding the lethal and sublethal effects, merthiocarb and spinosad killed all female offspring in less than 24 h; lambda-cyhalothrin and sulfur reduced the number of parasitized eggs; and acrinatrin, deltamethrin and azoxystrobin affected the emergence rate. After that, we can recommend the use of chlorantraniliprole and *B. thuringiensis* to control Lepidoptera, cyromazine to control Diptera, pirimicarb to control Homoptera, hexythiazox to control mites and azoxystrobin can be used as fungicide in an integrated pest management program with mass released of *T. achaeae*.

Key words: pesticide, integrated pest management, egg parasitoid, toxicity, side effect

A combination of biological and chemical control approaches is very important for a successful integrated pest management (IPM) programs (Musser et al. 2006, Ghorbani et al. 2016). IPM programs use a combination of strategies, such as chemical, biological, and mechanical control, to maintain pest populations below economic thresholds (Roubos et al. 2014, Khan et al. 2015). The use of pesticides that are effective against target pests but have little or no negative effects on their natural enemies are desirable (Bastos et al. 2006, Preetha et al. 2010). Although biological control is very important in pest control, chemical control is still needed when there is a pest outbreak that produces an economic loss.

The negative effects of pesticides on natural enemies induce the necessity to study their side effects to adjust chemical and biological control programs and reduce the negative impacts of pesticides on non-target organisms (Souza et al. 2014, Ghorbani et al. 2016). Pesticides have lethal and sublethal effects on arthropods, as they can kill these organisms or negatively affect various biological parameters (Desneux et al. 2007, Garcia et al. 2009, Cordeiro et al. 2010, Castro et al. 2012).

Tomato (*Solanum lycopersicum* L.) is a major agricultural crop, with an estimated production of 170,751 MT year^{-1} (FAOSTAT 2014). Tomato crops are exposed to numerous pest organisms that cause economic losses, and the use of pesticides is necessary when they reach the economic threshold.

Tuta absoluta (Meyrick; Lepidoptera: Gelechiidae) is considered one of the most important pests for tomato production and results in serious damage in infested areas in different countries of the world (Desneux et al. 2011). The egg parasitoid *Trichogramma achaeae* (Nagaraja and Nagarkatti; Hymenoptera: Tricogrammatidae) was identified as an agent for the biological control of *T. absoluta* in the

Palaearctic region (Polaszek et al. 2012). Nevertheless, one method for the protection of these parasitoids is to use selective insecticides and fungicides, as well as other chemical and/or biological agents that are harmless to these organisms.

A combined use of parasitoid releases and safe pesticide products will maximize the impact of their use in pest control as tomato cultivation requires pesticides to be applied for protection against other pests and diseases, such as flies, whiteflies, spider mites and fungi (Momanyi et al. 2012).

Regardless of literature available on the effects of pesticides on *Trichogramma* spp. (Cônsoli et al. 2001, Garcia et al. 2009, Blibech et al. 2015), there is information on the adverse effects of the most commonly used pesticides regularly used in tomato crops on the eggs parasitoid *T. achaeae,* which are used in mass releases to control *T. absoluta*. Several studies have shown that *Trichogramma* spp. are very vulnerable to a large variety of pesticides (Hassan et al. 1998, Vieira et al. 2001, Khan et al. 2015).

This study aimed to evaluate the negative effects of 22 commonly used pesticides (20 insecticides or acaricides and 2 fungicides) in greenhouse tomato crops in Azores, on biological parameters of *T. achaeae*. We also analyzed the sublethal effects on the offspring.

Materials and Methods

Insects

Tr. achaeae were originally collected from eggs of *Thysanoplusia orichalcea* (Fabricius) and *Chrysodeixis calcites* (Esper; Lepidoptera: Noctuidae) found at Chã da Macela, São Miguel Island, Azores (Portugal) (37° 75′ 87″ N, 25° 54′ 12″ O, at 253 m altitude) and maintained in laboratory conditions on *Ephestia kuehniella* (Zeller; Lepidoptera: Pyralidae) eggs for 5–7 generations. Parasitoids were reared on *E. kuehniella*, glued onto yellow cardboard (0.8 × 8.5 cm) with the aid of 30 % (w/v) gum Arabic, in glass tube (10.0 × 1.0 cm diameter) containing honey droplets as a source of food for emerged adult. The glass tubes were closed with cotton wool and maintained at 21 ± 1°C of temperature, 70 ± 5% relative humidity (RH) and with a natural photoperiod (11.9 ± 3.6 h Light).

Pesticides

All tests were performed with fresh solutions of commercial pesticides prepared in water. Twenty insecticides and two fungicides were tested at the concentrations recommended by the manufacturer as indicated in Table 1. Pesticides were selected based on their use for the control of different pests in tomato greenhouses in the Azores archipelago.

Toxicity of Dried Pesticide Residues to *Tr. achaeae* Adults

For analysis of the effect of the various pesticides on the mortality of females and on other biological parameters of *T. achaeae*, yellow cards (1.5 × 0.8 cm) were prepared, each with 500 UV-killed *E. kuehniella* eggs glued to the card with a 30% solution of Arabic glue. The experiment design follows the guidelines of the International Organisation for Biological and Integrated Control (IOBC) for exposure of parasitoids to pesticides. The assay was performed in two different periods of time (group A and group B), with one control for each group. Every treatment was repeated four or five times, depending on the mortality of the females, and there were 15 replicates, corresponding to 15 host egg cards, sprayed with one of the 22 pesticides or with water (n = 60–75 per treatment). Each set of host egg cards was sprayed with 6 ml of an aqueous suspension of one of the pesticides, using Potter tower equipment (Burkard, Rickmansworth, United Kingdom) at 2 bars. Controls were sprayed with distilled water. This resulted in homogeneous spray coverage of 9.52 ± 2.17 µl (mean ± SD) of fluid cm^{-2}, corresponding to the concentrations recommended by the manufacturer (1,000 l ha^{-1} = 10 µl cm^{-2}). After treatment, egg cards were air-dried in an open plastic box for 24 h, and then they were offered to one isolated *T. achaeae* female, with less than 24 h of life, into the glass tube. The female was supplied with a honey solution (50%) and allowed to parasitize until her death. Females were observed daily until death to calculate

Table 1. Pesticides tested and their used and recommended field rates used in the experiment

Active ingredient (a.i.)	Chemical group	Trade name with formulation	Used	Company	Dose/cm^2
Abamectin	Avermectin	Bermectine 1.8 EC	Insecticide/acaricide	SIPCAM Portugal	0.005 µl
Acetamiprid	Neonicotinoid	Epik 20 SG	Insecticide	SIPCAM Portugal	0.005 mg
Acrinatrin	Pyrethroid	Rufast Avance 75 EW	Insecticide	SELECTIS	0.006 µl
Azoxystrobin	Strobilurin	Ortiva 23.1 SC	Fungicide	SYNGENTA	0.010 µl
Bacillus thuringiensis subsp. Kurstaki	Microbiological	Bactil X2 6.4 WP	Insecticide	SIPCAM Portugal	0.005 mg
Chlorantraniliprole	Anthranilic diamide	Altacor 35 WG	Insecticide	Bayer CropScience Portugal	0.001 mg
Chlorpyrifos	Organophosphate	Destroyer 480 EC	Insecticide	Nufarm Portugal	0.020 µl
Cyromazine	Triazine	Trigard 75 WP	Insecticide	SYNGENTA	0.003 mg
Deltamethrin	Pyrethroid	Decis 25 EC	Insecticide	Bayer CropScience Portugal	0.005 µl
Emamectin Benzoate	Avermectin	Affirm 0.85 SG	Insecticide	SYNGENTA	0.015 mg
Hexythiazox	Carboxamide	Nisorum 10 WP	Acaricide	SIPCAM Portugal	0.005 mg
Imidacloprid	Neonicotinoid	Confidor 200 SL	Insecticide	Bayer CropScience Portugal	0.005 µl
Lambda-cyhalothrin	Pyrethroid	Atlas 9.5 SC	Insecticide	SELECTIS	0.003 µl
Lufenuron	Benzoylurea	Match 0.50 EC	Insecticide	SYNGENTA	0.020 µl
Merthiocarb	Carbamate	Mesurol 200 SC	Insecticide	Bayer CropScience Portugal	0.050 µl
Methomyl	Carbamate	Lannate 25 WP	Insecticide	DU PONT-SAPEC	0.013 µl
Neem oil	Limonoid	Neem EC	Insecticide	BIAGRO	1.000 µl
Pirimicarb	Carbamate	Pirimor 50 WG	Insecticide	SYNGENTA	0.005 mg
Spinosad	Spinosyn	Spintor 480 SC	Insecticide	Lusosem	0.003 µl
Sulfur	Inorganic	Stullun 80 WG	Fungicide	SAPEC Agro Portugal	0.040 mg
Thiamethoxam	Neonicotinoid	Actara 25 WG	Insecticide	SYNGENTA	0.003 mg
Vegetable oils	-	Vegetable oil soap	Insecticide	Natur^{chemis}	1.000 µl

the longevity of adults in contact with the treated eggs. For the two groups, after offspring emergence, development time (egg to adult), number of parasitized eggs (host eggs that turned black), emergence rate (number of host eggs with a hole made by *Trichogramma*/total number of parasitized eggs) and sex ratio of offspring (number of females/total number of adults) were quantified for each treatment.

All tests were performed under semi-controlled conditions, at 26 ± 1°C for group A and 23 ± 1°C for group B, in 70 ± 5% RH and with a natural photoperiod (13.3 ± 1.3 h Light).

Effects on the First Generation

After emergence, female offspring were isolated in a glass tube, and a yellow card with untreated *E. kuehniella* eggs and a small drop of honey solution was added. Parasitism was allowed for 7 d. Then, the female was removed, and the mortality rate was recorded. Once the progenitor was removed, the egg cards were kept until the end of development in the same conditions. The estimated parameters were progenitor mortality rate, development time, number of parasitized eggs, emergence rate, and sex ratio of the offspring.

Statistical Analysis

Differences between treatments and the water control in female longevity, parasitoid development times, and number of parasitized host eggs that turned black were determined by a T-test using √(x + 0.5)-transformed data. Adult emergence rates and sex ratio were analyzed by T-test using arcsine √ x-transformed data to normalize their distribution (Zar 1996). Female infertility rates and female offspring mortality 7 d after emergence were analyzed by chi-square (χ^2) tests for comparison of proportions ($P < 0.05$). All analyses were performed using the statistical software SPSS 23.0 (IBM SPSS 2015).

Furthermore, the reduction in *T. achaeae* parasitism as related to the control treatment was calculated by the following equation: $E(\%) = (1 - Vt/Vc) \times 100$, in which $E(\%)$ is the percent reduction in parasitism, Vt is the median parasitism for the treatment tested, and Vc is the average parasitism observed for the control treatment (Carmo et al. 2010). The chemicals were classified according to the IOBC standards as follows: class 1, harmless ($E < 30\%$); class 2, slightly harmful ($30\% \le E \le 79\%$); class 3, moderately harmful ($80\% \le E \le 99\%$); and class 4, harmful ($E > 99\%$) (Hassan 1992).

Results

Toxicity of Dried Pesticide Residues to *Tr. achaeae* Adults

Among all the tested products, chlorpyrifos was the most poisonous; it significantly reducing the female's longevity and completely prevented the attainment of offspring (Table 2).

Most of the products tested affected the longevity of the females after contact with the eggs treated 24 h earlier, with the exception of chlorantraniliprole, lufenuron, *B. thuringiensis*, cyromazine and azoxystrobin, and eight of them reduced the longevity of females to less than 2 d (Table 2). The percentage of females that did not exhibit black host eggs (female infertility) was variable, and the most harmful products were chlorpyrifos, merthiocarb, spinosad, acrinatrin,

Table 2. Mean (± SE) female longevity, female infertility rate, development time (egg to adult), number of parasitized host eggs that turned black, emergence rate and female sex ratio of offspring of *T. achaeae*, when parasitism occurred 24 h after the host eggs were treated with 23 pesticides

Treatment (a.i.)	Female longevity (days)	Female infertilily (%)#	Development time (days)	Parasitized egg (*n*)	Emergence rate (%)	Female sex ratio (%)
Group A						
Abamectin	2.45 ± 0.2*	38.2*	9.1 ± 0.1*	9.4 ± 1.3*	54.6 ± 3.4*	71.0 ± 5.5
Acetamiprid	6.6 ± 0.4*	20.0	9.5 ± 0.1	16.6 ± 1.7*	89.8 ± 2.4	72.4 ± 3.3
Acrinatrin	1.6 ± 0.2*	68.0*	10.0 ± 0.2	0.6 ± 0.1*	90.1 ± 5.9	83.8 ± 6.2*
B. thuringiensis	7.2 ± 0.5	21.8	9.5 ± 0.1	21.7 ± 2.1	94.0 ± 0.9*	61.5 ± 4.1
Chlorantraniliprole	7.2 ± 0.5	14.5	9.5 ± 0.1	22.4 ± 2.3	94.1 ± 1.0*	71.4 ± 3.5
Chlorpyrifos	<1.0 ± 0.0*	-	-	0.0 ± 0.0*	-	-
Deltamethrin	6.5 ± 0.5*	23.6	10.2 ± 0.2	5.8 ± 0.8*	90.9 ± 2.0	90.3 ± 3.3*
Emamectin Benzoate	1.9 ± 0.1*	49.3*	9.3 ± 0.1	7.3 ± 1.0*	84.7 ± 2.3	74.5 ± 3.6
Hexythiazox	6.8 ± 0.4*	10.9	9.4 ± 0.1	22.4 ± 1.8	92.1 ± 2.2	62.8 ± 4.0
Imidacloprid	1.8 ± 0.1*	38.7*	9.0 ± 0.1*	11.0 ± 1.2*	89.3 ± 1.3	74.8 ± 2.9
Lambda-cyhalothrin	5.3 ± 0.5*	30.9	10.1 ± 0.2	4.6 ± 0.6*	94.2 ± 1.52*	78.6 ± 5.1*
Lufenuron	7.3 ± 0.5	10.9	9.5 ± 0.2	23.5 ± 2.1	49.2 ± 4.6*	70.9 ± 4.1
Merthiocarb	1.0 ± 0.0*	77.3*	10.2 ± 0.3	0.5 ± 0.1*	36.3 ± 10.0*	62.0 ± 8.6
Methomyl	1.2 ± 0.1*	42.7*	9.2 ± 0.1	5.1 ± 0.7*	86.9 ± 2.2	73.9 ± 4.9
Neem oil	5.9 ± 0.5*	16.4	9.8 ± 0.2	14.6 ± 1.3*	88.2 ± 1.8	75.4 ± 3.9
Pirimicarb	6.1 ± 0.5*	16.4	9.4 ± 0.2	18.5 ± 1.5	93.6 ± 1.5*	66.8 ± 3.7
Spinosad	1.1 ± 0.0*	74.7*	10.0 ± 0.3	1.8 ± 0.4*	14.9 ± 3.9*	66.4 ± 9.7
Thiamethoxam	2.2 ± 0.2*	52.0*	9.1 ± 0.1*	6.9 ± 1.0*	86.8 ± 2.0	75.8 ± 2.8*
Control	8.2 ± 0.4	16.0	10.0 ± 0.4	26.7 ± 1.7	89.4 ± 1.5	66.3 ± 3.2
Group B						
Azoxystrobin	6.5 ± 0.4	8.3	12.6 ± 0.1	36.4 ± 2.3	89.9 ± 1.2	64.4 ± 4.1
Cyromazine	7.4 ± 0.4	5.0	12.5 ± 0.1	34.0 ± 2.14	88.2 ± 2.0*	63.5 ± 3.8
Sulfur	1.9 ± 0.1*	5.0	12.7 ± 0.1*	18.9 ± 1.2*	89.9 ± 1.3	74.8 ± 4.0*
Vegetable oils	3.5 ± 0.3*	6.7	12.7 ± 0.1*	22.4 ± 1.4*	87.2 ± 1.6*	68.9 ± 4.2
Control	7.6 ± 0.4	8.3	12.4 ± 0.1	40.0 ± 2.36	93.2 ± 1.1	62.7 ± 3.9

Treatments differing significantly (T-test or # Chi-square (χ^2) test for comparison of proportions; $P < 0.05$) from the water control are indicated by an asterisk (*).

and thiamethoxam, which induced an infertility percentage greater than 50% (Table 2).

The results showed that only three pesticides significantly reduced the development time (abamectin, thiamethoxam and imidacloprid) (Table 2). Several tested pesticides caused a significant reduction in the number of parasitized host eggs, with the exception of chlorantraniliprole, lufenuron, pirimicarb, *B. thuringiensis*, hexythiazox, cyromazine and azoxystrobin, which resulted in rates of parasitisation equal or higher than the controls (Table 2). Merthiocarb, acrintrin, and spinosad resulted in less than two parasitized egg per female and chlorpyrifos none at all.

The emergence rate was significantly reduced by spinosad, followed by merthiocarb, lufenuron, and abamectin (Table 2). The three pyrethroids significantly affected the female sex ratio, with an increase in the number of females in the population (Table 2).

Consistent with these results, chlorpyrifos was the most toxic pesticide, reducing parasitism to zero, and was classified as harmful (class 4). Merthiocarb, methonil, lambda-cyhalothrinon, acrinatrin, and spinosad were moderately harmful (class 3). Only chlorantraniliprole, lufenuron, *B. thuringiensis,* hexythiazox, and cyromazine had no significant effect on the number of parasitized eggs (class 1) (Table 3). Data from the fungicide results showed that sulfur was classified as slightly harmful (class 2) and azoxystrobin as harmless (class 1) to *T. achaeae* (Table 3).

Effects on the First Generation

Analysis of the effects of merthiocarb, chlorpyrifos, and spinosad treatments was not performed, as the number of offspring females was very low, and they died in less than 24 h.

Table 3. Placement of the pesticide into IOBC survival side-effect classes based on the number of parasitized host eggs by *T. achaeae* that turned black, when parasitism occurred 24 h after the host eggs were treated with 22 compounds

Pesticide	*E* (%)	Class	Classification
Abamectin	65	2	Slightly harmful
Acetamiprid	38	2	Slightly harmful
Acrinatrin	98	3	Moderately harmful
Azoxystrobin	9	1	Harmless
Bacillus thuringiensis	19	1	Harmless
Chlorantraniliprole	16	1	Harmless
Chlorpyrifos	100	4	Harmful
Cyromazine	15	1	Harmless
Deltamethrin	78	2	Slightly harmful
Emamectin Benzoate	73	2	Slightly harmful
Hexythiazox	16	1	Harmless
Imidacloprid	59	2	Slightly harmful
Lambda-cyhalothrin	83	3	Moderately harmful
Lufenuron	12	1	Harmless
Merthiocarb	98	3	Moderately harmful
Methomyl	81	3	Moderately harmful
Neem oil	45	2	Slightly harmful
Pirimicarb	31	2	Slightly harmful
Spinosad	93	3	Moderately harmful
Sulfur	53	2	Slightly harmful
Thiamethoxam	74	2	Slightly harmful
Vegetable oils	44	2	Slightly harmful

E (%) = (1 – Vt/Vc) × 100, in which E is the effect of the pesticide on parasitism viability reduction compared to the control, Vt is the parasitism viability for the treatments and Vc is the parasitism viability for the control. Class 1- harmless (E < 30%); 2- slightly harmful (30% < E < 79%); 3- moderately harmful (80% < E < 99%); and 4- harmful (E < 99%).

Regarding the mortality of the females, the most dangerous products were emamectin benzoate, imidacloprid, lambda-cyhalothrin, acrinatrin, and neem oil, which caused more than 95% female mortality during the first 7 d. However, lufenuron, acetamiprid, *B. thuringiensis*, cyromazine, vegetable oils, and azoxystrobin did not affect this parameter (Table 4). The development time was significantly reduced by chlorantraniliprole, abamectin, lufenuron, pirimicarb, emamectin benzoate, acrinatrin, hexythiazox, vegetable oils, and sulfur, while the emamectin benzoate increased significantly this parameter (Table 4).

Only emamectin benzoate, imidacloprid, lambda-cyhalothrin, and sulfur significantly reduced the mean number of parasitized eggs. The emergence rate was significantly affected, with an increase induced by imidacloprid, lambda-cyhalothrin, acrinatrin, and deltamethrin, not very relevant in biological terms, and a reduction by azoxystrobin (Table 4). The offspring female sex ratio was also significantly affected by some pesticides, with a reduction in the number of females caused by chlorantraniliprole, emamectin benzoate, vegetable oils and by the three pyrethroids (lambda-cyhalothrin, acrinatrin and deltamethrin) and an increase caused by azoxystrobin (Table 4).

Discussion

Tr. achaeae is a suitable biological agent for use in pest management programs against *T. absoluta*, an introduced pest species that is difficult to manage with insecticides alone in tomato greenhouses.

Most laboratory and field studies have shown that *Trichogramma* wasps are highly susceptible to broad-spectrum insecticides (Li et al. 1986). Consequently, use of insecticides and *Trichogramma* has historically been considered incompatible.

The pesticide concentrations tested were equivalent to those recommended for field application. The toxic effects were assessed by measuring different biological parameters of *T. achaeae* exposed to residues on host eggs, 24 h after treatment.

A high degree of variation in the effects was found among classes of insecticides and among insecticides of the same class. Some classes of insecticides, such as the organophosphates, some carbamates, some pyrethroids and spinosad, showed little or no selectivity to the parasitoid species tested. Our study showed that different insecticides possessed significantly different risks to the *T. achaeae* adults, which may provide useful information for integration of biological control with pesticide control.

In the literature, organophosphate, carbamate, and synthetic pyrethroid insecticides are generally considered highly hazardous to biological control agents due to their broad-spectrum activity (Carmo et al. 2010, Cloyd 2012, Amaro et al. 2015).

Chlorpyrifos, an organophosphate, is one of the most widely used insecticides worldwide (Delpuech and Meyet 2003). Residual amounts of chlorpyrifos on the treated eggs are very toxic to *T. achaeae* as they caused high mortality in the wasps (100 %) in the 24 h after its application and reduced the parasitisation rate to zero. Therefore is classified as harmful (class 4). Amaro et al. (2015) also found a high mortality and a negative effect on the behavior of *T. pretiosum*. These results were expected because organophosphate compounds have been described as highly harmful to parasitoids (Hassan et al. 1998, Moura et al. 2005, Moura et al. 2006), and its use in IPM should be avoided (Amaro et al. 2015).

Other neurotoxic insecticides, such as carbamates (pirimicarb, methiocarb and methomyl), may be directly and indirectly more harmful to natural enemies than non-nerve toxin-type pesticides (Cloyd 2012). The present study showed that merthiocarb and

Table 4. Female mortality rate after 7 d of parasitism, mean (± SE) development time (egg to adult), number of parasitized host eggs that turned black, emergence rate and female sex ratio of offspring of *T. achaeae*, obtained from eggs treated with 19 pesticides

Treatment (a.i.)	Female mortality (%)#	Development time (days)	Parasitized egg (*n*)	Emergence rate (%)	Female sex ratio (%)
Group A					
Abamectin	86.7*	9.1 ± 0.0*	22.3 ± 2.9	91.8 ± 1.6	60.2 ± 5.8
Acetamiprid	60.0	9.3 ± 0.1	23.3 ± 2.6	92.9 ± 1.1	54.1 ± 5.5
Acrinatrin	100.0*	9.1 ± 0.1*	19.9 ± 5.4	99.3 ± 0.7*	23.0 ± 10.9*
B. thuringiensis	75.6	9.3 ± 0.1	24.9 ± 2.5	93.8 ± 0.8	55.9 ± 4.4
Chlorantraniliprole	91.1*	9.2 ± 0.1*	28.78 ± 2.6	91.4 ± 1.1	49.0 ± 4.9*
Deltamethrin	93.3*	9.3 ± 0.1	30.3 ± 2.7	97.6 ± 0.6*	11.4 ± 4.7*
Emamectin Benzoate	95.6*	10.0 ± 0.1*	18.8 ± 2.9*	88.4 ± 3.5	31.9 ± 5.9*
Hexythiazox	93.3*	9.2 ± 0.1*	25.4 ± 3.2	94.2 ± 1.0	58.6 ± 4.0
Imidacloprid	97.8*	9.3 ± 0.1	18.8 ± 2.7*	96.4 ± 0.9*	71.0 ± 6.3
Lambda-cyhalothrin	96.4*	9.3 ± 0.2	15.0 ± 3.5*	96.6 ± 1.3*	5.9 ± 4.1*
Lufenuron	75.6	9.1 ± 0.1*	31.6 ± 2.0	94.6 ± 0.7	62.5 ± 4.8
Methomyl	88.9*	9.3 ± 0.1	27.2 ± 4.7	92.8 ± 1.5	51.9 ± 8.2
Neem oil	100.0*	9.3 ± 0.1	24.9 ± 2.6	92.7 ± 1.5	55.0 ± 5.3
Pirimicarb	86.7*	9.1 ± 0.1*	32.9 ± 2.6	93.7 ± 0.9	65.2 ± 3.4
Thiamethoxam	88.9*	9.2 ± 0.1*	32.2 ± 3.0	94.3 ± 9.0	52.2 ± 6.2
Control	57.8	9.6 ± 0.2	27.5 ± 2.7	93.6 ± 0.8ab	67.0 ± 3.9c
Group B					
Azoxystrobin	9.0	13.6 ± 0.1	53.9 ± 2.3	91.2 ± 1.0*	60.6 ± 4.4*
Cyromazine	8.0	13.6 ± 0.1	60.5 ± 2.1*	95.6 ± 0.5	52.4 ± 4.8
Sulfur	41.0*	12.7 ± 0.2*	20.9 ± 4.2*	89.9 ± 1.3*	22.8 ± 8.8
Vegetable oils	19.0	13.2 ± 0.1*	48.9 ± 2.8	95.1 ± 0.5	36.4 ± 5.1
Control	11.0	13.5 ± 0.1	52.3 ± 2.9	95.2 ± 0.8	39.2 ± 5.5

Treatments differing significantly (T-test or # Chi-square (χ^2) test for comparison of proportions; $P < 0.05$) from the water control are indicated by an asterisk (*).

methomyl were moderately harmful, and pirimicarb was slightly harmful (class 2) based on their reduction of parasitisation. All of them severely affected the longevity of the females and the number of host eggs parasitized and significantly reduced the longevity of offspring populations. Merthiocarb and methomyl also increased female infertility rate. Different studies have reported that pirimicarb did not affect the biological parameters of *T. pretiosum* (Carvalho et al. 2001), however, other authors found that this product caused high mortality when adults of other parasitoids were exposed to dry residues or the direct spray method (Desneux et al. 2004, Moens et al. 2012). Bueno et al. (2008) demonstrated that methomyl significantly reduced parasitism viability when applied in the *T. pretiosum* immature stages.

Pyrethroids are the second most widely used family of insecticides (Delpuech and Delahaye 2013), however their application is not compatible with biological control methods, as shown in different studies (Beserra and Parra 2005, Carmo et al. 2010, Ksentini et al. 2010). In our study, pyrethroids were moderately harmful (class 3) to *T. achaeae* and notably reduced the longevity of females after contact with treated host eggs and the number of parasitized eggs, and acrinatrin significantly increased the percentage of infertile females. We also observed lethal and sublethal effects in the offspring. Beserra and Parra (2005), observed that different formulations of lambda-cyhalothrin, when applied before contact of the females with the host eggs, were harmful to *T. pretiosum* and Wang et al. (2012) showed that pyrethroids (cyhalothrin, fenpropathrin and lambda-cyhaltrin) are highly toxic to *T. ostriniae* adults. Very little is known about the effect of acrinatrin on *Trichogramma* species; however, the same author reported that acrinatrin is harmful to different parasitoid species. Concerning the effect of deltamethrin residues on *Trichogramma* adults, Ksentini et al. (2010) found them to be very harmful towards all *Trichogramma* species tested, and the same was observed by Bastos et al. (2006), Blibech et al. (2015), and Thubru et al. (2016).

Nowadays, neonicotinoids are the most widely used insecticidal class in the world (Jeschke and Nauen 2008, Simon-Delso et al. 2015), and are among the most effective insecticides for control of sucking and chewing herbivores (Tomizawa and Casida 2003). Some authors have suggested that some neonicotinoids may be used in combination with natural enemies in IPM programs (Millar and Denholm 2007, Pisa et al. 2017). However, in our study, we detected a significant reduction in the longevity of the females after contact with eggs treated with the assessed neonicotinoids and in the number of parasitized eggs, in treatments with thiamethoxam and imidacloprid. In this study, these neonicotinoids were classified as slightly harmful. Some authors have shown that thiamethoxam was harmful to other *Trichogramma* species, such as *T. chilonis*, *T. platneri*, and *T. pretiosum* (Brunner et al. 2001, Williams and Price 2004, Preetha et al. 2009). Imidacloprid has been found to be harmful to *T. chilonis*, *T. cacoeciae*, and *T.* nr. *brassicae* (Hewa-Kapuge et al. 2003, Saber 2011). Conversely, other authors indicated that acetamiprid is safe for *T. pretiosum* (Moura et al. 2005) and could be recommended in association with this species in tomato pest management programs (Moura et al. 2006).

As we observed, the harmlessness of chlorantraniliprole was also reported for different *Trichogramma* species, such as *T. pretiosum*, *T. dendrolimi*, *T. chilonis*, and *T. japonicum* and other species of parasitoid eggs (Preetha et al. 2009, Brugger et al. 2010, Uma et al. 2014).

Additionally, avermectin produced a high mortality of the females, increased the infertility of the females, and reduced the number of parasitized host eggs, and abamectin reduced the emergence rate of *T. achaeae*. The lethal and sublethal effect on the offspring population was evident in the female mortality rate, and when emamectin benzoate was used, we observed a reduction in the number of females in the population. Araújo et al. (2013) observed a reduction in the number of parasitized host eggs and placed abamectin in Class 2 for *T. pretiosum* in eggs of *Sitotroga cerealella* (Oliver; Lepidoptera:

Gelechiidae), and Khan et al. (2015) reported that it was highly toxic to the same species. Hussain et al. (2010) found that abamectin and emamectin benzoate were harmful to *T. chilonis* adults after 24 h of exposure.

Lufenuron is classified as harmless; however, it affects the emergence rate of *T. achaeae*. This is a benzoylphenylurea compound and acts by inhibiting the production of chitin, showing some selectivity across insect taxonomic orders (Carmo et al. 2010). It was reported to have only mild side effects on *Trichogramma* species, such as *T. dendrolimi* (Takada et al. 2001), and *T. cacoeciae* (Hassan and Haves 1998). However, the emergence rate of *T. achaeae* was significantly reduced, as observed by Carmo et al. (2010) in a study with the egg parasitoid *Telenomus remus* (Nixon; Hymenoptera: Scelionidae).

Concerning spinosad, the high mortality values and the negative effect on biological parameters of *T. achaeae* could be explained by its mode of action, similar to the observed with the neonicotinoids pesticides, which excites the nervous system by activating the nicotinic receptors of the acetylcholine, causing paralysis of the insects (Cônsoli et al. 2001). Similar results were observed by Ksentini et al. (2010) and Thubru et al. (2016), among others, in studies with different species of *Trichogramma*.

Our results showed that hexythiazox and cyromazine could be considered harmless to this egg parasitoid, consistent with the observations by Nadimi et al. (2008) in a study to evaluate the effect of hexythiazox (Nisorun, EC 10%) on *Phytoseiulus persimilis* (Evans; Acari: Phytoseiidae). Lira et al. (2015) reported a low acute toxicity of hexythiazox on *Tamarixia radiata* (Waterston; Hemiptera: Eulophidae), an important ectoparasitoid of *Diaphorina citri* (Kuwayama; Hemiptera: Liviidae). Hassan (1994) also reported the harmless effect of cyromazine on *T. cacoeciae*.

Based on a lack of toxicity and no apparent lethal and sublethal effects, it seems acceptable to assume that *B. thuringiensis* products, soap and oil could be successfully integrated into a biological control program unless these products are used in conjunction with small parasitoids (Brunner et al. 2001). Numerous studies have shown that *B. thuringiensis* is harmless to *Trichogramma* species (Vieira et al. 2001, Zhu et al. 2009, Thrubru et al. 2016). Among all 22 pesticides evaluated, neem oil reduced the longevity of the females and the number of parasitized host eggs by *T. achaeae*, and caused a very high mortality of their offspring. El-Wakeil et al. (2006) reported that neem products reduced the parasitism rate of *T. pretiosum* and had minor side effects on *T. minutum*, and Khattak and Mamoon-ur-Rashid (2006) observed that neem oil affected the parasitization of bollworm eggs by *T. chilonis*. Burnner et al. (2001) observed mortality of *T. platneri* when exposed to sprays of soap and suggested that was due to physical properties of the formulations, and mortality was caused by the adhesion of wings, legs, and antennae to the body.

In our study, both fungicides tested were harmless or only slightly harmful to *T. achaeae*, as observed by other authors for the same genus (Pratissoli et al. 2010, Silva and Bueno 2015) or in other egg parasitoids. According to Pratissoli et al. (2010), the fungicide azoxystrobin was selective to *T. atopovirilia* and can be used in integrated management of diseases in cucurbitaceous crops. Nevertheless, sulfur was slightly harmful to *T. achaeae*, with negative effects on female longevity and in the number on parasitized host eggs and lethal and sublethal effects on the offspring. Thomson et al. (2001) considered sulfur harmful to adults and to immature stages of two *Trichogramma* species (*T. carverae*, *T. funiculatum*) and suggested that to reduce the impact on resident *Trichogramma*, other chemicals will need to be used.

According to our results, we suggest the used of chlorantraniliprole and *B. thuringiensis* to control Lepidoptera as *T. absoluta* and caterpillars, cyromazine to control Diptera as *Liriomyza huidobrensis* (Blanchard; Diptera: Agromyzidae) and other miners, pirimicarb to control Homoptera as aphids, hexythiazox to control mites and azoxystrobin to control fungus in an IPM program with mass released of *T. achaeae*. However, the insecticides usually used to control with flies, one of the major pests in tomato crops, showed to be toxic to *T. achaeae*.

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