



Lethal and parasitism effects of selected novel pesticides on the immature stages of *Trichogramma chilonis* (Trichogrammatidae: Hymenoptera)

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Abstract

Selectivity of pesticides to the natural enemies in an agroecosystem is required for more effective integrated pest management. *Trichogramma chilonis* (Ishii) is an important natural enemy of lepidopteran pests, and is often exposed to pesticides. Effects of selected pesticides on acute mortality and parasitism when applied to parasitoids in egg, larval and pupal stages in their hosts were evaluated at 1x (field dose in Pakistan), 2x (double field dose) and 0.5x (half of field dose) doses. The parasitized host eggs were dipped in formulated solutions of pesticides when parasitoids were in different life stages. Parasitoid emergence from hosts treated with acetamiprid, fipronil and abamectin in the egg treatment and with spinetoram in all immature treatments were $\leq 33.9\%$. Treatment with acetamiprid (≤ 83.1 and $\leq 60.9\%$), fipronil (≤ 42.3 and $\leq 72.7\%$), and abamectin (≤ 17.2 and $\leq 52.6\%$) yielded emergence in larval and pupal stage treatments, respectively. Spirotetramat, chlorantraniliprole, spiromesifen, haloxypop-p-methyl, bispyribac sodium, nicosulfuron, chlorothalonil + procymidone, myclobutanil, pyraclostrobin + metiram, and trifloxystrobin + tebuconazole produced parasitoid emergence $\geq 80.1\%$. Parasitoids emerged from hosts treated with spirotetramat, chlorantraniliprole, spiromesifen, bispyribac sodium, pyraclostrobin + metiram and trifloxystrobin + tebuconazole (except at $2 \times$ dose in egg treatment) produced $\geq 84\%$ parasitism in all treatments. Myclobutanil treatment of egg, and nicosulfuron and haloxypop-p-methyl treatments of larvae and pupae, yielded $> 90\%$ parasitism. Acetamiprid and fipronil treatment of larvae and pupae, and abamectin treatment of pupae produced $\leq 78.46\%$ and $\leq 10.76\%$ parasitism, respectively. Over half of the pesticides caused no significant mortality to immature stages or exhibited little to no adverse impacts on parasitism and are promising for integration with these parasitoids.

Keywords Immature stages · *Trichogramma chilonis* · Mortality · Emergence · Parasitism

Introduction

Characterizing pesticide effects on beneficial insects is important for developing pest management strategies that integrate pesticides and natural enemies (van den Bosch and Stern 1962; Fishel 2013; You et al. 2016; de Paiva et al. 2020), and seek to minimize the adverse effects of pesticides by using selective compounds, or altering the dosage or schedule of pesticide application (Way 1986; Hassan et al.

1994; Martinson et al. 2001; Khan et al. 2014). Chemicals and biological control agents are being successfully integrated under integrated pest management (IPM) programs (Akhtar et al. 2021).

Pesticides contribute to pest control in agroecosystems (Engindeniz and Engindeniz 2006). Efficient.

integration of chemical and biological controls lessens the input of chemicals in agroecosystem (Jiang et al. 2018). Selective pesticides contribute to maintain pest population under economic threshold levels (de Paiva et al. 2020). Pesticides with selective modes of action contribute to 1) enhanced ecosystem services by conserving natural enemies (Jacas and Urbaneja 2009; Cheng et al. 2018; Tores and Bueno 2018), 2) reduced environmental degradation from broad spectrum insecticides (Gurr et al. 2000; Landis et al. 2000; Snyder 2019), 3) reduced likelihood of pest resurgence, (Hutchison et al. 2004; Mahankuda et al.

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2019), and 4) potentially delayed development of insecticide resistance in arthropod pests (Ruberson et al. 1998; Stark et al. 2007; Khan et al. 2015b; Khan 2017).

The safe, effective and sustainable management of insect pests demands use of strategies that encourage biological control (Lou et al. 2013). Biological control agents or natural enemies play a key role in agricultural production and can help to minimize the use of synthetic chemicals (Petersen 1993; Thomson and Hoffmann 2010). Parasitoids are important and often play critical roles as natural enemies in crop production due to their capacity to suppress various host populations (Wajnberg and Hassan 1994; Bale et al. 2008; Bompard et al. 2013; Bellows 2001; Jiang et al. 2019). However, pesticides adversely affect parasitoids' efficacy against their hosts (Brown 1989; Desneux 2004, 2006; Biondi et al. 2013, 2015; Wang et al. 2016, 2017; Parreira et al. 2018). Both parasitoids and predators are typically more susceptible to pesticides than the pest insects (Gill and Garg 2014). Nevertheless, parasitoids are generally more susceptible to pesticides compared to predators (Croft 1990; Hassan 1989, 1992; Biondi et al. 2012; Khan and Ruberson 2017). Insect parasitoids come into contact with insecticides through direct exposure spray droplets or residues on crop foliage (Sheng et al. 2021). Pesticides may induce direct mortality of natural enemies or inflict sublethal effects on their reproduction, behaviour, foraging or movement (Jepson 1989; Croft 1990; Desneux 2007; de Paiva et al. 2018; Wahengbam et al. 2018).

Trichogramma species play an important role in IPM of many crops by parasitizing eggs of many lepidopterans worldwide (Hassan et al. 1998; Hassan and Abdelgader 2001; Khan et al. 2015b; Cheng et al. 2018; Willow et al. 2019). They are successfully used in inundative and inoculative biological control programs worldwide for management of insect pests in corn, rice, cotton, sugar beet, tomatoes, vegetables, and orchards (Hassan 1993; Smith 1996; Wang et al. 2012; Nascimento et al. 2018). There are around 210 described species of *Trichogramma* distributed globally, of which 25 species are used for pest management in 34 crops in 30 countries, which manage more than 400 pest species (Wajnberg and Hassan 1994; Parra and Zucchi 1997; Hassan et al. 1998; Pinto 2006; Zucchi et al. 2010; Polaszek 2010; Goulart et al. 2011).

Trichogramma chilonis has been reared and successfully used in augmentation worldwide (Pinto and Stouthamer 1994; Lingathurai et al. 2015) for controlling several lepidopteran insect pests on corn (*Zea mays* L.), cotton (*Gossypium hirsutum* L.), and vegetables (Chang et al. 2001; Ballal et al. 2009; Wang et al. 2012). It is an important egg parasitoid of lepidopteran pests (Sattar et al. 2011; Khan et al. 2014), including rice leaf folder *Cnaphalocrocis medinalis* (Guenée) in Pakistan (Sagheer et al. 2008).

Trichogramma spp. wasps are highly susceptible to most broad-spectrum pesticides (Bull and Coleman 1985; Wang et al. 2012), which reduce their efficacy against host pests (Brar et al. 1991; Consoli et al. 1998; Schuld and Schmuck 2000; Wang et al. 2012; Khan et al. 2015b; Pontes et al. 2020), creating compatibility problems in integrated pest management. Therefore, the current study is focused on the integration of *Trichogramma chilonis* with pesticides for more effective control of lepidopteran pests.

The current study evaluated effects of selected pesticides, including insecticides, miticides, herbicides and fungicides on 1) the emergence of *T. chilonis* from hosts treated when parasitoids were in different life stages, and 2) parasitism of eggs of the angoumois grain moth, *Sitotroga cerealella* Olivier by female *T. chilonis* emerged from eggs of *S. cerealella* treated with pesticides when parasitoids were in the egg, larval and pupal stages. *S. cerealella* has typically been used for rearing *Trichogramma* species because of low rearing costs and ease of mass production (Hassan 1997). Pesticides were selected because 1) they are commonly and widely used chemicals worldwide, and have novel chemistries, and 2) almost all of these chemicals specifically target Lepidopteran in the agroecosystem.

Materials and methods

Rearing of *Sitotroga cerealella*

An electric suction apparatus was used to collect adults of *Sitotroga cerealella* Olivier from infested wheat grains and transfer them into a plastic jar (10 × 15 cm) with mesh (size 35 to 40) affixed to the bottom for holding wheat flour to allow moths to lay eggs for 24 h. Subsequently, eggs were collected from the flour using sieves (mesh no 50, 70). All moth stages were reared on wheat grains in plastic jars (10 × 15 cm) and was maintained in the Entomology laboratory of Nuclear Institute for Food and Agriculture (NIFA), Tarnab, Peshawar, under ambient laboratory conditions of 24 ± 6 °C, 65 ± 10% RH, and 16:8 h (L:D), until adult emergence.

Rearing of *Trichogramma chilonis*

Approximately 800 to 1000 fresh eggs of *S. cerealella* (< 24 h old) were glued (with Arabic gum) on paper card (4 × 7 cm), and were exposed to newly emerged *T. chilonis* (approximately 30 to 50 pairs) in a glass jar (5 × 12 cm) for 24 h under the same laboratory conditions used for rearing of *S. cerealella*. The likely parasitized exposed egg card was then transferred to another glass jar and was incubated at the 23 ± 3°C, 70 ± 10% (RH), and 14:10 (L:D) until adult parasitoid emergence.

Experimental design and setup/toxicity testing

Commercial formulations of 14 pesticides (Table 1) were mixed with water to prepare three doses, namely the field dose (x) used in Pakistan, double the field dose (2x), and half the field dose (0.5x) to determine their effects on the immature stages of *T. chilonis* in the experiments conducted in the Entomology laboratory (NIFA) under the same laboratory conditions used for rearing *S. cerealella*.

Eggs of *S. cerealella* containing eggs (24 h after exposure to parasitoids), larvae (72 h after exposure to parasitoids) and pupa (144 h after exposure to parasitoids) of *T. chilonis* were treated with pesticides by dipping 10 card strips (0.8 × 8 cm), each containing 10–15 parasitized host eggs, in each of the pesticide solutions or in water (untreated control) for 1–2 s. One complete treatment of pesticide used approximately 300–450 host eggs. Each card was air dried at room temperature for 1 h, and subsequently transferred to a glass

vial (1 × 10 cm), was incubated at laboratory conditions used for rearing of *T. chilonis* until adult emergence.

After parasitoids emerged, single cards containing approximately 250 to 300 fresh eggs of *S. cerealella* were each exposed to 4–6 female *T. chilonis* (depending on the number of host eggs on the card) in a vial for 24 h. Each exposed card was transferred to a separate vial and was incubated under the stated conditions. The number of parasitoid adults emerged as well as numbers of pupae that failed to yield adults (blackened host eggs containing parasitoid pupae) were counted and percentage of emergence and parasitism relative to controls calculated.

Data calculation and statistical analysis

The raw data obtained were converted into percent emergence/parasitism and mean parasitism for each treatment before analysis.

$$\text{Percent emergence/Mean Parasitism} = \frac{\text{Total no. emerged adults/parasitized host eggs in each treatment}}{\text{Total no. paras. host eggs/female parasitoids used in each treatment}}$$

$$\text{Percent parasitism relative to control} = \frac{\text{Total no. parasitized host eggs in each treatment}}{\text{Total no. parasitized host eggs in each control}} \times 100$$

Table 1 Label descriptions of the pesticides used in the experiments

Trade name and formulation	Type of chemical	Active ingredient	Chemical class	Field rate /acre
Coragen 200SC	insecticide	Chlorantraniliprole	Anthranilic diamide	80-mL
Movento 240SC	insecticide	Spirotetramat	Tetramic acids	125 mL + Adju. 250-mL
Radiant 120SC	insecticide	Spinetoram	Spinosyns	100-mL
Mospilon 20SP	insecticide	Acetamiprid	Neonicotinoid	125-gm
Regent 50SC	insecticide	Fipronil	Phenylpyrazol	480- mL
Oberon 240SC	miticide	Spiromesifen	Tetronic acids	250-mL
Abamectin 1.8EC	miticide	Abamectin	Avermectin or Glycoside	480-mL
Clover 200WP	herbicide	Bispyribac Sodium (20%w/w) + Adjuvant (Poly- ethoxylated Fatty alcohol, 99.50%w/w)	Pyrimidinylthiobenzo-ates	80-gm
Sun 750WDG	herbicide	Nicosulfuron	Sulfonylurea	30-gm
Percept 10.8EC	herbicide	Haloxypop-p-methyl	Aryloxyphenoxyprop-ionate	350- mL
Systhane 20EW	herbicide	Myclobutanil	Triazole	35-mL
Nativo 750WG	fungicide	Trifloxystrobin (25%w/w) + Tebu- conazole (50% w/w)	Mandelamide + Triazole	65-gm
CabrioTop 600WDG	fungicide	Pyraclostrobin (5% w/w) + Meti- ram (55% w/w)	methoxy-carbamates + Ethylene bisdithiocarbamates	250-gm
Protocol 500WP	fungicide	Chlorothalonil (33.3%w/w) + Pro- cymidone (16.7% w/w)	Organochlorine + Dicarboximide	500-gm

WP wettable powder, EC emulsifiable concentrate, SP soluble powder, WDG water-dispersable granule, SC soluble concentrate, WG wettable granule, EW emulsifiable concentrate in water

$$\text{Percent emergence relative to control} = \frac{\text{Total no. emerged adults in each treatment}}{\text{Total no. emerged adults in each control}} \times 100$$

The Shapiro–Wilk tests for all data analyzed indicated that none of these data were normally distributed, with all *p*-values (<0.001). Therefore data were analyzed with a rank-based nonparametric tests: Aligned ranks transformation ANOVA (ART-ANOVA: one way, see, e.g., Beasley and Zumbo 2009; or Higgins and Tashtoush 1994). The R package (R statistical software: R Core team 2013) called ARTool (Kay and Wobbrock 2015) is developed by Wobbrock et al. (2011) is used to cover ART-ANOVA. For all tests the alpha level was set at $\alpha = 0.001$ (also denoted as ***). Multiple comparison between pesticides (Table 1: Active ingredients), by doses were conducted using post hoc Kruskal–Wallis tests based on the R agricolae (de Mendiburu 2020) package. The alpha level was adjusted with the Benjamin & Hochberg method (Benjamini and Hochberg 1995) (which is much less conservative than the Bonferroni correction). Multiple comparison between doses, by pesticides were conducted using the aligned Friedman rank test from the scmamp R packages (Calvo and Santafe 2016).

The percentage reduction in emergence or parasitism compared to controls was calculated by the formula: $E(\%) = (1 - Et/Ec) \times 100$, where “E” is measured as either reduction of parasitism rate or adult emergence rate compared to controls. “Et” is the parasitism or emergence rate observed in each pesticide treatment, and “Ec” is the parasitism or emergence rate observed in the untreated control (Manzoni et al. 2007).

Rates of emergence or parasitism relative to the controls were characterized using toxicity categories of International Organization for Biological Control (IOBC)/West Palaearctic Regional Section (WPRS) (Hassan 1994; Sterk et al. 1999): 1 = harmless ($E < 30\%$ emergence or parasitism); 2 = slightly harmful ($30 \leq E \leq 79\%$); 3 = moderately harmful ($79 < E \leq 99\%$); 4 = harmful ($> 99\%$ emergence or parasitism), where “E” is the effect of the pesticide on the biological control agent being measured as the reduction in percentage of emergence or parasitism compared to the control.

Results

Effect of pesticides on parasitoid emergence

Egg stage treatment

The R package called Artool (ART-ANOVA) indicated a significant main effects for pesticides ($F = 427.24$, $df = 13/126$, $p < 0.001$) and doses ($F = 843.80$, $df = 3/378$, $p < 0.001$), as well as interaction between pesticides and doses ($F = 139.28$, $df = 39/378$, $P < 0.001$).

Table 2 presents the mean percentage emergence of *T. chilonis* from host eggs treated at egg stage of parasitoids with different insecticides, miticides, herbicides and fungicides at 2x, x and 0.5x doses and from untreated (control) eggs. Figure 1 shows the mean percentage parasitoid emergence relative to the controls for all pesticide treatments of parasitoids in the egg stage. Emergence in the treatments with the insecticides acetamiprid, spinetoram and fipronil and the miticide abamectin was $\leq 42.1\%$ (Fig. 1). The field dose (x) of fipronil was harmful, those of spinetoram and abamectin were moderately harmful and acetamiprid at dose x was slightly harmful for emergence of parasitoids from host eggs (IOBC classification, Table 2). Spiromesifen, with 68.1%–81.5% emergence relative to the controls (Fig. 1), was harmless at both x and 0.5x, but slightly harmful for emergence at 2x dose (IOBC classification, Table 2). Spirotetramat and chlorantranilprole, with $\geq 88.5\%$ emergence relative to the controls, along with all herbicides and fungicides tested against the parasitoid egg stage were harmless for emergence of parasitoids at used all doses (Fig. 1; IOBC classification, Table 2).

Larval stage treatment

The ART-ANOVA indicated a significant main effects for pesticides ($F = 594.46$, $df = 12/117$, $p < 0.001$) and doses ($F = 1184.74$, $df = 3/351$, $p < 0.001$), as well as an interaction between pesticides and doses ($F = 167.71$, $df = 36/351$, $p < 0.001$).

Table 3 shows the mean percentage emergence of *T. chilonis* from host eggs treated at larval stage of

parasitoids with different insecticides, miticides, herbicides and fungicides at 2x, x and 0.5x doses and from untreated (control) eggs. Figure 2 shows the mean percentage parasitoid emergence relative to the controls for all pesticide treatments of parasitoids in the larval stage. Emergence in the treatments with the insecticides acetamiprid, spinetoram and fipronil and the miticide abamectin was $\leq 68.7\%$, except 0.5x dose of acetamiprid (Fig. 2). The field doses of spinetoram, fipronil and abamectin were moderately harmful, and acetamiprid at x dose was slightly harmful for emergence of parasitoids from host eggs (IOBC classification, Table 3). The remaining pesticides including spirotetramat, chlorantranilprole, 2x dose of acetamiprid and spiromesifen were harmless, as were all doses of the herbicides and fungicides with $> 73\%$ emergence relative to the controls (Fig. 2; IOBC classification, Table 3). The data regarding effect of chlorothalonil + procymidone on emergence in the larval stage treatments are not included due to fungus attack on the parasitized cards.

Table 2 Percentage emergence (mean ± SE) of *T. chilonis* from host eggs (*S. cerealella*) treated with different pesticides when parasitoids were in egg stage (Post Hoc Kruskal–Wallis tests, P=0.001 or 0.1%), and IOBC ranking of toxicity (based on % reduction in emergence relative to control)

Pesticides	(Doses and toxicity ranking); Emergence (mean ± SE)						
	2x	C	x	C	0.5x	C	Control
Spirotetramat	85.4 ± 2.99 cd	1	85.5 ± 5.79 c	1	85.2 ± 4.38 cd	1	85.6 ± 7.78 efg
Chlorantraniliprole	80.1 ± 7.28 e	1	86.2 ± 3.87 bc	1	86.5 ± 3.47 bc	1	86.6 ± 4.69 def
Acetamiprid	5.4 ± 1.28 f	3	25.0 ± 3.31 de	2	34.0 ± 5.13 ef	2	80.7 ± 8.50 g
Spinetoram	0.7 ± 0.46 g	4	9.2 ± 3.01 ef	3	28.6 ± 3.93 fg	2	90.9 ± 3.31 c
Fipronil	0.4 ± 0.37 g	4	0.0 ± 0.00 g	4	0.7 ± 0.52 h	4	92.2 ± 3.35 bc
Abamectin	1.1 ± 0.52 g	3	3.4 ± 0.91 fg	3	7.6 ± 1.26 gh	3	91.2 ± 3.31 c
Spiromesifen	60.9 ± 7.19 f	2	65.2 ± 7.42 d	1	72.9 ± 5.71 e	1	89.4 ± 8.06 cde
Haloxyfop-p-methyl	88.4 ± 3.50 bc	1	89.3 ± 2.53 b	1	86.9 ± 3.32 bc	1	89.6 ± 3.55 cde
Bispyribac sodium	90.8 ± 2.78 b	1	93.4 ± 2.93 a	1	95.1 ± 2.75 a	1	96.7 ± 3.33 ab
Nicosulfuron	83.3 ± 4.82 de	1	83.5 ± 2.86 c	1	83.3 ± 4.08 d	1	83.6 ± 5.54 fg
Myclobutanil	95.2 ± 1.51 a	1	94.3 ± 2.89 a	1	95.3 ± 2.06 a	1	95.6 ± 2.30 ab
Chlorothalonil + Procymidone	89.2 ± 1.87 bc	1	84.0 ± 4.07 c	1	89.7 ± 2.46 b	1	90.0 ± 2.12 cd
Pyraclostrobin + Metiram	79.2 ± 6.36 e	1	86.1 ± 7.91 bc	1	82.8 ± 4.18 d	1	89.5 ± 3.55 cde
Trifloxystrobin + Tebuconazole	96.5 ± 1.60 a	1	93.9 ± 3.23 a	1	96.2 ± 2.45 a	1	100 ± 0.00 a

Means followed by the same letter within a column are not significantly different (Post Hoc Kruskal–Wallis tests, p > 0.001). “C” indicates toxicity class based on IOBC

Pupal stage treatment

The ART-ANOVA showed a significant main effects for pesticides (F=221.18, df= 12/117, p<0.001) and doses (F=1777.87, df= 3/351, p<0.001) as well as an interaction (F=227.51, df= 36/351, p<0.001) between pesticides and doses.

Table 4 indicates the mean percentage emergence of *T. chilonis* from host eggs treated at pupal stage of parasitoids with different insecticides, miticides and herbicides at 2x,

x and 0.5x dose rates and from untreated (control) eggs. Figure 3 shows the mean percentage parasitoid emergence relative to the controls for all pesticide treatments of parasitoids in the pupal stage. Emergence in the treatments with the insecticides spinetoram, acetamiprid, and fipronil and the miticide abamectin were ≤ 32.4% at 2x dose, and the latter three products led to emergence ranging from 61% to 82.8% at 0.5x dose (Fig. 3). The field dose (x) of acetamiprid and abamectin were slightly harmful, those of spinetoram and

Fig. 1 Percent emergence (mean) relative to control of *T. chilonis* from host eggs (*S. cerealella*) treated with different pesticides when parasitoids were in egg stage

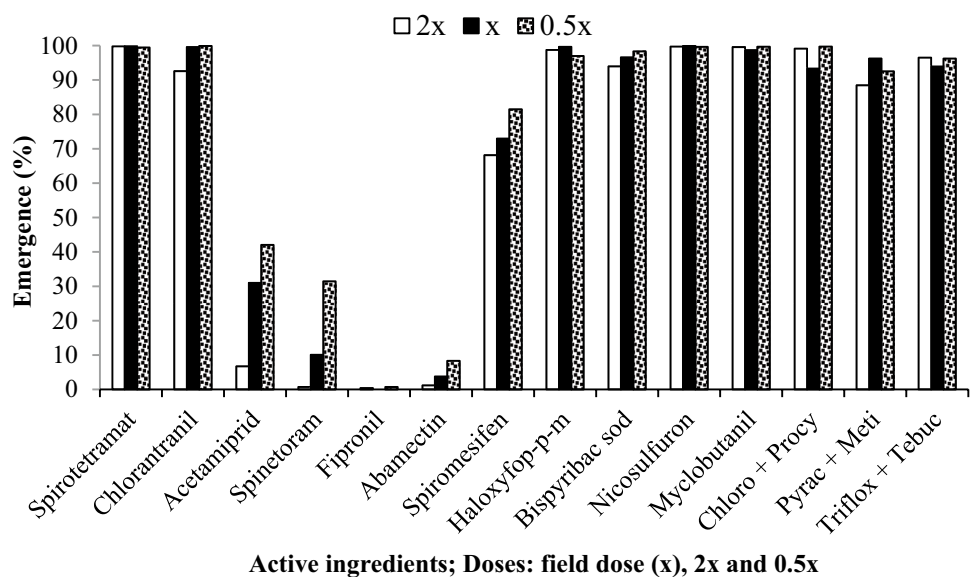


Table 3 Percentage emergence (mean±SE) of *T. chilonis* from the host eggs (*S. cerealella*) treated with different pesticides when parasitoids were in larval stage (Post Hoc Kruskal–Wallis tests, p=0.001 or 0.1%), and IOBC ranking of toxicity (based on % reduction in emergence relative to control)

Pesticides	(Doses and toxicity ranking); Emergence (mean ± SE)						
	2x	C	x	C	0.5x	C	Control
Spirotetramat	63.0±1.82 e	1	82.8±5.54 c	1	83.0±4.54 cd	1	86.0±6.28 c
Chlorantraniliprole	95.3±3.48 a	1	94.0±0.83 a	1	95.2±1.92 a	1	95.4±1.84 a
Acetamiprid	47.2±5.01 f	2	59.7±2.90 ef	2	83.1±4.00 d	1	86.9±3.90 c
Spinetoram	5.4±3.27 fg	3	5.0±1.05 gh	3	11.6±2.95 f	3	90.9±6.73 abc
Fipronil	0.0±1.39 h	4	11.4±2.81 fg	3	42.3±4.88 ef	2	90.2±6.73 c
Abamectin	2.9±0.89 gh	3	4.3±2.36 h	3	17.2±3.10 f	3	90.9±3.29 bc
Spiromesifen	65.0±7.48 e	1	71.4±4.06 d	1	80.4±3.31 d	1	88.6±3.08 c
Haloxyfop-p-methyl	69.7±6.61 d	1	69.2±8.07 de	1	75.1±7.21 de	1	89.5±7.88 c
Bispyribac sodium	88.8±5.11 bc	1	84.0±6.06 c	1	89.1±3.82 b	1	89.7±6.28 c
Nicosulfuron	89.2±1.44 b	1	89.2±2.33 b	1	89.3±3.09 b	1	89.7±6.28 c
Myclobutanil	94.7±2.92 a	1	93.3±2.84 a	1	94.8±2.27 a	1	95.1±1.99 c
Pyraclostrobin + Metiram	85.5±10.00 c	1	84.3±6.18 c	1	86.8±8.83 bc	1	89.5±7.88 ab
Trifloxystrobin + Tebuconazole	88.3±3.06 bc	1	88.2±2.77 b	1	88.5±1.57 b	1	88.6±3.35 c

Means followed by the same letter within a column are not significantly different (Post Hoc Kruskal–Wallis tests, p>0.001). “C” indicates toxicity class based on IOBC

fipronil were moderately harmful and harmless for emergence, respectively (IOBC classification, Table 4). Abamectin and acetamiprid at dose x were slightly harmful (Table 4) for emergence of parasitoids with ≤33.9% emergence relative to control (Fig. 3). Spirotetramat, chlorantraniliprole and spiromesifen with ≥91.4% emergence relative to the controls, and all the herbicides and fungicides (except chlorothalonil + procymidone) tested were harmless for emergence at used all doses, (Fig. 3; IOBC classification, Table 4). Data on chlorothalonil + procymidone were not included because of fungal attack on the egg cards.

Effect of pesticides on parasitism

Parasitism by females treated in the egg stage

The ART-ANOVA demonstrated a significant main effects for pesticides (F=183.386, df=10/99, p<0.001) and doses (F=684.198, df=3/297, p<0.001), as well as an interaction (F=48.046, df=30/297, p<0.001) between pesticides and doses.

Table 5 presents the mean percentage parasitism by female *T. chilonis* emerged from host eggs treated with

Fig. 2 Percent emergence (mean) relative to control of *T. chilonis* from host eggs (*S. cerealella*) treated with different pesticides at larval stage of parasitoids

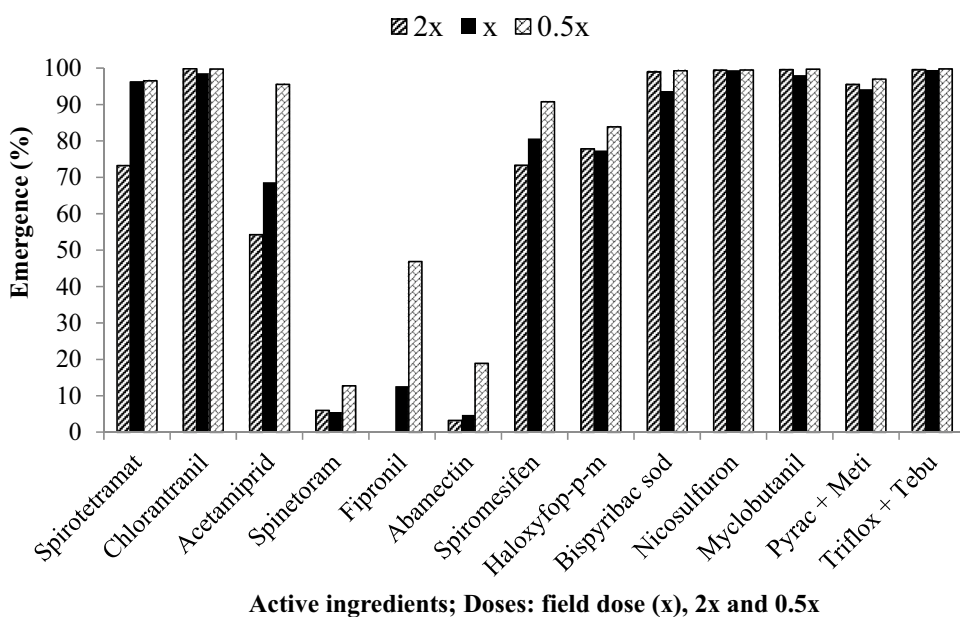


Table 4 Percentage emergence (mean ± SE) of *T. chilonis* adults from the host eggs (*S. cerealella*) treated with different pesticides at pupal stage of parasitoid (Post Hoc Kruskal–Wallis tests, p=0.001 or 0.1%), and IOBC ranking of toxicity (based on % reduction in emergence relative to control)

Pesticides	(Doses and toxicity ranking); Emergence (mean ± SE)							
	2x	C	X	C	0.5x	C	Control	
Spirotetramat	89.3 ± 2.11 ab	1	89.0 ± 2.47 b	1	89.4 ± 2.47 abc	1	89.4 ± 3.32 abc	
Chlorantranilprole	86.4 ± 5.20 cd	1	86.4 ± 7.91 cde	1	86.6 ± 5.18 de	1	86.7 ± 4.46 cd	
Acetamiprid	6.4 ± 2.18 hi	3	29.0 ± 3.43 hi	2	60.9 ± 5.82 gh	1	85.5 ± 5.36 d	
Spinetoram	1.8 ± 0.83 i	3	2.1 ± 0.77 j	3	6.4 ± 10.00 h	3	86.2 ± 8.39 d	
Fipronil	28.4 ± 5.27 fg	2	69.4 ± 4.21 gh	1	72.7 ± 5.10 fg	1	87.9 ± 5.85 bcd	
Abamectin	19.7 ± 2.43 gh	2	19.1 ± 3.26 ij	2	52.6 ± 6.56 gh	2	86.1 ± 8.39 d	
Spiromesifen	87.6 ± 3.94 bc	1	89.8 ± 2.33 b	1	90.1 ± 1.87 ab	1	90.5 ± 3.92 abc	
Haloxyfop-p-methyl	82.0 ± 5.38 e	1	88.7 ± 5.37 bc	1	88.3 ± 4.17 bcd	1	89.4 ± 3.32 abc	
Bispyribac sodium	93.1 ± 2.73 a	1	93.3 ± 6.18 a	1	93.2 ± 2.24 a	1	93.4 ± 2.04 a	
Nicosulfuron	85.1 ± 3.25 d	1	85.2 ± 5.08 def	1	90.6 ± 2.64 ab	1	90.7 ± 4.36 ab	
Myclobutanil	82.0 ± 7.19 e	1	81.8 ± 5.52 fg	1	83.8 ± 4.65 ef	1	85.2 ± 3.86 d	
Pyraclostrobin + Metiram	79.8 ± 3.03 ef	1	83.5 ± 4.02 ef	1	85.8 ± 3.45 e	1	87.2 ± 5.18 bcd	
Trifloxystrobin + Tebuconazole	87.3 ± 4.06 cd	1	87.4 ± 3.68 bcd	1	87.0 ± 9.68 cde	1	87.4 ± 3.47 bcd	

Means followed by the same letter within a column are not significantly different (Post Hoc Kruskal–Wallis tests, p > 0.001). “C” indicates toxicity class based on IOBC

various insecticides, miticides, herbicides and fungicides at x, 2x and 0.5x doses when parasitoids were in the egg stage and from control eggs. Figure 4 shows the percentage parasitism relative to controls by female *T. chilonis* emerged from host eggs treated when parasitoids were in the egg stage. Spinetoram, fipronil and abamectin were toxic to parasitoids in the egg stage with ≤ 28.6% emergence (Table 2) at all doses. Consequently, very few adults emerged or those that emerged were unable to parasitize the

host eggs. These products are considered harmful for parasitism and are, therefore, are not included in Table 5 and Fig. 4. According to Fig. 4 and the IOBC classification in Table 5, acetamiprid was harmful for parasitism at 2x and x (0 parasitism), and moderately harmful at 0.5x (2 mean parasitism) dose. Nicosulfuron and chlorothalonil + procymidone were slightly harmful for parasitism (Fig. 4; IOBC, Table 5) at 2x, while the other pesticides were harmless for parasitism at all doses.

Fig. 3 Percent emergence (mean) relative to control of *T. chilonis* from host eggs (*S. cerealella*) treated with different pesticides when parasitoids were in pupal stage

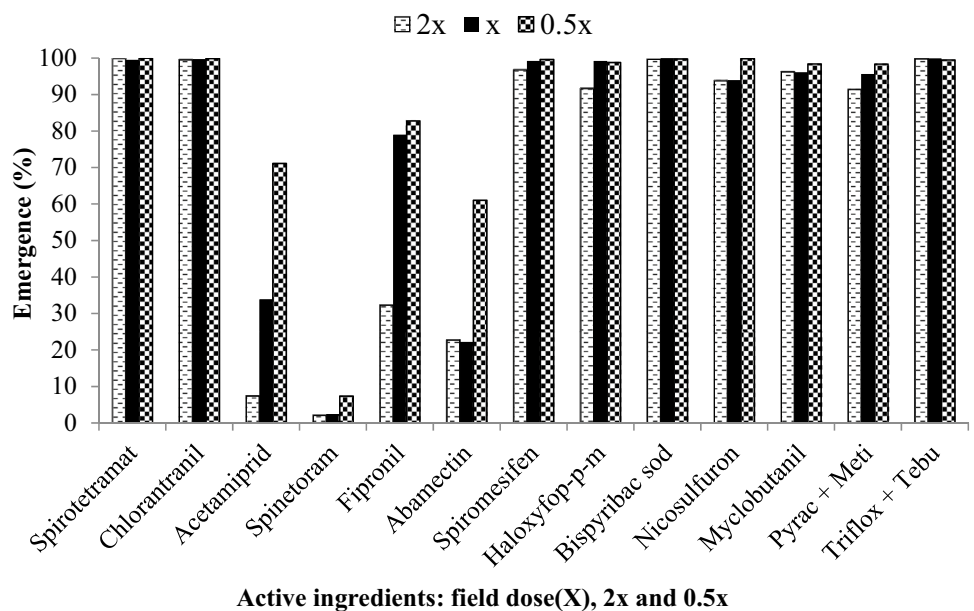


Table 5 Parasitism (mean \pm SE) by *T. chilonis* emerged from the host eggs (*S. cerealella*) treated at egg stage of parasitoid with different pesticides (Post Hoc Kruskal–Wallis tests, $p=0.001$ or 0.1%), and IOBC ranking of toxicity (based on % reduction in parasitism relative to control)

Pesticides*	(Doses and toxicity ranking); Parasitism (mean \pm SE)						
	2x	C	x	C	0.5x	C	Control
Spirotetramat	24.4 \pm 3.34 a	1	23.7 \pm 2.26 ab	1	25.4 \pm 3.16 b	1	25.5 \pm 8.77 abc
Chlorantranilprole	24.7 \pm 2.51 a	1	24.4 \pm 1.98 a	1	24.0 \pm 2.16 b	1	24.9 \pm 2.88 bcd
Acetamidrid	0.0 \pm 0.00 n f	4	0.0 \pm 0.00 d	4	2.0 \pm 0.21 d	3	23.1 \pm 4.44 de
Spiromesifen	22.3 \pm 9.76 b	1	22.3 \pm 6.61 bc	1	21.7 \pm 7.25 d	1	22.4 \pm 4.96 e
Haloxyfop-p-methyl	22.0 \pm 2.93 b	1	22.2 \pm 1.86 c	1	22.4 \pm 2.78 cd	1	28.7 \pm 4.23 cde
Bispyribac sodium	21.1 \pm 1.28 bc	1	21.0 \pm 1.08 c	1	23.2 \pm 2.03 bc	1	23.6 \pm 0.86 cde
Nicosulfuron	16.3 \pm 0.91 d	2	17.5 \pm 2.21 d	1	23.5 \pm 2.42 b	1	24.3 \pm 7.71 bcde
Myclobutanil	24.3 \pm 1.83 a	1	24.3 \pm 0.83 a	1	24.3 \pm 1.63 b	1	24.4 \pm 0.99 bcd
Chlorothalonil + Procymidone	13.4 \pm 1.36 e	2	21.0 \pm 1.70 c	1	22.1 \pm 2.38 cd	1	23.6 \pm 1.70 cde
Pyraclostrobin + Metiram	24.3 \pm 4.10 a	1	27.2 \pm 2.53 a	1	27.8 \pm 4.48 a	1	28.8 \pm 4.23 a
Trifloxystrobin + Tebuconazole	18.5 \pm 1.46 cd	1	21.6 \pm 2.15 c	1	24.0 \pm 3.28 b	1	25.7 \pm 4.37 ab

Means followed by the same letter within a column are not significantly different (Post Hoc Kruskal–Wallis tests, $p>0.001$). “C” indicates toxicity class based on IOBC

* some of the pesticides were not included as their parasitism data are not available because of toxicity of these products to parasitoid eggs stage, very few adults emerged or those emerged successfully were unable to parasitize the host eggs

Parasitism by females treated in the larval stage

The ART-ANOVA indicated a significant main effects for pesticides ($F=106.773$, $df=10/99$, $p<0.001$) and doses ($F=346.785$, $df=3/297$, $p<0.001$), as well as an interaction ($F=31.734$, $df=30/297$, $p<0.001$) between pesticides and doses.

Table 6 presents the mean percentage parasitism by female *T. chilonis* emerged from host eggs treated with various insecticides, miticides, herbicides and fungicides

at x, 2x and 0.5x doses when parasitoids were in the larval stage and from control eggs. Figure 5 shows the percentage parasitism relative to controls by female *T. chilonis* emerged from host eggs treated when parasitoids were in the larval stage. Spinetoram and abamectin were toxic to parasitoid larval stage with $\leq 17.2\%$ emergence from the treated host eggs at all doses. Very few adults emerged or those that emerged were unable to parasitize the host eggs. These products are considered harmful for parasitism and are, therefore, are not included in Table 6 and Fig. 5.

Fig. 4 Percent parasitism (mean) relative to control of *S. cerealella* eggs by *T. chilonis* emerged from host eggs (*S. cerealella*) treated at egg stage of parasitoids

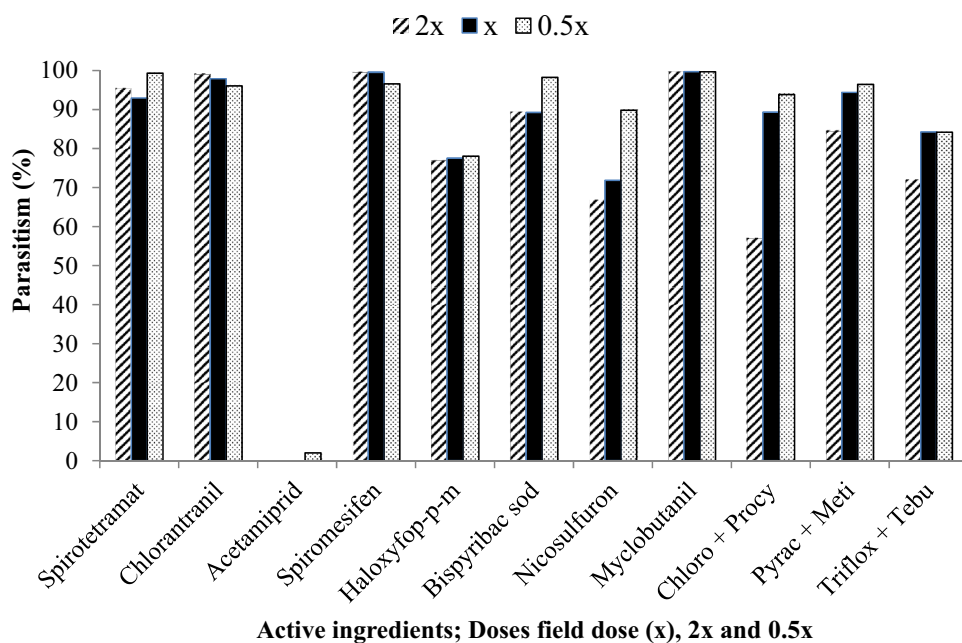


Table 6 Parasitism (mean ± S.E) by *T. chilonis* emerged from eggs of *S. cerealella* treated with different pesticides at larval stage of parasitoid (Post Hoc Kruskal–Wallis tests, p=0.001 or 0.1%), and IOBC ranking of toxicity (based on % reduction in parasitism relative to control)

Pesticides*	(Doses and toxicity ranking); Parasitism (mean ± SE)							
	2x	C	x	C	0.5x	C	Control	
Spirotetramat	20.8 ± 1.94 ef	1	23.1 ± 3.99 de	1	23.1 ± 3.97 c	1	24.2 ± 1.07 de	
Chlorantranilprole	21.7 ± 2.02 de	1	22.0 ± 2.59 ef	1	22.5 ± 4.45 cd	1	23.3 ± 0.96 ef	
Acetamiprid	10.2 ± 5.96 hi	2	20.6 ± 2.36 fg	2	23.1 ± 3.23 c	1	29.5 ± 3.92 a	
Fipronil	0.0 ± 0.00 i	-	7.8 ± 1.31 g	2	10.0 ± 1.99 e	2	21.5 ± 5.29 f	
Spiromesifen	19.7 ± 2.31 fg	1	19.5 ± 2.23 g	1	19.9 ± 3.25 de	1	22.2 ± 1.74 ef	
Haloxypop-p-methyl	23.1 ± 3.70 bc	1	23.9 ± 9.02 cd	1	24.3 ± 8.60 bc	1	25.5 ± 5.19 cd	
Bispyribac sodium	23.3 ± 2.07 cd	1	26.9 ± 1.55 a	1	26.2 ± 1.66 ab	1	27.0 ± 1.07 bc	
Nicosulfuron	26.1 ± 2.00 ab	1	26.3 ± 2.99 abc	1	26.8 ± 4.01 ab	1	26.9 ± 1.07 bc	
Myclobutanil	18.3 ± 2.35 gh	2	22.0 ± 1.47 ef	1	28.0 ± 2.30 a	1	28.2 ± 1.65 ab	
Pyraclostrobin + Metiram	26.4 ± 3.56 a	1	26.3 ± 5.07 ab	1	24.1 ± 6.28 bc xxxxxxx	1	26.4 ± 5.18 bcd	
Trifloxystrobin + Tebuconazole	22.5 ± 2.15 cd	1	23.9 ± 1.70 bcd	1	24.1 ± 1.63 bc	1	24.2 ± 0.94 de	

Means followed by the same letter within a column are not significantly different (Post Hoc Kruskal–Wallis tests, p > 0.001). “C” indicates toxicity class based on IOBC

*some of the pesticides were not included as their parasitism data are not available as aforementioned in the Table 1 footnote

Acetamiprid was slightly harmful for parasitism at 2x and x (≤ 69.9% parasitism relative to control) doses (Fig. 5, Table 6), and harmless at 0.5x. Female wasps emerged from myclobutanil treated eggs parasitized hosts at a rate of 65%–78.2% at 2x and x doses. The remaining pesticides yielded parasitism of ≥ 85.6% at all doses.

Parasitism by females treated in the pupal stage

The ART-ANOVA indicated a significant main effects for pesticides (F = 212.166, df = 11/108, p < 0.001) and doses

(F = 417.209, df = 3/324, p < 0.001), as well as an interaction (F = 51.223, df = 33/324, p < 0.001) between pesticides and doses.

Table 7 presents the mean percentage parasitism by female *T. chilonis* emerged from host eggs treated at pupal stage of parasitoids with various insecticides, miticides, herbicides and fungicides at x, 2x and 0.5x doses and from control eggs. Figure 6 shows the percentage parasitism relative to controls by female *T. chilonis* emerged from host eggs treated at the pupal stage of parasitoids. Acetamiprid, fipronil and abamectin were toxic to the parasitoid

Fig. 5 Percent parasitism (mean) relative to control of *S. cerealella* eggs by *T. chilonis* emerged from host eggs (*S. cerealella*) treated with different pesticides when parasitoids were in larval stage

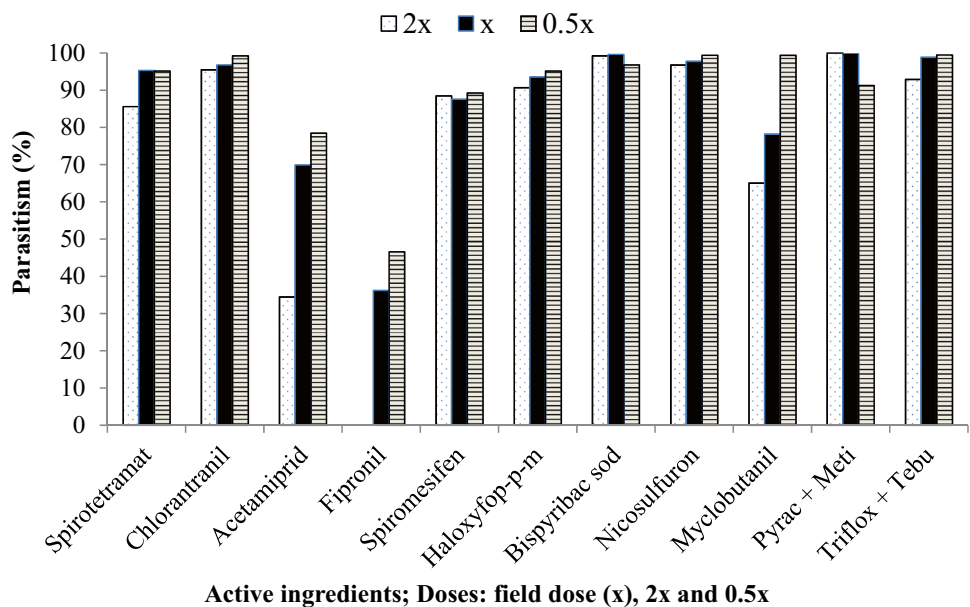


Table 7 Parasitism (mean \pm S.E) of *S. cerealella* eggs by *T. chilonis* emerged from host eggs (*S. cerealella*) treated with different pesticides at pupal stage of parasitoid (Post Hoc Kruskal–Wallis tests, $p=0.001$ or 0.1%), and IOBC ranking of toxicity (based on % reduction in parasitism relative to control)

Pesticides*	(Doses and toxicity ranking); Parasitism (mean \pm SE)							
	2x	C	x	C	0.5x	C	Control	
Spirotetramat	23.9 \pm 4.81 de	1	24.3 \pm 2.76 cd	1	24.3 \pm 3.26 d	1	24.6 \pm 2.08 cde	
Chlorantraniliprole	27.2 \pm 2.83 ab	1	26.8 \pm 2.75 ab	1	26.8 \pm 1.95 a	1	27.2 \pm 4.83 ab	
Acetamiprid	0.5 \pm 0.18 h	3	11.3 \pm 2.88 fg	2	12.5 \pm 2.17 ef	2	22.7 \pm 2.10 def	
Fipronil	5.4 \pm 1.33 gh	3	13.7 \pm 2.69 fg	2	15.0 \pm 1.97 ef	2	26.3 \pm 2.51 bc	
Abamectin	0.0 \pm 0.00 h	4	0.8 \pm 0.15 g	3	2.4 \pm 1.15 f	3	22.4 \pm 0.95 ef	
Spiromesifen	25.3 \pm 2.89 bcd	1	25.6 \pm 1.92 abc	1	25.1 \pm 4.33 bcd	1	25.9 \pm 2.23 bc	
Haloxypop-p-methyl	24.5 \pm 6.08 cd	1	24.9 \pm 3.76 cd	1	25.0 \pm 3.97 cd	1	25.0 \pm 3.73 cd	
Bispyribac sodium	25.9 \pm 2.18 abc	1	24.0 \pm 2.21 de	1	25.8 \pm 0.46 abc	1	26.6 \pm 2.43 ab	
Nicosulfuron	21.0 \pm 1.25 ef	1	20.9 \pm 1.97 ef	1	21.5 \pm 2.01 e	1	22.2 \pm 2.04 f	
Myclobutanil	19.9 \pm 5.31 fg	1	24.5 \pm 2.58 cd	1	24.6 \pm 0.27 d	1	24.6 \pm 2.56 cd	
Pyraclostrobin + Metiram	27.5 \pm 1.78 a	1	28.3 \pm 1.05 a	1	27.6 \pm 0.92 ab	1	28.6 \pm 1.10 a	
Trifloxystrobin + Tebuconazole	23.7 \pm 2.18 de	1	25.4 \pm 5.03 bcd	1	25.2 \pm 2.56 cd	1	25.7 \pm 2.69 bc	

Means followed by the same letter within a column are not significantly different (Post Hoc Kruskal–Wallis tests, $p > 0.001$). “C” indicates toxicity class based on IOBC

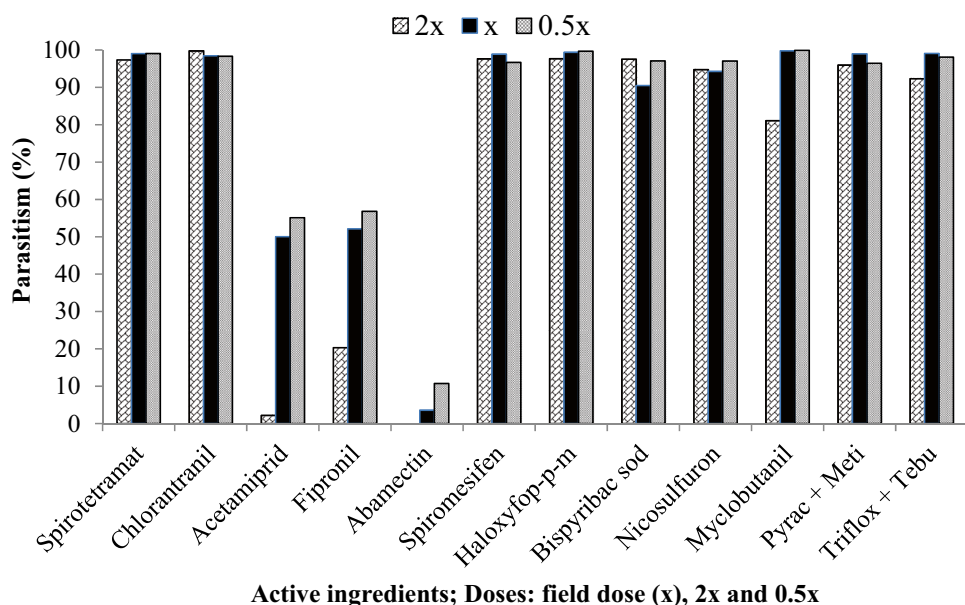
*some of the pesticides were not included as their parasitism data are not available because of less emergence or emerged parasitoids were unable to parasitize the host eggs

pupal stage with $\leq 56.8\%$ parasitism relative to control at all doses. Few adults emerged ($\leq 6.4\%$) from the spinetoram treated host eggs, and those that emerged were unable to parasitize the host eggs. These products are considered harmful for parasitism and are, therefore, not included in Table 7 and Fig. 6. The other pesticides showed $\geq 81\%$ parasitism relative to control and were harmless at all doses (Fig. 6, Table 7).

Discussion

Most effective IPM programmes require that biological control agents and applied pesticides be compatible with each other (Stark et al. 2007; Desneux et al. 2007) so that the chemicals have minimal influence on the activity of natural enemies (Singh and Varma 1986; Guedes et al. 1992; Suinaga et al. 1996). In the current study pesticides

Fig. 6 Percent parasitism (mean) relative to control of *S. cerealella* eggs by *T. chilonis* emerged from the host eggs (*S. cerealella*) treated with different pesticides when parasitoids were in pupal stage



were tested against the egg, larval, and pupal stages of *T. chilonis* to evaluate adverse effects of selected pesticides on parasitoid emergence, and females that emerged from treated eggs were evaluated for their parasitism efficacy. The pesticides used (Table 1) are relatively novel chemistries with novel modes of action compared to older, conventional broad-spectrum pesticides. They are commercially available and formulated products of inert materials and active ingredients, so there may be a contribution of inert materials in the overall effects of pesticides. More than half of tested pesticides were relatively selective and caused no significant.

x adverse impact on emergence of and parasitism by the parasitoids.

All herbicides tested were post emergence products and therefore are sprayed during the cropping season.

Insecticides, miticides, herbicides and fungicides were included in the study to achieve more effective integration of chemicals and biological controls to address multiple pest problems in the agroecosystem.

Very limited information is available on potential effects of pesticides on natural enemies (Firake et al. 2017), and on the effect of many of the newer pesticides on *Trichogramma* spp. (Lingathurai et al. 2015). The present results could not exclude harmful lethal effects of certain pesticides in general, but only at doses that are likely to get into contact with the parasitoid. Acetamiprid, spinetoram, fipronil, and abamectin caused high mortality of immature parasitoids and more adversely affected parasitism compared to the remaining pesticides used in the present study. Nevertheless, acetamiprid, fipronil, and abamectin were less adverse for emergence when treatments shifted from the egg stage toward the pupal stage, as well as from 2x toward 0.5x dose (Tables 2, 3 and 4; Figs. 1, 2 and 3). The tendency among those exhibiting stage-dependent effects regarding emergence was for treatment of larval and pupal stages to yield greater parasitoid emergence (Tables 3 and 4). Acetamiprid (all stages treated within the host eggs), fipronil (both larval and pupal stages treated), and abamectin (pupal stage treated) adversely affected parasitism and showed stage variable and dose dependent effect, as parasitism rate decreased when dose shifted from the 0.5x through 2x dose (Tables 5, 6 and 7; Figs. 4, 5 and 6). The high rate of toxicity of such pesticides for emergence and parasitism are due to the high rates of penetration into the cuticle of host eggs (Yu 1998; de Paiva 2018). Stage-dependent effects suggest that the parasitoid egg and larval stages were more sensitive/susceptible to these pesticides compared to the pupal stage (Varma and Singh 1987; Consoli et al. 1998; Biondi et al. 2015; Khan and Ruberson 2017), and moreover, dose affects the level of adverse effects on the organism as the dose makes the poison.

The toxicity of the aforementioned pesticides to the immature stages of *T. chilonis* was supported by previous findings on lethal effects of pesticides on the immature stages of *Trichogramma pretiosum* (Khan and Ruberson 2017). The findings also concur with previous results on adult *T. chilonis* (Khan 2020) that acetamiprid, spinetoram, fipronil and abamectin were very harmful pesticides for adult *T. chilonis* exposed to the dried residues on glass, as well as subsequent parasitism by the exposed female parasitoids. Acetamiprid was less adverse for emergence and parasitism when dose declined from 2x to 0.5x dose for all immature stages. The toxic effects of acetamiprid on emergence are supported by Hewa-Kapuge et al. (2003), who reported that acetamiprid was toxic for emergence of *T. chilonis*. Moura et al. (2006) also treated (sprayed) host eggs (*Anagasta kuehniella*) with acetamiprid and found acetamiprid harmless to larvae, but slightly harmful to pupae of *T. pretiosum*. However, the current study demonstrated that acetamiprid is considerably more damaging to both larval and pupal stages of *T. chilonis*. This may be due to different methodology: Moura et al. (2006) sprayed parasitized eggs, whereas in the current experiment the parasitized eggs were immersed in the solution for 1–2 s, which would have increased coverage and possibly permeation into the host egg. Similarly, both acetamiprid and abamectin were slightly to moderately toxic to adult parasitoids *T. dendrolimi*, *T. ostrinae* and *T. chilonis* when exposed to dry residues in glass vials (Cheng et al. 2018).

Abamectin had a greater adverse effect when parasitoids were treated in the egg and larval stages (Tables 2, 3; Figs. 1, 2) than in the pupal stage (Table 4; Fig. 3). Similar observations were made by Cónsoli et al. (1998), who reported that abamectin was harmful to slightly harmful for emergence of *T. chilonis*, when parasitoids were treated in different immature stages. Hussain et al. (2010) concluded that abamectin significantly adversely affected emergence of *T. chilonis* from the treated host eggs of *S. cerealella*. Carvalho et al. (2003) also found abamectin adversely affected emergence of *T. pretiosum* from host eggs treated when parasitoids were in egg, larval and pupal stages. Similarly, Carvalho et al. (2003) found that abamectin-treated host eggs at the parasitoids' pupal stage led to significantly reduced parasitism by emerged female *T. pretiosum*.

Fipronil significantly reduced emergence of and parasitism by parasitoids. Fipronil was slightly to moderately harmful for emergence of *T. chilonis* at field recommended concentration (FRC) in the larval and pupal stage treatments, respectively (Tables 3, 4). This result was supported by Ghorbani et al. (2016), who observed that FRC of fipronil was slightly harmful for emergence of *T. brassicae* from the treated larvae, prepupae and pupae. Similarly, fipronil negatively affected parasitism by females treated in the pupal stage at both x and 0.5x doses.

Spinetoram was moderately harmful for parasitoids emerging after treatment in larval and pupal stages at all used doses (Tables 3, 4). The adverse effect of spinetoram in the egg treatment declined from harmful to slightly harmful as the dose decreased from 2x to 0.5x (Table 2). However, parasitoids treated in host eggs at egg, larval and pupal stages with spinetoram did not parasitize host eggs (Tables 5, 6 and 7). The current study is supported by Khan and Ruberson (2017) who found spinetoram adversely affected immature stages of *T. pretiosum*. Similarly, Khan et al. (2015a) found abamectin was moderately harmful, while spinetoram, fipronil and acetamiprid were slightly harmful for parasitism at field dose by *T. chilonis* of previously treated host eggs of *Sitotroga cerealella*.

The remaining pesticides (spirotetramat, chlorantraniliprole, spiromesifen, haloxyfop-p-methyl, bispyribac sodium, nicosulfuron, chlorothalonil + procymidone, myclobutanil, pyraclostrobin + metiram, and trifloxystrobin + tebuconazole) were harmless for parasitoid emergence at all doses and all parasitoid stages treated, except spiromesifen, which was slightly harmful for emergence when host eggs were treated at parasitoid egg stage at 2x dose (Table 2). The miticide spiromesifen generally did not adversely affect emergence of parasitoids. The pesticides found to be harmless to the parasitoids in immature stages might be due to that fact that 1) the pesticide could not penetrate through the egg-skin of *S. cerealella*, or their mode of action simply implies no harmful interaction with the parasitoids within the host, for example herbicides and fungicides or/and 2) the immature stages of parasitoids could easily degrade the pesticides so that pesticides have no adverse impact on the parasitoids (Yu 1998; de Paiva 2018). This result corresponded with previous results with immature stages of *T. pretiosum* (Khan and Ruberson 2017). Chlorantraniliprole was found harmless for emergence of *T. chilonis*, which was confirmed by Hussain et al. (2012), who demonstrated that the same chemical resulted in maximum emergence of *T. chilonis* from the host eggs treated 8 days after parasitism and showed minimum effect on parasitoid emergence from eggs treated 1, 3, 5 and 7 days after parasitism. Wahengbam et al. (2018) found both chlorantraniliprole and spiromesifen did not adversely affect *T. chilonis* and *T. pretiosum* when they were treated in the pupal stage. Chlorantraniliprole was harmless for parasitism by *T. pretiosum* (de Paiva et al. 2018).

Spirotetramat demonstrated $\geq 82.8\%$ emergence and is classified as harmless for emergence of *T. chilonis* at all doses and all stages treated, except 2x dose in larval treatment (Table 3). This is supported by Tabebordbar et al. (2020) who concluded the mean emergence rate of *T. evanescens* when exposed to the recommended field concentrations of spirotetramat was 84%. The rating of spirotetramat as harmless for parasitism is supported by Bruck

et al. (2009), who found that spirotetramat was harmless for parasitism by *T. cryptophlebiae* in citrus. According to Moens et al. (2012), limited research has been conducted to assess the side effects of spirotetramat on natural enemies. Furthermore, an earlier finding by Khan and Ruberson (2017) demonstrated that both chlorantraniliprole and spirotetramat caused no significant mortality to immature stages of *T. pretiosum*.

Herbicides and fungicides were harmless for emergence (Tables 2, 3 and 4), nevertheless, they have some dose based adverse impacts on parasitism in the present study: all the herbicides, namely haloxyfop-p-methyl, bispyribac sodium, nicosulfuron, and fungicides, namely myclobutanil, chlorothalonil + procymidone, pyraclostrobin + metiram and trifloxystrobin + tebuconazole did not adversely affect emergence or parasitism at all doses and all stages treated, except the 2x dose of both nicosulfuron and chlorothalonil + pyraclostrobin which adversely affected parasitism by parasitoids treated at egg stage (Table 5), and the 2x dose of myclobutanil adversely affected parasitism by parasitoids treated at larval stage (Table 6). Thus, dose wise effects of pesticides were observed for both groups of pesticides in some cases.

There are few studies of the effects of herbicides and fungicides on *Trichogramma*. However, the literature demonstrated fungicides are generally harmless for emergence of *Trichogramma* (Hagley and Laing 1989; Stark and Banken 1999; Jalali and Singh 1993; Vieira et al. 2001), particularly, when applied to parasitoid pupae (Hassan 1994). In the current study, the herbicide bispyribac sodium was harmless for emergence as well as for parasitism at all doses and immature stages treated. This was supported by Khan et al. (2015a) who concluded that bispyribac sodium was harmless for parasitism by *T. chilonis* of previously treated host eggs at x, 2x and 0.5x doses. Moreover, Khan (2020) found that bispyribac sodium was slightly harmful for adult *T. chilonis* exposed to dried field dose residues 1 and 5 days after application, and harmless after 10 and 15 days of drying in glass vials. Khan (2020) also rated bispyribac sodium as slightly harmful for parasitism by females exposed to the 1-day dried residue in glass vials and harmless in older residual treatments. The difference in effects of the bispyribac sodium between the current study and previous studies is likely due to methodology differences, and stages of parasitoid tested.

The mixture of trifloxystrobin + tebuconazole and of pyraclostrobin + metiram did not adversely affect either emergence of, or parasitism by *T. chilonis* in the current study. Similarly, myclobutanil was deemed harmless for emergence of, as well parasitism by *T. chilonis* (except for parasitism at 2x dose in the larval treatment: Table 6). Khan and Ruberson (2017) found that myclobutanil, pyraclostrobin, and trifloxystrobin + tebuconazole did not adversely affect immature stages of *T. pretiosum*. Similarly, the results of Carmo et al. (2010) also supports that many fungicides, including triflox-

ystrobin, pyraclostrobin and tebuconazole do not significantly affect emergence of and parasitism by *Telonomus remus* compared to an untreated control. Bueno et al. (2008) treated egg, larval and pupal stages of *T. pretiosum* with myclobutanil under laboratory conditions and found myclobutanil harmless for emergence of *T. pretiosum*. Literature on the effect of myclobutanil on parasitism by *Trichogramma* emerged from host eggs treated with the myclobutanil is not available. However, Khan and Ruberson (2017) found the same chemical had a negligible impact on the foraging behavior, including stinging of host eggs, of *T. pretiosum*.

The present result revealed that pyraclostrobin + metiram, and chlorothalonil + procymidone (except 2 × dose in egg treatment: Table 5) had no adverse impact on parasitism by *T. chilonis*, supporting Petersen (1995), who concluded that metiram had no significant effect on percent reduction in egg production or hatchability by rove beetle *Alleochara bilineata*, and further concluded that chlorothalonil and procymidone had no adverse impact on predation by the beetle. Haloxypop-p-methyl was harmless for parasitism by parasitoids in the current study. This is supported by Peterson (1995) that haloxypop-R was found safe for egg production (%) of the beetle *Alleochara bilineata*. Khan et al. (2015a) found that pyraclostrobin + metiram, chlorothalonil + procymidone and haloxypop-p-methyl were harmless for parasitism by *T. chilonis* of previously treated host eggs at x, 2x and 0.5x doses. Furthermore, Khan (2020) also demonstrated that both chlorothalonil + procymidone and haloxypop-p-methyl were harmless for parasitism by *T. chilonis* exposed to the dried residues at field dose in a glass vial 1, 5, 10, and 15 days after treatment of the glass.

Harmlessness of nicosulfuron and chlorothalonil + procymidone for emergence of *T. chilonis* is supported by earlier findings that nicosulfuron had no negative impact on the development and emergence of *T. pretiosum* (Khan and Ruberson 2017). Nicosulfuron and chlorothalonil + procymidone showed increased toxicity for parasitism from harmless at x dose to slightly harmful at 2x dose in the parasitoid egg stage (Table 5), while the same chemicals were found harmless for parasitism at all used doses when host eggs were treated at larval and pupal stages of parasitoids (Tables 6 and 7). Leite et al. (2017) also described nicosulfuron as "harmless" for parasitism by *Trichogramma* of previously treated host eggs of *Anagasta kuehniella*.

Products that are harmful at field rate (x) and even at half dose (0.5x) are not compatible with IPM. Further, the compatibility of field rate/dose to all stages of parasitoids is required for more successful integration of biological and chemical control in agroecosystem. Even if parasitoids larvae or pupae were slightly less susceptible, the high toxicity to eggs of parasitoids means that such products should preferably not be used in IPM as at any given time, there

will be a mixture of parasitoid eggs, larvae and pupa in the host eggs and one cannot time pesticide application to only expose larvae or pupae.

The pesticides tested in the current study have different modes of action. Pesticides found harmless in the laboratory will most probably be harmless to the natural enemies in the field. However, pesticides evaluated and found harmful in the laboratory may be less harmful in the field and therefore should be further tested (Hassan 1977; Steiner 1977), because of possible environmental degradation of pesticides or coverage differences.

Conclusion

Selected pesticides, including insecticides, miticides, herbicides, and fungicides were tested against the immature stages (eggs, larvae and pupae) of *Trichogramma chilonis* to determine their effects on emergence of and parasitism by parasitoids emerged from the treated eggs of *Sitotroga cerealella*. Acetamiprid, spinetoram, fipronil and abamectin exhibited relatively high adverse effects on emergence of *T. chilonis* from hosts exposed at field recommended dose, and at 2x and 0.5x doses for all immature stages. However, the impact of fipronil and abamectin varied with parasitoid life stage and pesticide dose. Most of the remaining pesticides yielded > 80% parasitoid emergence at all doses and stages treated.

Acetamiprid, fipronil and abamectin adversely affected parasitism by parasitoids emerging from treated host eggs. Nevertheless, the adverse impact declined when dose was reduced from 2x through 0.5x. Field trials are recommended to determine if their negative effect on parasitoid emergence and parasitism persist under field conditions. The remaining pesticides yielded ≥ 84% parasitism at field dose across all parasitoid stages. Therefore, most of the used pesticides exhibited good compatibility with parasitoids for both emergence and parasitism. The current results should be largely extrapolatable to other lepidopteran pests and real world situations.

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Declarations

Conflict of interest I am the sole author of the manuscript and declares no conflict of interest with any person, institution or organization.

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