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ORIGINAL RESEARCH ARTICLE

Insecticidal toxicity to *Trichogramma chilonis* Ishii (Hymenoptera: Trichogrammatidae) and subsequent effects on parasitic efficiency and adult emergence rate of descendant generation

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Abstract

Trichogramma chilonis (Ishii) is an important egg parasitoid, widely used as biological control agent of lepidopteran pests. To understand the risks associated with specific insecticide use towards the parasitoid, we studied the effect of 10 insecticides sprayed at recommended field dosages (RFD) on factitious host, *Corcyra cephalonica* eggs with parasitoid in different development stages, egg (one-day after parasitism), larval (3-days after parasitism), pre-pupal (5-days after parasitism) and pupal (7-days after parasitism). The emerging F_1 adults were further evaluated for parasitic efficiency and reduction in adult emergence of F_2 generation. All the tested insecticides significantly reduced the parasitism of surviving *T. chilonis* female wasps with varying rates (15.2 to 43.9%). Against pre-imaginal stages, insecticides were comparatively more harmful to the egg and larval stages of parasitoid as compared to pre-pupal and pupal stages. Compared to control values, the parasitism rate of *T. chilonis* adults and emergence of F_2 adults were also significantly reduced in all the tested insecticides. Chlorantraniliprole, methoxyfenozide and flubendiamide were quite safer to all the developmental stages of the parasitoid, while chlorpyriphos was found to be highly toxic according to the classification given by International Organization for Biological Control (IOBC). Further studies need to be carried out to verify the effect of these insecticides on *T. chilonis* under semi-field and field conditions.

Keywords Insecticides · *Trichogramma* · Disruptive effect · Integrated pest management

Introduction

Egg parasitoids belonging to the family Trichogrammatidae are among the most widely used biological control agents worldwide (Hoffmann et al. 2001). The tiny hymenopteran wasps are known to parasitize eggs of insect-pests from diverse insect orders mostly Lepidoptera, few on Coleoptera, Diptera and Neuroptera (Zucchi et al. 2010; Polaszek 2010; Sarwar and Salman 2015). More than 800 living species from 90 genera are known to occur in the family Trichogrammatidae across the globe. Among these, *Trichogramma* Westwood (173 living species) and *Oligosita* Walker (95 living species) genera are highly speciose (Noyes 2019). The trichogrammatids fauna in India is represented by 151 species from 31 genera. Thirty two species of Genus *Trichogramma* are available in India (Begum and Anis 2014). These species of *Trichogramma* have played a key role in regulating insect-pest population in nature and have been consequently utilized commercially in biological control programs against lepidopteran pests of various economically important agricultural, vegetable, horticultural and ornamental crops (Pratissoli and Parra 2001; Sithanantham et al. 2001; Singh et al. 2007; Shera et al. 2017a, b; Sigsgaard et al. 2017; Navik and RichaVarshney 2018; Sangha et al. 2018; Shera et al. 2017a, b; Laurentis et al. 2019).

In India, *Trichogramma chilonis* Ishii is the most common species (Navik and RichaVarshney 2018), widely utilized in inundative release programs against important lepidopteran pests in sugarcane (Shehnmar et al. 2003; Geetha et al. 2009; Padmasri and Sudhrani 2014; Srikanth et al. 2016), paddy (Kaur and Brar 2008; Sangha et al. 2018), maize (Shera et al. 2017a, b), cotton (Brar et al. 2002) and vegetable crops (Suresh et al. 2007; Lad et al. 2009; Khan et al. 2011;

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Jalali et al. 2016). Therefore, it is important to understand the parasitoid-insecticide interaction, so as to exclude those chemicals that have adverse effects in agro-ecosystems.

Current crop production systems are subjugated by extensive use of broad-spectrum insecticides which severely hindered the contribution of natural enemies for pest suppression. An understanding on the compatibility of natural enemies with insecticides is indispensable for utilizing chemical insecticides prudently along with biological control programs (Stark et al. 2007). Hence, insecticides need to be used selectively in integrated pest management (IPM) systems to ensure sustainable crop protection and environmental stability (Greathead 1995; Haseeb et al. 2000; Khan and Ruberson 2017). Insecticides exert a wide array of disruptive effects on natural enemies such as mortality, changes in parasitism rate, development, fecundity, consumption rate or behaviour etc. (Saber 2011; Sohrabi et al. 2013; Karmakar and Shera 2018).

The International Organization for Biological Control (IOBC) has developed a procedure for assessing the impacts of pesticides on natural enemies (Hassan 1989, 1998). Table 1 summarizes the characteristics (chemical groups, modes of action, target pests) of some insecticides commonly used in sugarcane, rice, maize, cotton and vegetable crops against different insect pests. Negative

Table 1 Characteristics, formulations and doses of insecticides tested in bioassays on immature stages and adults of *Trichogramma chilonis*

Insecticide	Commercial Formulation	Insecticidal Group	Mode of Action (IRAC*)	Formulation	Technical grade	Recommended field dose	
						Active ingredient (g a.i. ha^{-1})	Formulation (g or mL/ ha^{-1})
Chlorantraniliprole	Coragen® 18.5 SL	Anthranilic diamide	Ryanodine receptor modulators	Soluble liquid	18.5% w/w	27.75	150 mL
Chlorpyriphos	Dursban™ 20EC	Organophosphate	Acetyl cholinesterase (AChE) inhibitors	Emulsifiable concentrate	20% w/w	175	875 mL
Imidacloprid	Confidor® 17.8SL	Neonicotinoid	Nicotinic acetylcholine receptor (nAChR) competitive modulators	Soluble liquid	17.8% w/w	20.03	112.5 mL
Triazophos	Fulstop® 40EC	Organophosphate	Acetyl cholinesterase (AChE) inhibitors	Emulsifiable concentrate	40% w/w	600	1500 mL
Bifenthrin	Talstar® 10EC	Pyrethroids	Sodium channel modulators	Emulsifiable concentrate	10% w/w	100	1000 mL
Fipronil	Regent® 5SC	Phenylpyrazoles	GABA-gated chloride channel blockers	Suspension concentrate	5% w/w	100	2000 mL
Flubendiamide	Fame® 480 SL	Diamide	Ryanodine receptor modulators	Soluble liquid	39.35% w/w	39.35	100 mL
Clothianidin	Clutch® 50WDG	Neonicotinoid	Nicotinic acetylcholine receptor (nAChR) competitive modulators	Water dispersible granules	50% w/w	125	250 g
Thiamethoxam	Actara® 25WG	Neonicotinoid	Nicotinic acetylcholine receptor (nAChR) competitive modulators	Water dispersible granules	25% w/w	25	100 g
Methoxyfenozide	Intreprid™ 24SC	Diacylhydrazines	Ecdysone receptor agonists	Suspension concentrate	24% w/w	20	500 mL

*According to IRAC (2019)

effects of insecticides on the *Trichogramma* populations have been reported (Jalali and Singh 1993; Wang et al. 2012; Abdulhay and Rathi 2014; Souza et al. 2014; Khan and Ruberson 2017), however little is known about effects on various developmental life stages of the parasitoid, *T. chilonis* and subsequent impact on surviving/emerging adults for parasitic efficiency and emergence of second generation adult population. A better understanding of these impacts could lead to develop an effective integrated pest management strategy in different agro-ecosystems with minimum disruptive influence to this parasitoid.

Materials and Methods

Insects

The culture of host insect, *Corcyra cephalonica* (Stainton) was maintained on sorghum grains. *Corcyra* rearing boxes measuring 43 × 23 × 12 cm made of medium density fibreboard (Rescholar Equipment, India) were filled with 2.5 kg of milled sorghum. After charging with *Corcyra* eggs @ 0.20 cc per box (Sharma et al. 2016), they were covered with perforated lids having iron mesh (20 mesh) both externally and internally. The moths emerging from these boxes were collected daily and transferred to the specially designed oviposition cages (35 × 25 × 18 cm; Rescholar Equipment, India). The eggs collected from these moths were utilized for the rearing of parasitoid *T. chilonis*.

Collected eggs were passed through 15, 30 and 40 mesh sieves and further run over a slope of paper to eliminate the dust particles. The cleaned eggs were deep frozen for 12–14 h to prevent hatching of *Corcyra* larvae. These eggs were glued on white cards of size 15 × 10 cm to prepare sentinel *Corcyra* cards. The eggs were exposed to adult female of *T. chilonis* for 24 h in glass jars for parasitism. The parasitized eggs turned black on the fourth day. Emergence of the parasitoid started after seventh day of parasitism. Hence, new cards were prepared after seven days for maintaining the pure culture, which was further used in this study.

Insecticides

Ten insecticides, chlorantraniliprole (Coragen® 18.5 SL), chlorpyriphos (Dursban™ 20EC), imidacloprid (Confidor® 17.8SL), triazophos (Fulstop® 40EC), bifenthrin (Talstar® 10EC), fipronil (Regent® 5SC), flubendiamide (Fame® 480 SL), clothianidin (Clutch® 50WDG), thiamethoxam (Actara® 25WG), methoxyfenozide (Intreprid™ 24SC), belonging to different insecticidal groups recommended for the control of various insect pests in the sugarcane ecosystem were assessed on different development stages of *T. chilonis*. The insecticides and dosages used in the bioassays are listed in Table 1.

Bioassays

All bioassays were conducted under laboratory conditions in an environmental chamber at 27 ± 2 °C temperature, 70 ± 5% relative humidity and 14L: 10D photoperiod (Macro Scientific Works Ltd, India).

Toxicity of insecticides to pre-imaginal developmental stages (egg, larval, pre-pupal and pupal stages) of *Trichogramma chilonis*

In order to test the effects of various insecticides on the immature stages of *T. chilonis*, 55 sentinal cards (size 15 × 10 cm) were prepared, each having 20,000 *C. cephalonica* eggs glued to it. Each egg card was placed in separate glass jar and exposed to *T. chilonis* females for 24 h. After 24 h, these parasitized cards were removed from jars and cut into 20 small strips (size 0.5 × 1.5 cm). Twenty strips with *C. cephalonica* eggs, 24 h after parasitization (parasitoid in egg stage), were sprayed with selected insecticides dissolved in distilled water at their recommended field dosages using a hand compressor sprayer (ASPEE, India). Controls were sprayed with distilled water. After insecticide treatment, the cards were air dried at room temperature and placed inside separate glass tubes covered with muslin. Egg card strips, 3-days (parasitoid in larval stage), 5-days (parasitoid in pre-pupal stage) and 7-days (parasitoid in pupal stage) after parasitization were treated similarly. The number of adults emerged from these parasitized eggs were recorded and per cent adult emergence was worked out. Each treatment was replicated four times and there were five replicate strips in each treatment (n = 20 egg card strips per treatment). The toxicity of insecticides was categorized as per IOBC class based on reduction in adult emergence (Sterk et al. 1999): class 1 – harmless (< 30%), class 2—slightly harmful (30–79%), class 3—moderately harmful (80–99%) and class 4—harmful (> 99%).

Parasitism rate of *T. chilonis* F_1 adults emerged from insecticidal treated *Corcyra eggs* with parasitoid in different development stages and adult emergence of F_2 adults

The adults emerging from insecticidal treated *Corcyra* eggs with parasitoid in pre-imaginal stages (egg, larval, pre-pupal and pupal) were released inside separate test tubes containing strips with 100 fresh *C. cephalonica* eggs glued on it. These glass tubes were provided with honey streak on a paper strip as a source of food for the adult parasitoids. Black coloured eggs were considered as the parasitized eggs, on the basis of which, per cent parasitization was determined. The parasitized eggs were inspected daily to record the emergence of F_2 adults. The reduction in parasitism capacity was calculated using the formula: R = [1-(P/p)]*100, where R = percentage of reduction in parasitism capacity, P = mean value of parasitism for each insecticide, and p = mean parasitism observed for the control treatment (Biondi et al. 2015).

Statistical analysis

The data on the parasitism and adult emergence rates were analysed though one way analysis of variance (ANOVA) for completely randomized design to compare different treatments ($P < 0.05$). Prior to analysis, the percentage parasitism and emergence data were transformed using arcsin $\sqrt{x}$ to resolve non-normal distributions of the percentage data. All statistical tests were carried out using IBM SPSS 22.0 for Windows (IBM Corp. 2013).

Results

Insecticidal exposure to immature stages

All the insecticides applied at different developmental stages, egg (24 h after parasitization), larval (3-days after parasitization), pre-pupal (5-days after parasitization) and pupal (7-days after parasitization) significantly affected the adult emergence rate of the parasitoid as compared to unsprayed parasitized eggs (control) Fig. 1.

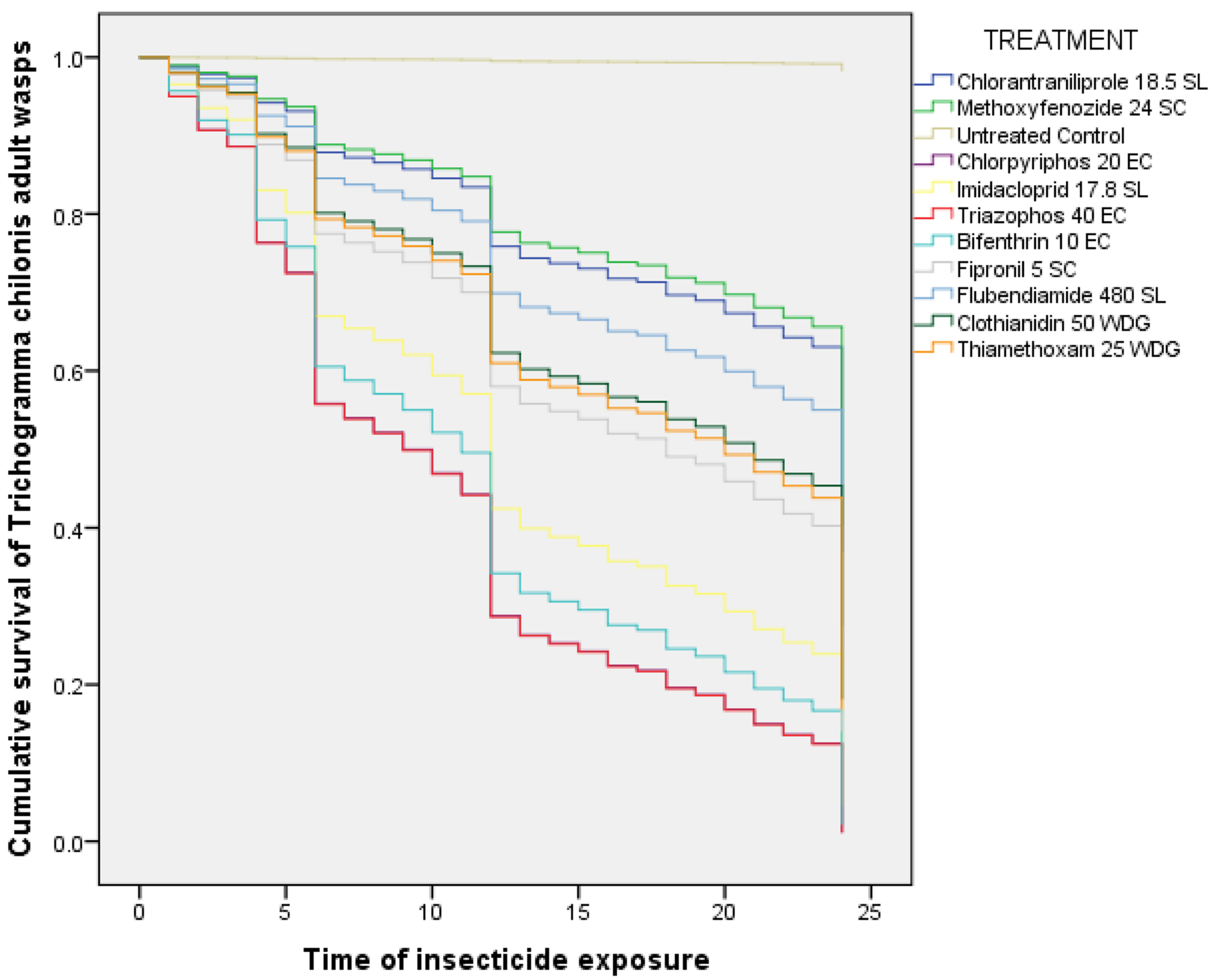

Fig. 1 Cumulative proportion of surviving Trichogramma adults exposed to different insecticidal treatments over 24 h. Data (n = 100) were analysed with Cox regression survival analysis

Table 2 Reduction in emergence percentage of F_1 *Trichogramma chilonis* adults from insecticide treated *Corcyra cephalonica* eggs with parasitoid in different development stages and, class of toxicity according to IOBC

Treatments	Mean reduction in adult emergence ± SE (%)							
	Egg stage	[a]IOBC Toxicity class	Larval stage	[a]IOBC Toxicity class	Pre-pupal stage	[a]IOBC Toxicity class	Pupal stage	[a]IOBC Toxicity class
Chlorantraniliprole 18.5 SL	6.10 ± 0.58a	1	11.51 ± 0.20b	1	9.47 ± 0.29a	1	3.85 ± 0.12a	1
Chlorpyriphos 20 EC	85.88 ± 0.28j	3	72.40 ± 0.46i	2	66.65 ± 0.29i	2	78.32 ± 0.27j	2
Imidacloprid17.8 SL	33.94 ± 0.28e	2	30.55 ± 0.20d	2	19.60 ± 0.15d	1	10.28 ± 0.13d	1
Triazophos 40 EC	40.90 ± 0.19 g	2	46.23 ± 0.31 g	2	59.65 ± 0.09 h	2	76.39 ± 0.11i	2
Bifenthrin 10 EC	60.07 ± 0.27i	2	44.44 ± 0.24f	2	55.01 ± 0.16 g	2	74.79 ± 0.20 h	2
Fipronil 5 SC	52.92 ± 0.22 h	2	59.73 ± 0.24 h	2	29.68 ± 0.33f	1	27.66 ± 0.12 g	1
Flubendiamide 480 SL	20.64 ± 0.15d	1	30.34 ± 0.38d	2	23.54 ± 0.30e	1	21.34 ± 0,19f	1
Clothianidin 50 WDG	18.68 ± 0.38c	1	26.50 ± 0.19c	1	19.52 ± 0.14d	1	13.02 ± 0.13e	1
Thiamethoxam 25 WDG	35.36 ± 0.33f	2	42.24 ± 0.25e	2	18.70 ± 0.10c	1	8.75 ± 0.23b	1
Methoxyfenozide 24 SC	10.05 ± 0.07b	1	10.83 ± 0.09a	1	12.57 ± 0.21b	1	11.44 ± 0.12c	1
F value	3350.82		4968.21		6312.57		19,521.04	
df	9,30		9,30		9,30		9,30	
P value	< 0.0001		< 0.0001		< 0.0001		< 0.0001	

Mean (± SE) within the same column followed by different lowercase letters are statistically different at $P < 0.05$ level

[a]IOBC class based on reduction in adult emergence as per Sterk et al. (1999); class 1—harmless (< 30%), class 2—slightly harmful (30–79%), class 3—moderately harmful (80–99%) and class 4—harmful (> 99%). Untransformed data have been presented

Upon treatment in egg stage, chlorantraniliprole, methoxyfenozide, clothianidin and flubendiamide were categorized as harmless (IOBC class 1; Table 2). The reduction in adult emergence was lowest in chlorantraniliprole, while chlorpyriphos was highly toxic ($F = 3350.82$, $df_{9,30}$; $p < 0.0001$). At larval stage, methoxyfenozide, chlorantraniliprole and clothianidin reduced the adult emergence by 10.8%, 11.5% and 26.5%, respectively and were categorized as harmless (IOBC class 1), whereas remaining seven insecticides were categorized as slightly harmful with 30.3 to 72.4% reduction in adult emergence ($F = 4968.21$, $df_{9,30}$; $p < 0.0001$). The effect of insecticides on pre-pupal and pupal stages was almost similar. All the test insecticides except chlorpyriphos, triazophos and bifenthrin were quite safe to pre-pupal ($F = 6312.57$, $df_{9,30}$; $p < 0.0001$) and pupal stages ($F = 19{,}521.04$, $df_{9,30}$; $p < 0.0001$) of *T. chilonis.*

Parasitism rate of *T. chilonis* F_1 adults emerging from insecticide treated pre-imaginal stages and adult emergence of F_2 adults

Insecticide treatment in egg stage

Significant differences were found in the parasitism rate of *T. chilonis* adults emerged from insecticide exposed *Corcyr*a eggs having parasitoid in egg stage ($F = 507.59$, $df_{10,33}$; $p < 0.0001$) and subsequent adult emergence of second generation adults ($F = 203.37$, $df_{10,33}$; $p < 0.0001$). The highest parasitism and adult emergence rates were observed in chlorantraniliprole and were not significantly different from methoxyfenozide. Significantly lower parasitism and adult emergence was observed in chlorpyriphos (Table 3). Based on the reduction in adult emergence, chlorantraniliprole, flubendiamide, clothianidin and methoxyfenozide were found to be harmless (IOBC class 1); imidacloprid, triazophos, bifenthrin, fipronil and thiamethoxamas slightly harmful (IOBC class 2), while chlorpyriphos was categorized as moderately harmful (IOBC class 3).

Insecticide treatment in larval stage

There were significant differences in the parasitism rate of *T. chilonis* adults emerged from insecticide treated *Corcyr*a eggs with parasitoid in larval stage ($F = 2907.09$, $df_{10,33}$; $p < 0.0001$). The reduction in parasitism was significantly higher in chlorpyriphos, while it was lowest in chlorantraniliprole. Significant differences were found in adult emergence rate among the different insecticidal treatments ($F = 5659.13$, $df_{10,33}$; $p < 0.0001$). Chlorantraniliprole and methoxyfenozide were rated as harmless (IOBC class 1), while all other insecticides were found to be slightly harmful (IOBC class 2) to second generation when parasitoid larval stage was exposed to insecticides (Table 4).

Table 3 Mean parasitism (%) by *Trichogramma chilonis* adults of F_1 generation emerged from insecticide treated *Corcyra cephalonica* eggs with parasitoid in egg stage (1st day of development) and adult emergence (%) of F_2 adults along with class of toxicity according to IOBC

Insecticides	Mean parasitism ± SE (%)	Parasitism reduction (%)	Adult emergence rate ± SE (%)	Adult emergence reduction (%)	[a]IOBC Toxicity class
Chlorantraniliprole 18.5 SL	80.32 ± 0.21b	9.84	75.49 ± 0.13b	10.00	1
Chlorpyriphos 20 EC	1.50 ± 0.96 g	98.31	2.50 ± 1.44 g	97.01	3
Imidacloprid17.8 SL	77.92 ± 0.09bc	12.53	54.34 ± 0.16e	35.21	2
Triazophos 40 EC	23.12 ± 0.27f	74.04	40.00 ± 0.11f	52.31	2
Bifenthrin 10 EC	29.70 ± 0.30e	66.66	37.35 ± 0.54f	55.47	2
Fipronil 5SC	52.11 ± 0.30d	41.50	61.05 ± 0.30e	27.21	2
Flubendiamide 480 SL	75.57 ± 0.07c	15.17	70.19 ± 0.26 cd	16.32	1
Clothianidin 50 WDG	74.82 ± 0.05c	16.01	65.32 ± 0.12de	22.12	1
Thiamethoxam 25 WDG	76.49 ± 0.12c	14.14	51.47 ± 0.15f	38.63	2
Methoxyfenozide 24 SC	78.06 ± 0.20bc	12.38	71.01 ± 0.14bc	15.34	1
Untreated control	89.09 ± 0.13a	-	83.88 ± 0.22a	-	
F value	507.59		203.37		
df	10,33		10,33		
P value	< 0.0001		< 0.0001		

Mean (± SE) within the same column followed by different lowercase letters are statistically different at $P < 0.05$ level

[a]IOBC class based on reduction in parasitism and adult emergence as per Sterk et al. (1999); class 1—harmless (< 30%), class 2—slightly harmful (30–79%), class 3—moderately harmful (80–99%) and class 4—harmful (> 99%). Untransformed data have been presented

Insecticide treatment in pre-pupal stage

The parasitism rates ($F = 4098.61$, $df_{10,33}$; $p < 0.0001$) of F_1 adults emerged from insecticide treated *Corcyr*a eggs with parasitoid in pre-pupal stage and subsequent emergence of F_2 adults ($F = 23{,}424.86$, $df_{10,33}$; $p < 0.0001$) in all the insecticidal treatments were significantly inferior to the control values (Table 5). Among different insecticides, the parasitism and adult emergence was higher in methoxyfenozide and chlorantraniliprole. Based on IOBC classification, all the insecticides were rated as harmless(IOBC class 1) except triazophos and bifenthrin (slightly harmful), while insecticide chlorpyriphos was categorized as harmful(IOBC class 4) to second generation adults.

Table 4 Mean parasitism (%) by *Trichogramma chilonis* adults of F_1 generation emerged from insecticide treated *Corcyra cephalonica* eggs with parasitoid in larval stage (3rd day of development) and adult emergence (%) of F_2 adults along with class of toxicity according to IOBC

Insecticides	Mean parasitism ± SE (%)	Parasitism reduction (%)	Adult emergence rate ± SE (%)	Adult emergence reduction (%)	[a]IOBC Toxicity class
Chlorantraniliprole 18.5 SL	73.39 ± 0.21b	15.24	70.84 ± 0.09b	14.03	1
Chlorpyriphos 20 EC	23.50 ± 0.79 k	72.86	26.81 ± 0.29 k	67.46	2
Imidacloprid17.8 SL	46.65 ± 0.21 g	46.12	43.77 ± 0.21 g	46.88	2
Triazophos 40 EC	37.24 ± 0.21j	56.99	30.85 ± 0.18j	62.56	2
Bifenthrin 10 EC	41.05 ± 0.36 h	52.59	33.97 ± 0.27i	58.77	2
Fipronil 5SC	48.86 ± 0.30f	43.57	51.91 ± 0.36f	37.01	2
Flubendiamide 480 SL	58.71 ± 0.18e	32.19	53.28 ± 0.17e	35.34	2
Clothianidin 50 WDG	59.40 ± 0.05d	31.40	57.63 ± 0.11d	30.06	2
Thiamethoxam 25 WDG	38.95 ± 0.32i	55.01	34.73 ± 0.25 h	57.85	2
Methoxyfenozide 24 SC	70.03 ± 0.20c	19.12	67.92 ± 0.13c	17.58	1
Untreated control	86.59 ± 0.16a	-	82.41 ± 0.31a	-	
F value	2907.09		5659.13		
df	10,33		10,33		
P value	< 0.0001		< 0.0001		

Mean (± SE) within the same column followed by different lowercase letters are statistically different at $P < 0.05$ level

[a]IOBC class based on reduction in parasitism and adult emergence as per Sterk et al. (1999); class 1—harmless (< 30%), class 2—slightly harmful (30–79%), class 3—moderately harmful (80–99%) and class 4—harmful (> 99%). Untransformed data have been presented

Table 5 Mean parasitism (%) by *Trichogramma chilonis* adults of F_1 generation emerged from insecticide treated *Corcyra cephalonica* eggs with parasitoid in pre-pupal stage (5th day of development) and adult emergence (%) of F_2 adults along with class of toxicity according to IOBC

Insecticides	Mean parasitism ± SE (%)	Parasitism reduction (%)	Adult emergence rate ± SE (%)	Adult emergence reduction (%)	[a]IOBC Toxicity class
Chlorantraniliprole 18.5 SL	75.12 ± 0.12c	10.55	70.27 ± 0.25c	13.32	1
Chlorpyriphos 20 EC	2.50 ± 0.41 k	97.02	0.00 ± 0.00 k	100.0	4
Imidacloprid17.8 SL	62.02 ± 0.14 h	26.14	64.75 ± 0.30 g	20.13	1
Triazophos 40 EC	28.99 ± 0.36j	65.47	36.34 ± 0.42j	55.17	2
Bifenthrin 10 EC	39.41 ± 0.28i	53.07	43.15 ± 0.18i	46.77	2
Fipronil 5SC	71.01 ± 0.11d	15.44	64.59 ± 0.12 h	20.32	1
Flubendiamide 480 SL	70.66 ± 0.12e	15.86	65.50 ± 0.09f	19.20	1
Clothianidin 50 WDG	65.66 ± 0.10f	21.81	67.57 ± 0.11d	16.65	1
Thiamethoxam 25 WDG	63.12 ± 0.10 g	24.83	66.59 ± 0.20e	17.86	1
Methoxyfenozide 24 SC	78.42 ± 0.23b	6.62	72.54 ± 0.16b	10.52	1
Untreated control	83.98 ± 0.16a	-	81.07 ± 0.13a	-	
F value	4098.61		23,424.86		
df	10,33		10,33		
P value	< 0.0001		< 0.0001		

Mean (± SE) within the same column followed by different lowercase letters are statistically different at P < 0.05 level

[a]IOBC class based on reduction in parasitism and adult emergence as per Sterk et al. (1999); class 1—harmless (< 30%), class 2—slightly harmful (30–79%), class 3—moderately harmful (80–99%) and class 4—harmful (> 99%). Untransformed data have been presented

Insecticide treatment in pupal stage

All the ten insecticides significant reduced the parasitism rate of *T. chilonis* adults emerged from insecticide exposed *Corcyr*a eggs having parasitoid in pupal stage ($F = 4738.09$, $df_{10,33}$; $p < 0.0001$) and subsequent adult emergence of second generation adults ($F = 19{,}283.54$, $df_{10,33}$; $p < 0.0001$). The reduction in parasitism and percentage adult emergence were lower in chlorantraniliprole, methoxyfenozide and flubendiamide. Chlorpyriphos reduced the parasitism and adult emergence rates by almost 99% and 100%, respectively. As per IOBC classification, all the test insecticides were rated as harmless except bifenthrin (slightly harmful), triazophos

Table 6 Mean parasitism (%) by *Trichogramma chilonis* adults of F_1 generation emerged from insecticide treated *Corcyra cephalonica* eggs with parasitoid in pupal stage (7th day of development) and adult emergence (%) of F_2 adults along with class of toxicity according to IOBC

Insecticides	Mean parasitism ± SE (%)	Parasitism reduction (%)	Adult emergence rate ± SE (%)	Adult emergence reduction (%)	[a]IOBC Toxicity class
Chlorantraniliprole 18.5 SL	81.97 ± 0.17b	6.69	86.03 ± 0.12b	2.54	1
Chlorpyriphos 20 EC	1.00 ± 0.29 k	98.86	0.00 ± 0.00 k	100.0	4
Imidacloprid17.8 SL	71.72 ± 0.07 g	18.36	75.17 ± 0.06 g	14.85	1
Triazophos 40 EC	14.45 ± 0.48j	83.55	29.74 ± 0.20j	66.31	3
Bifenthrin 10 EC	30.00 ± 0.20i	65.85	38.53 ± 0.66i	56.35	2
Fipronil 5SC	71.47 ± 0.24 h	18.64	76.85 ± 0.03f	12.94	1
Flubendiamide 480 SL	78.58 ± 0.12d	10.55	80.93 ± 0.11d	8.32	1
Clothianidin 50 WDG	73.69 ± 0.06e	16.11	74.46 ± 0.06 h	15.65	1
Thiamethoxam 25 WDG	72.60 ± 0.13f	17.35	77.48 ± 0.17e	12.23	1
Methoxyfenozide 24 SC	79.81 ± 0.05c	9.15	82.06 ± 0.08c	7.04	1
Untreated control	87.85 ± 0.14a	-	88.28 ± 0.07a	-	
F value	4738.09		19,283.54		
df	10,33		10,33		
P value	< 0.0001		< 0.0001		

Mean (± SE) within the same column followed by different lowercase letters are statistically different at P < 0.05 level

[#]IOBC class based on reduction in parasitism and adult emergence as per Sterk et al. (1999); class 1—harmless (< 30%), class 2—slightly harmful (30–79%), class 3—moderately harmful (80–99%) and class 4—harmful (> 99%). Untransformed data have been presented (moderately harmful) and chlorpyriphos (harmful) to second generation when parasitoid in pupal stage was exposed to insecticides (Table 6).

Discussion

One of the major focuses of IPM is to integrate chemical and biological control tactics for sustainable insect pest management. Natural enemies have a strong tendency of susceptibility to insecticides as compared to their herbivore hosts/prey. The differential susceptibility of natural enemies to pesticides creates serious compatibility problems for integration of pesticides and bioagents in IPM programs (Stark et al. 2007). Accordingly, insecticides need to be selectively used for sustainable crop protection and environmental permanence (Greathead 1995; Haseeb et al. 2000). Depending on the development stage of the parasitoid, the effect of an insecticide can vary from harmless to harmful (Santos et al. 2006). The topical application of insecticides on host containing parasitoid development stage will determine the impact of insecticide residues upon adult exit (Mgocheki and Addison 2009). Our findings showed that insecticides were more harmful to the egg-larval stages of parasitoid as compared to pre-pupal-pupal stages. This may be due to higher activity of larval stage as compared to latter stages as suggested by Souza et al. (2014). The toxicity of insecticides to immature stages showed that chlorantraniliprole, methoxyfenozide and clothianidin were found to be comparatively safer to all the developmental stages as compared to other insecticide treatments, while imidacloprid, fipronil and flubendiamide were also relatively safer to pre-pupal and pupal stages of *T. chilonis*. The insecticides like chlorpyriphos, triazophos and bifenthrin were relatively toxic to all the immature stages of the parasitoid. The selectivity of insecticides might be due to the presence of the parasitoid within the host egg where the chorion protects the parasitoid from the insecticide (Stecca et al. 2016). Moreover, the ability of an insecticide to penetrate an insect egg chorion may vary depending on the physico-chemical properties of an insecticide, because relatively high molecular weight chemicals have a greater difficulty in penetrating the chorion (Stock and Holloway 1993). The insecticides might not have penetrated the outer chorion of host egg thereby not affecting the developing stage and/or may have partially degraded thus reducing insecticide sensitiveness. The presence of chorion of host eggs may also explain the tolerance of insecticides to immature stages as compared to parasitoid adults. Our results corroborate with findings of Hussain et al. (2012) who also reported that chlorantraniliprole and flubendiamide had minimum effect on the emergence of *T. chilonis.* The harmlessness of flubendiamide to the pupal stage of *T. pretiosum* (Carvalho et al. 2005) and *T. atopovirila* (Rezende et al. 2005) has also been reported earlier. Our findings on methoxyfenozide are in conformity with Suh et al. (2000) who also reported no adverse effect of this growth regulator on the pre-imaginal development and adult survival in *T. exigum*. The highest toxicity of chlorpyriphos and bifenthrin to *T. chilonis* when all immature stages (egg, larvae, pre-pupae, early pupae and pupae) in the host eggs of *Sitotroga cerealella* were exposed to insecticides (Hussain et al. 2010) are in conformity with our results. Zhao et al. (2012) also reported that chlorpyriphos and triazophos) exhibited the highest intrinsic toxicity to *T. japonicum.*

The direct effects of insecticides on the natural enemies for example mortality or survival are important, but, what are actually more significant are the indirect or delayed effects, which may inhibit development time, adult emergence, longevity, predation and/or parasitism by the natural enemy (Cloyd 2012). The adults emerging from insecticidal treated *Corcyra* eggs with parasitoid in pre-imaginal stages (egg, larval, pre-pupal and pupal) were studied for sub-lethal effects in subsequent generation of *T. chilonis*, i.e. reduction in parasitism and adult emergence rate. Overall, chlorantraniliprole, methoxyfenozide and flubendamide resulted in lower reduction in parasitism and higher adult emergence percentage as compared to other insecticides, while chlorpyriphos was highly toxic with highest parasitism reduction and lowest adult emergence rate. The disparity in reduced parasitism and adult emergence in different insecticides may be due to variation in toxicity of these insecticides at different development stage of the parasitoid. Our findings showed that the effect of insecticides on parasitism rate and adult emergence of descendant generation was more when host eggs were exposed to insecticides with parasitoid in egg or larval stage as compared to pre-pupal or pupal stage which is in accordance with Vianna et al. (2009) who reported that pupal stage was very tolerant to insecticides and the second generation adults of *T. pretiosum* showed no significant difference in parasitization and adult emergence.

Better understanding of the threats associated with specific insecticide use to natural enemies is critical when they are integrated into IPM programs. Based on lethal and sub-lethal effects, we conclude that insecticides, chlorantraniliprole (anthranilic diamide), methoxyfenozide (insect growth regulator) and flubendamide (diamide) could be recommended for use in sugarcane ecosystem. The use of these insecticides in IPM programs for sugarcane borers could facilitate biologically-based pest management in sugarcane production system. The plausibility of the contention that adults emerging from eggs dipped for varying duration in field recommended doses/technical grade needs elaborate experimentations. Further studies under field/semi field conditions howeverare required to enhance our knowledge of these insecticides' side effects on trichogrammatids in India.

Declarations

Conflict of Interest The authors declare that the authors have no conflict of interest.

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