Cross-resistance and biochemical mechanism in an insecticide-resistant population of *Trichogramma chilonis* Ishii (Hymenoptera: Trichogrammatidae) and its parasitizing efficiency against invasive fall armyworm *Spodoptera frugiperda* (J.E. Smith)

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Trichogramma chilonis **is an egg parasitoid of lepidopteran pests and widely used in biological control and integrated pest management (IPM) programmes. In this study, the cross-resistance in multiple insecticide-tolerant strain of *T. chilonis* was evaluated against chlorantraniliprole 18.5% SC, spinetoram 11.7% SC and thiamethoxam 12.6% + lambda-cyhalothrin 9.5% ZC. Bioassay studies revealed the highest resistance level against chlorantraniliprole (8.83-fold resistance) over the susceptible population, followed by spinetoram (2.41-fold). Metabolic enzymes carboxylesterase and glutathione S-transferase showed major involvement in the resistant populations, with the highest activity observed against chlorantraniliprole, followed by spinetoram. The resistant population at field recommended doses of chlorantraniliprole (400 ppm), parasitized 52.92% and 44.10% of *Corcyra cephalonica* and *Spodoptera frugiperda* eggs respectively, compared to 15.48% and 9.6% parasitism by the susceptible population. Integration and utilization of resistant strains of *T. chilonis* in IPM programmes can provide improved control of insect pests under insecticide-sprayed conditions and may reduce the insecticide load on crops.**

Keywords: Biological agent, cross-resistance, egg parasitoid, *Spodoptera frugiperda*, *Trichogramma chilonis*.

EGG parasitoids of the genus *Trichogramma* (Hymenoptera: Trichogrammatidae) are one of the most commonly used natural enemies throughout the world for biological control programmes[1]. With the ease of mass rearing on factitious hosts, the various species of *Trichogramma* have been successfully released over 80 million acres annually to control lepidopteran pests infesting agricultural and forest crops[1–3]. Owing to the broader host range, these parasitoids are being released using the inundative method that involves a large number of parasitoid releases, basically acting as biopesticides[1]. The egg parasitoids are more significant in pest management because they kill the pest at the egg stage before it causes damage to the crops[4].

The recent invasion of fall armyworm (FAW) *Spodoptera frugiperda* (J.E. Smith) has caused significant damage to maize in various parts of India[5–7]. The outbreak and spread of FAW have forced farmers to use chemical insecticides to control this pest on an emergency basis[8]. Nevertheless, the concurrent occurrence of native parasitoids on FAW at the field level indicated the potential of these parasitoids in the exploration of pest management[5,9]. Of these, the egg parasitoid *Trichogramma chilonis* Ishii was found parasitizing a higher percentage of FAW eggs in maize and sugarcane fields[9,10]. This enables us to use this parasitoid to manage of FAW in maize and other cereal crops.

Currently, several chemical insecticides are being tested and used to control FAW in maize and other cereals[11]. The use of chemical insecticides is a regular pest control strategy to tackle invasive pests. In such a scenario, the trait of insecticide resistance in egg parasitoids such as *T. chilonis* can be well exploited to manage notorious pests like FAW[12]. Further, a study showed that the insecticide-resistant *Trichogramma* strain could potentially suppress the pest population under insecticide stressed conditions, to achieve better pest control with significantly reduced insecticide load on crops[13]. The insecticide-resistant population has enhanced metabolic enzymes, esterases (carboxylesterase), glutathione

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S-transferases (GSTs) and cytochrome P-450 monooxygenases (Cyt P-450) that help in the detoxifying/sequestering insecticides molecules inside the insect body before they exert any toxic effect[14,15]. These metabolic enzymes mediate resistance and may lead to cross-resistance of broad-spectrum insecticides having a different mode of action[16]. This insecticide resistance development is often associated with fitness costs[17].

Therefore, under the changing scenario of pesticide usage, it is feasible to test the enhanced insecticide resistance of *T. chilonis* against new insecticides used in the crop ecosystem. This is because the insecticide stressed conditions greatly influence and alter parasitism and the emergence of parasitoids. Hence, in the present study we assessed the cross-resistance in multiple insecticide-tolerant strains of *T. chilonis*. Futher, to validate the presence of cross-resistance, the major insecticide detoxifying enzymes were quantified. Alongside, the parasitizing efficiency of both resistant and susceptible populations of *T. chilonis* was tested against the most commonly used insecticides to determine its compatibility for integrated pest management (IPM) against FAW in the maize ecosystem.

Materials and methods

Insect cultures and chemical insecticides

The susceptible population and multiple insecticide-tolerant strains (MITs) of *T. chilonis* were reared and maintained on eggs of *Corcyra cephalonica* Stainton under laboratory conditions, as described by Nagaraja[18]. FAW was reared on a maize-based artificial diet, as described by Ballal *et al.*[19], to obtain sufficient number of eggs for experimentation. The Government of India (GoI), Ministry of Agriculture and Farmers' Welfare, Department of Agriculture, Cooperation and Farmers Welfare (DAC & FW, 2019) recommended insecticides were chosen and doses for the bioassay were fixed based on the field-recommended doses (Table 1).

Rearing of susceptible and MITs of T. chilonis

The susceptible populations of *T. chilonis* (more than 360 generations) were mass-reared by preparing paper tricho-cards (15 × 10 cm) consisting of 16 small subunits (demarcations/stamps). The ultraviolet (UV) treated eggs of *C. cephalonica* (16,000 eggs = 1 cm^3 eggs/card) were pasted on paper tricho-cards using 10% gum arabica and shade-dried for about 30 min. These tricho-cards were exposed to 8–12 h-old mated females of *T. chilonis* for 24–48 h for parasitism and adults were fed with 30% honey placed inside the rearing container. Thereafter, the parasitized cards were incubated under controlled conditions (temperature: 26° ± 1°C, RH 60% ± 5%, LD 14:10) until the parasitoids emerged. The emerged parasitoids were used in experimentation and one subunit of parasitized eggs was placed in a glass tube (15 × 5 cm) for continuous maintenance of the susceptible population of *T. chilonis*.

MITs of *T. chilonis* were procured from the Division of Genomic Resources, ICAR-National Bureau of Agricultural Insect Resources (NBAIR), Bengaluru, and maintained by exposing the emerging adults to the recommended dosage of various groups of insecticides, viz. organochlorines, synthetic pyrethroids, organophosphates, neonicotinoids and spinosyns for over 60 generations after the development of insecticides-resistant strains[13]. These adults were used for studying cross-resistance in the tolerant strain of *T. chilonis* by exposing them sequentially to the three novel insecticides, viz. chlorantraniliprole 18.5% SC, spinetoram 11.7% SC and thiamethoxam 12.6% + lambda-cyhalothrin 9.5% ZC for over 90 generations (2018–21) and further used for laboratory bioassay. The parasitized eggs of *C. cephalonica* after each bioassay were exploited as mother culture to maintain the resistant population.

Bioassay

The inner surface of the cylindrical glass tubes (15 × 5 cm) was first sprayed with different treatment concentrations of insecticides and water as the control (Table 1). These tubes were shade-dried with utmost care to avoid deterioration of the insecticides. The treatment concentrations were set by diluting the field-recommended dosage of insecticides to three doses above and three below for bioassay studies. The freshly emerged females were allowed to mate for 8–12 h and the unfed (starved for 8–12 h) adults of MITs of *T. chilonis* and susceptible populations were released inside the glass tubes separately, with approximately 200 adults in each tube[20]. Observation on per cent mortality against each insecticide was recorded at different time intervals of 2, 4, 6 and 24 h after exposure. The per cent mortality was determined based on the number of adults that survived to the total number of adults released in the tubes. At the sixth hour post-exposure, a long strip (11 × 1.5 cm) of freshly prepared tricho-card was placed in each hollow tube to facilitate egg-laying (parasitism) by the surviving females of *T. chilonis.* Such seven treatments were maintained, including the control and each treatment was replicated thrice to minimize experimental errors.

Biochemical analysis of metabolic detoxifying enzymes using enzyme assay

The mechanism of insecticide resistance in the resistant strains of *T. chilonis* was determined by quantifying the major detoxifying enzymes, viz. carboxylesterase, GST and Cyt P-450, through enzyme assays from adults exposed to chlorantraniliprole, spinetoram and thiamethoxam + lambda-cyhalothrin. The results were compared with the enzyme activity of the susceptible population. Enzyme assays were carried out by extracting sample homogenate by crushing

around 200 *T. chilonis* adults in ice-cold phosphate buffer solution (50 mM PBS). Estimation of total protein content present in the enzyme sample was done by the coomassie dye binding method, as shown by Bradford[21]. The quantitative analysis of carboxylesterase, GST and Cyt P-450 activities was performed following the standard protocols given by Van Asperen[22] and Kinoshita *et al.*[23] respectively.

Parasitization efficiency of resistant T. chilonis

The parasitization efficiency of resistant (MITs) and susceptible populations of *T. chilonis* was studied on factitious host eggs of *C. cephalonica* and FAW as a target host under controlled laboratory conditions. To study the parasitizing efficiency of *T. chilonis* on *C. cephalonica*, 200 adult wasps fed with honey were released into each hollow glass tube sprayed with insecticides spinetoram 11.7% SC, chlorantraniliprole 18.5% SC, thiamethoxam 12.6% + lambda-cyhalothrin 9.5% ZC respectively, at different treatment concentrations (Table 1) and shade-dried. Six hours after insecticide exposure, egg cards (11 cm × 1.5 cm dimension) sprinkled with UV-sterilized *C. cephalonica* were placed in each hollow tube for parasitism. After 24 h of exposure, the cards were transferred to fresh glass tubes (15 ml) and incubated under controlled conditions. After five days, the egg cards were observed for parasitism and the number of parasitized (eggs turn black in colour) and unparasitized eggs was counted. The parasitism rate was calculated using the following formula and expressed as percentage.

$$\text{Per cent parasitism} = \frac{\text{Number of eggs parasitized}}{\text{Total number of eggs exposed}} \times 100$$

The parasitizing efficiency of MITs of *T. chilonis* was studied on target host eggs of FAW under insecticide-sprayed conditions. About 200 adults of both resistant and susceptible populations of *T. chilonis* were exposed to hollow tubes sprayed with different concentrations of insecticides. Freshly laid FAW eggs (<24 h-old) were obtained from laboratory-reared moths on an artificial diet[19]. These eggs were then UV-sterilized for about 3 h under UV lamp at 26° ± 1°C, RH 60% ± 5%, LD 14 : 10 (with per cent hatching of larvae from <1). About 200 UV-treated eggs were glued in a single layer on the paper cards (5 × 1.5 cm) to reduce heterogeneity and experimental errors. These cards were kept in the hollow tubes containing adults of *T. chilonis*, which were exposed to different concentrations of insecticides (mentioned above) for 6 h. The FAW egg cards were exposed to each insecticide at different concentrations separately and replicated three times. After 24 h of exposure, the egg cards were removed from the hollow tubes, placed in fresh glass tubes (15 × 2.5 cm) and incubated under controlled conditions. Five days later, the number of parasitized eggs (that turned black) was counted under a stereo-zoom microscope and the per cent parasitism was estimated.

Statistical analysis

The data obtained on the bioassay of *T. chilonis* mortality were input to POLO PLUS© software version 2.0 (2002–20, LeOra Software) to determine the LC_{50} and fiducial limit values against the respective treatment insecticides. The resistance ratio was tested against each concentration dose by dividing the LC_{50} value of the susceptible population by the LC_{50} value of the resistant population. The enzyme activities obtained from various samples of resistant and susceptible populations of *T. chilonis* were subjected to a Student's *t*-test for testing the hypothesis and finding the difference between the sample means. Data obtained on the parasitizing efficiency of both MITs and susceptible *T. chilonis* were expressed in percentage and subjected to Student's *t*-test to determine the difference between the populations.

Results

Enhanced insecticide resistance in T. chilonis

Three insecticides were screened for enhanced resistance in the laboratory-reared resistant population of *T. chilonis* and the susceptible population. The insecticide thiamethoxam + lambda-cyhalothrin (LC_{50} of 99.99 ppm) showed higher toxicity to the resistant population of *T. chilonis* followed by spinetoram (LC_{50} of 218.96 and 184.63 ppm) and chlorantraniliprole (LC_{50} of 8561.1, 6676.1, 1028.64 and 893.26 ppm) at different periods of exposure (Table 2). Among three insecticides tested, the highest resistance ratio was recorded for chlorantraniliprole (8.83-fold), followed by spinetoram (2.4-fold), and the lowest for thiamethoxam + lambda-cyhalothrin (1.25-fold) in the resistance population of *T. chilonis* as compared to the susceptible population (Table 2). Chlorantraniliprole was comparatively safer on the parasitoids in comparison to the other insecticides.

Biochemical analysis of metabolic detoxifying enzymes

Biochemical analysis of the enzymes showed a significantly higher level of activity of detoxifying enzymes in the resistant population of *T. chilonis* compared to the susceptible population (Table 3). The results revealed that there was increased activity in two major detoxifying enzymes, i.e. carboxylesterase and GST, in the resistant population. The highest activity of enzymes was observed in the resistant population of *T. chilonis* exposed to chlorantraniliprole and showed a 4.8- and 4.18-fold increase in carboxylesterase and GST activity respectively, compared to the susceptible

Table 1. Details of insecticides used

Insecticide	Insecticide group	IRAC mode of action	Field-recommended dosage (ppm)	Concentration tested (ppm)	Trade name	Manufacturer
Spinetoram 11.7% SC	Spinosyn	Nicotinic acetylcholine receptor (nAChR) allosteric activator	600	75, 150, 300, 600, 1200, 2400, 4800	Delegate®	Dow agroscience
Chlorantraniliprole 18.5% SC	Diamide	Ryanodine receptor activator	400	50, 100, 200, 400, 800, 1600, 3200	Coragen®	Dupont
Thiamethoxam 12.6% + Lambda-cyhalothrin 9.5% ZC	Neonicotinoid + synthetic pyrethroid	Nicotinic acetylcholine receptor competitive modulator + sodium channel modulator	500	62.5, 125, 250, 500, 1000, 2000, 4000	Alika®	Sygenta

Table 2. Relative toxicity of insecticides to the resistant (MITs) and susceptible populations of *Trichogramma chilonis* at different time-scales of exposure

Insecticide	Period of exposure (h)	Population	LC_{50} (ppm)	95% Fiducial limit (ppm)		Slope ± SE	χ^2 value (d*f*)	*P**	Resistance ratio (RR)#	Mean RR
				Lower	Upper					
Chlorantraniliprole	2	Resistant	8561.1	5561.1	15574	0.87 ± 0.081	2.90 (5)	0.58	7.92	8.83
		Susceptible	1081.36	747.34	1749.12	0.47 ± 0.059	1.05 (5)	0.21		
	4	Resistant	6676.1	4558.7	11244	0.92 ± 0.085	2.32 (5)	0.46	10.1	
		Susceptible	663.69	453.05	992.27	0.55 ± 0.065	2.50 (5)	0.50		
	6	Resistant	1028.64	908.41	1161.43	1.91 ± 0.13	1.40 (5)	0.25	8.73	
		Susceptible	117.81	82.17	156.26	1.59 ± 0.099	8.81 (5)	1.76		
	24	Resistant	893.26	767.72	1022.78	1.88 ± 0.15	3.90 (5)	0.78	8.55	
		Susceptible	104.49	73.82	136.82	1.75 ± 0.19	7.34 (5)	1.46		
Spinetoram	2	Resistant	219.86	132.61	321.97	1.55 ± 0.084	27.57 (5)	5.51	2.41	2.40
		Susceptible	91.11	54.32	130.84	1.44 ± 0.088	16.55 (5)	3.31		
	4	Resistant	186.98	115.27	266.27	1.59 ± 0.096	16.48 (5)	3.29	2.39	
		Susceptible	77.95	33.72	127.92	1.47 ± 0.10	20.04 (5)	4.00		
Thiamethoxam + lambda-cyhalothrin	2	Resistant	99.99	64.69	135.70	1.87 ± 0.12	17.28 (5)	3.45	1.25	
		Susceptible	79.51	45.20	113.75	1.74 ± 0.12	16.85 (5)	3.37		

*$P \geq 0.05$ indicates a good fit between observed and expected regression lines in a probit analysis. SE, Standard error; d*f*, Degrees of freedom.
#Resistance ratio = LC_{50} of resistant *T. chilonis* population/LC_{50} of susceptible population.

Table 3. Differences in activity of detoxifying enzymes between resistant (MITs) and susceptible population of *Trichogramma chilonis*

Population	Carboxylesterase (μmol min^{-1} mg^{-1})	*P*-value	Glutathione S-transferase (μmol min^{-1} mg^{-1})	*P*-value
R – Chlorantraniliprole	0.039 ± 0.00014 (4.8)	$t = 43.30$; $P < 0.00001$	0.031 ± 0.0016 (4.18)	$t = 11.44$; $P < 0.00033$
R – Spinetoram	0.029 ± 0.0001 (3.58)	$t = 28.92$; $P < 0.00001$	0.015 ± 0.0011 (2.02)	$t = 4.95$; $P < 0.0078$
R – Thiamethoxam + lambda-cyhalothrin	0.021 ± 0.00027 (2.59)	$t = 15.72$; $P < 0.00009$	0.012 ± 0.00046 (1.62)	$t = 5.52$; $P < 0.0053$
S – Laboratory	0.0081 ± 0.00042	–	0.0074 ± 0.00047	–

Enzyme activity of the resistant population differs significantly from the susceptible population ($P < 0.05$) based on Student's *t*-test. Values in brackets depict the fold increase activity in resistant population compared to susceptible population. R, Resistant population; S, Susceptible population.

populations. The activity of carboxylesterase and GST in the resistant population of spinetoram was 3.54- and 2.01-fold, and for thiamethoxam + lambda cyhalothrin it was 2.59- and 1.62-fold respectively, over the susceptible population (Table 3). This confirmed the involvement of detoxifying enzymes in resistance development in MITs in *T. chilonis* over the susceptible populations. However, the involvement of Cyt P-450 was not prominent.

Parasitization efficiency of resistant T. chilonis

The parasitism levels by resistant *T. chilonis* were significantly different for chlorantraniliprole-sprayed conditions at various concentrations for the eggs of *C. cephalonica* and FAW compared to the susceptible population (Tables 4 and 5). However, the parasitism rate was not significantly different when resistant and susceptible populations were exposed under spinetoram and thiamethoxam + lambda-cyhalothrin-sprayed conditions ($P > 0.05$). The resistant population of *T. chilonis* parasitized 52.92% of *C. cephalonica* eggs and 44.1% of FAW eggs at the field-recommended dosage of chlorantraniliprole (0.4 ml/l) compared to the susceptible population that parasitized 15.48% of *C. cephalonica* and 6.9% of FAW eggs. No parasitism by resistant *T. chilonis* was recorded when *C. cephalonica* and FAW eggs were exposed to the field-recommended dose of spinetoram (0.6 ml/l) and thiamethoxam + lambda-cyhalothrin (0.5 ml/l). The resistant and susceptible populations of *T. chilonis* in untreated control parasitized 73.20% and 63.70% of *C. cephalonica* eggs respectively, and 60.83% and 51.78% of FAW eggs.

Discussion

Egg parasitoids of *Trichogramma* are widely used as potential biocontrol agents in biological control or as a component of IPM against lepidopteran pests infesting field crops[1]. However, the excessive use of insecticides in crop ecosystems significantly lowers their efficacy[24]. Hence, the development of insecticide-tolerant strains through insecticide-induced selection pressure has the potential to suppress insect pests under an insecticide-sprayed environment[13]. In the present study, field-collected, insecticide tolerant (MITs) *T. chilonis* was further subjected to selection pressure by exposing them to the field-recommended doses of three insecticides, viz. chlorantraniliprole, spinetoram and thiamethoxam + lambda-cyhalothrin. The resistant population of *T. chilonis* exhibited an enhanced level of resistance to chlorantraniliprole (8.83-fold) without experiencing multiple cycles of continuous exposure and was comparably safer to parasitoids than the other two insecticides. Similarly, the lowest effect of chlorantraniliprole on the survival of *Trichogramma* spp. has been reported from India[25] and China[26] in the rice ecosystem. Xie *et al.*[27] reported a 17.8-fold resistance rate of *T. japonicum* to imidacloprid, and 8.8-fold and 6.9-fold resistance rate of *T. chilonis* to imidacloprid and thiamethoxam respectively. Shankarganesh *et al.*[28] found that lambda-cyhalothrin was harmful to *T. chilonis.* In the present study, we found that the combination of thiamethoxam + lambda-cyhalothrin was toxic and caused a higher percentage of adult mortality. In nature, the resistance levels in parasitoids are low when compared to predators due to their non-continuous encounters with the selection pressure induced by insecticides under field conditions. Therefore, continuous exposure of parasitoids to the same/similar group of pesticides tends to develop resistance and such populations could be used in pest management in compatibility with insecticides. The lower LC_{50} values of the resistant populations of *T. chilonis* were greater than the field-recommended dosages, suggesting that populations of *T. chilonis* were able to tolerate the field doses of insecticides tested to induce resistance in the parasitoids. The lower toxicity of chlorantraniliprole suggested that it has relatively less effect on the survival of the parasitoids[26,29]. In field conditions, the ability of the resistant population to survive under insecticide stress conditions may be important to maintaining a population in order to achieve enhanced pest control.

Several metabolic enzymes are accountable for the detoxifying the insecticides in insects. Carboxylesterases, GST and Cyt P-450 are the major metabolic enzymes responsible for insecticide resistance[13–15]. In this study, carboxylesterase and GST showed elevated levels of activity in the resistant population of *T. chilonis* compared to the susceptible population. Further, the metabolic activity of carboxylesterase and GST was increased by 4.8- and 4.18-fold respectively, in a resistant population of the parasitoid. Jalali *et al.*[13] have reported that GST-conjugative activity in the tolerant strain was 2.13-fold higher than that of the susceptible strain. Similarly, carboxylesterase activity in the tolerant

Table 4. Parasitism of insecticide-resistant (MITs) and susceptible populations of *Trichogramma chilonis* on *Corcyra cephalonica* eggs under different insecticide-sprayed conditions

Chlorantraniliprole			Spinetoram			Thiamethaxam + lambda-cyhalothrin		
Concentration (ppm)	R-population	S-population	Concentration (ppm)	R-population	S-population	Concentration (ppm)	R-population	S-population
50	68.76	46.8	75	33.12	12.60	62.5	1.80	0.00
100	66.96	33.48	150	18.54	10.00	125	1.08	0.00
200	64.44	22.68	300	6.66	0.00	250	0.00	0.00
400	52.92	15.48	600	0.00	0.00	500	0.00	0.00
800	40.68	8.28	1200	0.00	0.00	1000	0.00	0.00
1600	25.56	1.08	2400	0.00	0.00	2000	0.00	0.00
3200	11.52	0.00	4800	0.00	0.00	4000	0.00	0.00
Untreated control	72.80	64.30	Untreated control	73.20	63.70	Untreated control	73.60	63.30
t test (0.05%)	$t = 2.33$; $P = 0.035$		*t* test (0.05%)	$t = 0.47$; $P = 0.64$		*t* test (0.05%)	$t = 0.14$; $P = 0.89$	

Table 5. Parasitism of insecticide resistant (MITs) and susceptible populations of *Trichogramma chilonis* on *Spodoptera frugiperda* eggs under different insecticide sprayed conditions

Chlorantraniliprole			Spinetoram			Thiamethaxam + lambda-cyhalothrin		
Concentration (ppm)	R-population	S-population	Concentration (ppm)	R-population	S-population	Concentration (ppm)	R-population	S-population
50	57.30	27.90	75	27.60	10.50	62.5	0.50	0.00
100	55.80	18.90	150	15.45	8.00	125	0.00	0.00
200	53.37	12.90	300	5.55	0.00	250	0.00	0.00
400	44.10	6.90	600	0.00	0.00	500	0.00	0.00
800	33.90	0.90	1200	0.00	0.00	1000	0.00	0.00
1600	21.30	0.00	2400	0.00	0.00	2000	0.00	0.00
3200	9.60	0.00	4800	0.00	0.00	4000	0.00	0.00
Untreated control	60.10	51.78	Untreated control	61.60	50.75	Untreated control	60.80	51.30
t test (0.05%)	$t = 2.95$; $P = 0.010$		*t* test (0.05%)	$t = 0.51$; $P = 0.61$		*t* test (0.05%)	$t = 0.12$; $P = 0.90$	

strain (MITs) was 1.5- to 9-fold higher than in the susceptible strain. Furthermore, these metabolic enzymes act together; however, the proportion of enzymatic activity differs with insecticides[30]. Besides, in this study, the involvement of Cyt P-450 was not prominent.

The parasitizing efficiency is an important parameter of the effectiveness of parasitoids on the target hosts[26]. In this study, the three insecticides at different concentrations influenced the parasitizing efficacy of *T. chilonis* on the laboratory host, *C. cephalonica* and the target host FAW. Chlorantraniliprole at the field dose (0.4 ml/l) did not show any negative effect on the parasitism of both resistant and susceptible populations of *T. chilonis* exposed to both hosts under laboratory conditions. The resistant population of *T. chilonis* parasitized a significantly higher percentage of *C. cephalonica* and FAW eggs under an insecticide stress environment. Khan[31] reported chlorantraniliprole as harmless to the adults of *T. chilonis* with less adverse effect, which parasitized >80% of *S. cerealella* eggs and 53.42% of *C. cephalonica* eggs[32] at field doses of insecticide. Similarly, several studies also showed chlorantraniliprole as a safe insecticide not affecting the parasitizing capacity of *Trichogramma* spp., with lower adult mortality at field-recommended concentration[33,34]. However, in the present study, spinetoram (0.6 ml/l) and thiamethoxam + lambda-cyhalothrin (0.5 ml/l) were potent and significantly reduced the parasitizing efficacy of resistant and susceptible *T. chilonis* when exposed to the eggs of *C. cephalonica* and FAW at field-recommended dosage. Earlier studies have also reported acute toxicity of spinetoram to the adults of *T. pretiosum*[33] and *T. chilonis*[31] that induced 98.8% and >95.3% mortality respectively. This adversely affected the parasitism rate of *T. pretiosum* and *T. chilonis*. Moreover, spinetoram has been known to cause an adverse effect on hymenopteran parasitoids[31]. The harmful effect of ready-mix insecticide formulations of thiamethoxam + lambda-cyhalothrin was also reported against the *Trichogramma galloi* on biological characteristics; thus it is not recommended for use in IPM programmes[35]. The use of the tolerant/resistant strain may reduce insecticidal load and as a component of IPM in various crops to manage pests in insecticide-sprayed conditions[36]. Hence, the present study demonstrates that the resistant population of *T. chilonis* could parasitize a higher percentage of *C. cephalonica* and FAW eggs than the susceptible population and would sustain better in insecticide stress conditions at the field level when integrated under IPM for management of notorious pests like FAW.

Conclusion

The MITs of *T. chilonis* confirmed the enhanced spectrum of resistance against chlorantraniliprole and parasitized a higher percentage of FAW eggs, indicating that this resistant strain can better withstand insecticide-stressed field conditions. Further, higher parasitizing efficiency and performance of the resistant population provide an opportunity to integrate with chlorantraniliprole at the field-recommended dosage to achieve better control against FAW. The survival and parasitism rate of the resistant population of *T. chilonis* in the chlorantraniliprole-sprayed conditions indicate the compatibility of biocontrol agents and chemicals. Hence, while formulating IPM programmes against pests, especially FAW, the resistant *T. chilonis* could be considered as potential biocontrol agents for pest management.

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