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Research Article

Genetic aspects of insecticide tolerance in *Trichogramma chilonis* Ishii (Hymenoptera: Trichogrammatidae) strains

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ABSTRACT: *Trichogramma* genus is one of the most important groups of biotic agents employed for the control of several lepidopterous pests. However, these parasitoids are also subjected to environmental stress such as insecticides, temperature etc. in the agricultural field. Selection of parasitoids tolerant to insecticides has been recognized as a potent method for enhancing their performance. In this regard, the present study was carried out to know insecticide tolerance level and the kind of inheritance in *Trichogramma chilonis* Ishii., strains on commonly used insecticides in the agricultural fields. Endosulfan, spinosad and lamda cyhalothrin tolerant strains along with a susceptible one were selected to carryout the crossing experiments. The LC_{50} values of endosulfan, spinosad and lamda cyhalothrin tolerant strains were found to be 278.03, 9.84 and 6.23 ppm respectively. This is in contrast to the respective susceptible strains to these insecticides which have exhibited LC_{50} values of 106.03, 4.78 and 3.45 ppm respectively. The endosulfan tolerance appeared to be recessive for the cross involving tolerant male parent and semi dominant for the tolerant female parent. The spinosad tolerance was found to be semi dominant for crosses involving both male and female tolerant parents. In contrast, the lamda cyhalothrin tolerance was completely dominant for crosses with the tolerant male and female parents.

KEY WORDS: *Trichogramma chilonis*; pesticide tolerance; degree of dominance.

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INTRODUCTION

World over several parasitoids and predators of crop pest have been successfully employed for pest management. Trichogrammatids are one of the most important groups of biotic agents for the suppression of many lepidopterous pests in India as well (Singh and Jalali, 1994). Being egg parasitoids, they play a vital role in destroying the early stages of the pest and reducing the use of insecticides that otherwise may contribute to environment pollution. However, these parasitoids are also subjected to environmental stress such as insecticides and temperature in the agricultural fields.

Acceptance of biological control agents in Indian agriculture has been limited despite the growing awareness about the hazards of indiscriminate use of pesticides on environment and human health. This is because biocontrol agents are perceived to be slow acting in nature and are susceptible to pesticides and other abiotic factors. In this regard, selection of parasitoid strains, that are resistant to insecticides is recognized as a potent method for enhancing their performance. Genetic improvement of natural enemies has produced pesticide resistant strains for several species of parasitoids and predators of insects and mites (Croft, 1990). *Trichogramma* is by far the most widely mass-produced and inundatively released biocontrol agent globally in agriculture and forestry ecosystems (Smith, 1996). The pattern and bases of inheritance on insecticide tolerance has been a matter of considerable interest among biocontrol researchers on *Trichogramma* elsewhere (Krukierek *et al*., (1975; Rosenheim and Hoy, 1988), while Devi *et al*.(2006) have examined this aspect in India recently, involving strains resistant to indoxycarb, spinosad and tebufenozide. It is in this context that the present study was carried out to compare with spinosad the kind of inheritance in *Trichogramma chilonis* Ishii., strains tolerant to endosulfan and lamdacyhalothrin which are commonly used in the agricultural fields of Karnataka state, India.

MATERIALS AND METHODS

Trichogramma chilonis strains

Three pesticide tolerant strains of *T. chilonis* along with a susceptible one were employed to carryout the crossing experiments. The strains tolerant to endosulfan (TcT1), spinosad (TcT4) and lamda cyhalothrin (TcT5) were obtained from the National Bureau of Agriculturally Important Insects (NBAII), Bangalore. Later, they were maintained in the Vector Biology Research Lab of Zoology Department at University of Mysore, Mysore upto three generations to carryout crossing experiments.

Crossing experiments

To carryout the crosses, the *Trichogramma* parasitised eggs of *Corcyra cephalonica* Stainton susceptible and pesticide tolerant strains were kept individually in glass vials (5 x 2 cm) for emergence and sexed under stereozoom microscope following the method of Devi *et al*. (2006).

The following crosses were made

a) susceptible male x TcT1 female
   susceptible female x TcT1 male (reciprocal crosses)

b) susceptible male x TcT4 female
   susceptible female x TcT4 male

c) susceptible male x TcT5 female
   susceptible female x TcT5 male

Egg card with about 50 eggs of *C. cephalonica* was placed inside the glass vial after ensuring the mating. The F1 progenies were inbred and the progenies of F2 generation were exposed to different insecticide concentration following the method of Devi *et al*. (2006).

Insecticide exposure

Solutions of the three insecticides, namely endosulfan (35% EC), spinosad (2.5% EC) and lamda cyhalothrin (5% EC) were prepared in serial dilution by taking dosages double than the field recommended doses and reducing dilution by half (Devi *et al*., 2006). Endosulfan concentrations used for exposure were 43.75, 87.5, 175, 350, 700 and 1400 ppm and water spray as control. For spinosad 1.87, 3.75, 7.5, 15, 30 and 60 ppm and for lamda cyhalothrin 0.9, 1.87, 3.75, 7.5, 15 and 30 ppm were employed.

Glass vials of 20 x 4 cm size, open at both the ends were used for pesticide exposure. Required insecticide concentration was sprayed with the help of an atomizer. After drying in shade one end of the vial was covered with a double layered black cloth and fastened with rubber band. From the other side about 100 adult parasitoids were released in to each of the glass tube. Mortality was recorded after 6 hours of constant exposure.

Statistical analysis

The data obtained on the mortality were subjected to probit analysis to calculate LC_{50} and fiducial limits. Chisquare and p-value were calculated to know the significance. The degree of dominance (D) was calculated by using the formula

$$D = \frac{2X_2 - X_1 - X_3}{X_1 - X_3}$$

where X_1 = logarithm of LC_{50} of tolerant parent.

X_2 = logarithm of LC_{50} of heterozygous F2 progeny.

X_3 = logarithm of LC_{50} of susceptible parent.

For D value, 1 indicates complete dominance, –1 indicates complete recessiveness and 0 to +1 indicates semi dominant level of resistance. The resistance factor was calculated by dividing LC_{50} value of tolerant parent by LC_{50} value of susceptible parent. These interpretations are on the basis of the formulae provided by Stone (1968).

RESULTS AND DISCUSSION

The results of the insecticide exposure experiments involving various crosses are furnished in Table 1. It was found that the LC_{50} values of endosulfan, spinosad and lamda cyhalothrin tolerant strains were 278.03, 9.84 and 6.23 ppm, respectively. These values are significantly higher ($p<0.05$) than that of the respective control values of 106.03, 4.78 and 3.45 ppm, respectively.

The F2 progeny from the endosulfan tolerant cross namely TcT1 male x susceptible female gave an LC_{50} value of 166.49 ppm and that of the reciprocal cross was 189.41 ppm. This shows an intermediate LC_{50} value with that obtained for the susceptible (106.03 ppm) and tolerant parent strains (278.3 ppm) (Table 1). The F2 progeny of the spinosad tolerant crosses, namely TcT4 male x susceptible female and the reciprocal crosses exhibited the LC_{50} values of 8.84 and 8.55 ppm, respectively, compared with that of susceptible (4.78 ppm) and tolerant parental strain (9.84 ppm). Similarly, F2 progenies from the lamda cyhalothrin tolerant cross have shown 6.86 ppm in case of male tolerant parent and 7.23 ppm in the reciprocal cross. These values are significantly higher than that of the tolerant parental strains alone exposed to pesticide (6.23 ppm) ($p<0.05$).

All the above three crosses have shown positive significant dose mortality responses, the higher the concentration, the lower the survival (Fig. 1, 2 and 3). The resistance factor for endosulfan tolerant strain (TcT1), F2 progenies of the crosses, TcT1 male x susceptible female and TcT1 female x susceptible male were 2.6, 1.5 and 1.7 respectively, over the susceptible one. The resistance factor of spinosad tolerant strain (TcT4), F2 progenies of the crosses TcT4 male x susceptible female

Table 1: Response of parental strains and F2 progenies of crosses involving susceptible and tolerant *Trichogramma chilonis* to different pesticides

Sl. No.	Treatment		N	LC_{50} (ppm)	Slope ± SE	Heterogeneity	Resistance Factor	Degree of dominance
	I. Endosulfan							
	Parental strains	Crosses						
1	Susceptible	–	400	106.03	2.4448 ± 0.3336	39.34*		
2	Tolerant	–	400	278.03	2.2706 ± 0.1610	17.98*	2.6	
3		Endosulfan tolerant male X	400	166.49	1.9859 ± 0.2129	36.53*	1.5	–0.063 – incomplete
4		Susceptible female						recessiveness
		Endosulfan tolerant female X Susceptible male	400	189.41	2.0322 ± 0.2108	35.59*	1.7	0.2 – semi dominant
	II. Spinosad							
1	Susceptible	–	400	4.78	2.4240 ± 0.2536	23.88*		
2	Tolerant	–	400	9.84	2.1733 ± 0.2004	29.89*	2.0	
3		Spinosad tolerant male X Susceptible female	400	8.84	2.4542 ± 0.0815	4.17	1.8	0.7 – semi dominance
4		Spinosad tolerant female X Susceptible male	400	8.55	2.2129 ± 0.0768	7.34	1.7	0.6 – semi dominance
	III. Lamda Cyhalothrin							
1	Susceptible	–						
2	Tolerant	–						
3			200	3.45	2.1852 ± 0.1069	3.76		
4			200	6.23	2.7965 ± 0.3286	18.64*	1.8	
		Lamda Cyhalothrin tolerant male X Susceptible female	200	6.86	2.2578 ± 0.2025	10.52*	1.9	1.3 – Complete dominance
		Lamda Cyhalothrin tolerant female X Susceptible male	200	7.23	1.7729 ± 0.2426	27.64*	2.0	1.5 – Complete

* Significant at 5% level ($p < 0.05$); N = number of susceptible, tolerant parents and F2 generation adults of the crosses used for insecticide exposure.

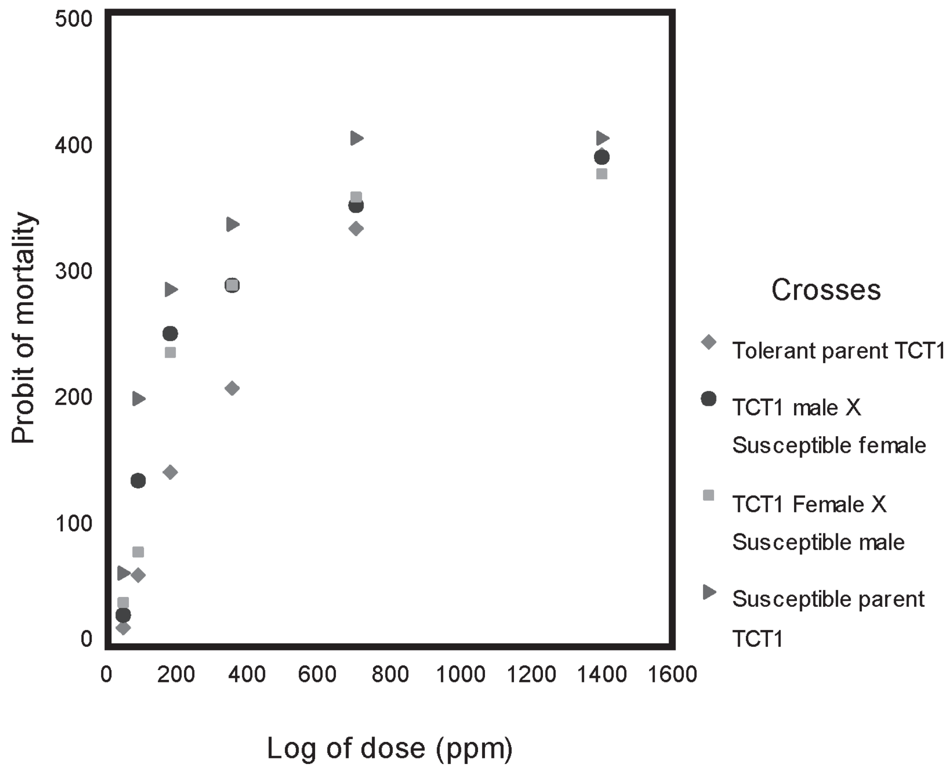

Fig. 1. Dose – mortality response of different concentration of endosulfan and the crosses

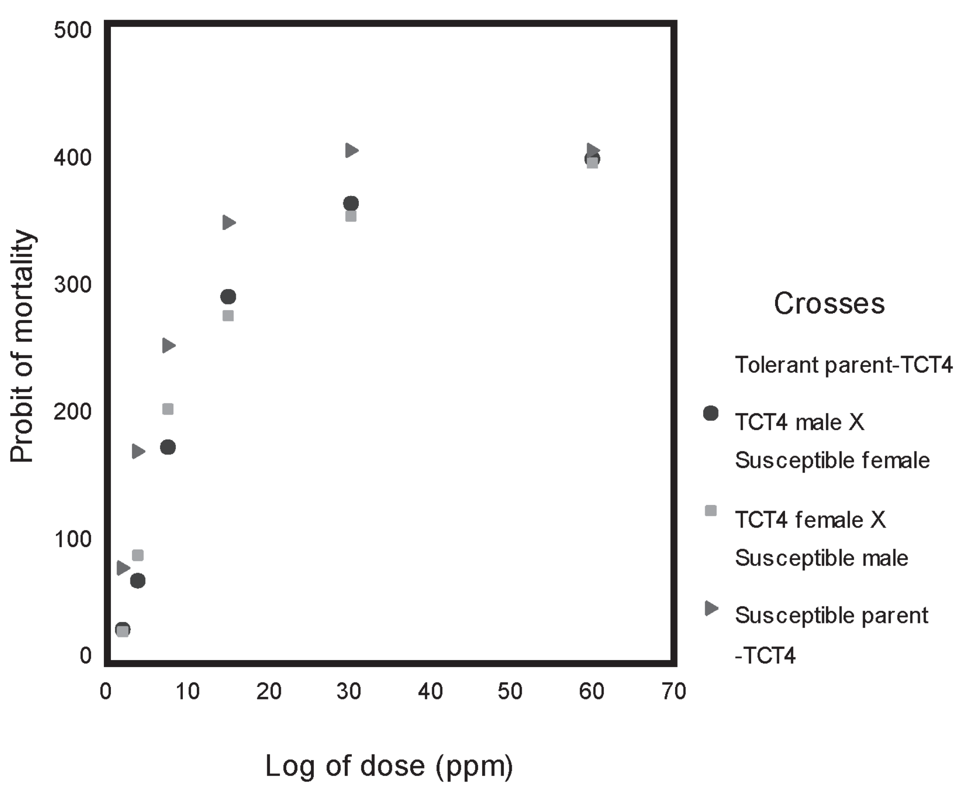

Fig. 2. Dose – mortality response of different concentration of spinosad and the crosses

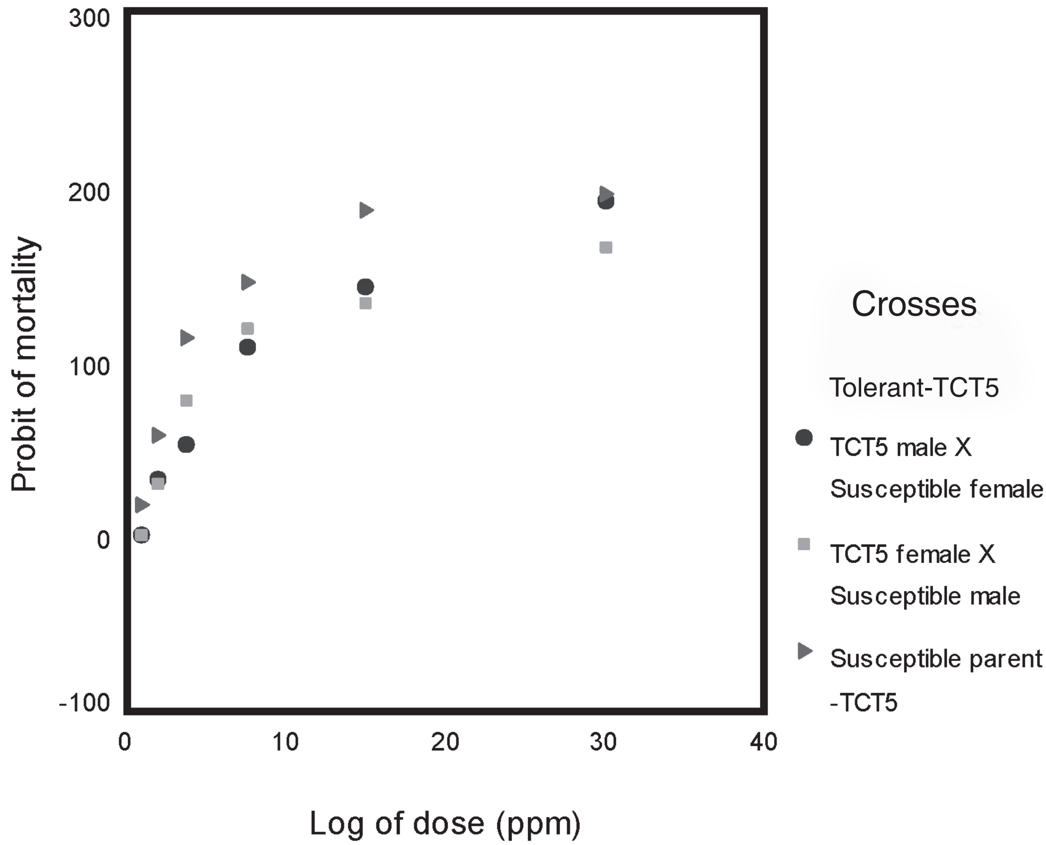

Fig. 3. Dose – mortality response of different concentration of lamda cyhalothrin and the crosses

and TcT4 female x susceptible male were 2.0, 1.8 and 1.7, respectively. Similarly, resistance factor for lamda cyhalothrin tolerant strain (TcT5), F2 progenies of the crosses TcT5 male x susceptible female and TcT5 female x susceptible male were 1.8, 1.9 and 2.0, respectively (Table 1).

The degree of dominance in endosulfan crosses showed –0.06 for TcT1 male, while that of the female it was 0.2. In case of spinosad resistant TcT4 crosses, the degree of dominance was 0.7 and 0.6 for male and female respectively, while for lamda cyhalothrin tolerant TcT5 crosses it was 1.3 and 1.5 for TcT5 male and female, respectively (Table 1).

The *T. chilonis* strains tolerant to endosulfan, spinosad and lamda cyhalothrin exhibited significantly higher LC_{50} values ($p<0.05$) compared to the respective susceptible strains (Table 1). These results are in agreement with the studies conducted by Devi *et al.*, (2006) with indoxycarb, tebufenozide and spinosad on *T. chilonis*. In the present study, endosulfan-treated cross the F2 progenies exhibited such LC_{50} values which are in between the values obtained for tolerant and susceptible parental strains. Similar observation was also made by Wirth *et al*. (2010) in *Culex quinquefasciatus* employing recombinant bacterial toxins. In contrast, spinosad-treated F2 crosses the LC_{50} values are found to be closer to the tolerant parent (Table 1). However, in lamda cyhalothrin treated cross it was higher than that of the tolerant parent strains. All the three crosses have shown significant dose mortality response ($p<0.05$) on the survival of *T. chilonis* adults in the present study.

The resistance factor for endosulfan-tolerant strain (TcT1), F2 progenies of the crosses involving tolerant male and females were increased 2.6, 1.5 and 1.7 folds respectively compared to the susceptible one. Similarly, in the spinosad tolerant strain (TcT4), F2 progenies of the crosses involving tolerant male and females were increased 2.0, 1.8 and 1.7 times respectively and in lamda cyhalothrin tolerant strain (TcT5) and its F2 progenies of the crosses involving tolerant male and females the resistance factors were increased by 1.8, 1.9 and 2.0 folds respectively in contrast to the susceptible strain. Such observations regarding the resistance factors were also made by Devi *et al*. (2006) on *T. chilonis* using different insecticides.

The data further reveals that endosulfan tolerance appeared to be recessive with the degree of dominance

(D) being –0.06 for the cross involved with the tolerant male parent and semi dominant for tolerant female parent with D value being 0.2 (Table 1). Further, the spinosad tolerance was found to be semi dominant for crosses involving both male and female tolerant parent with the D value being 0.7 and 0.6 respectively. However, the heterogeneity factors were non significant ($p>0.05$). In contrast, the lamda cyhalothrin tolerance was found to be completely dominant for crosses with the tolerant male and female parent with the D value being above 1. Thus, the results indicate that the mode of inheritance differed with the class of insecticide used in the tolerant strains of *T. chilonis*. Such observations may help in the establishment of stable and tolerant strains in the succeeding generations in the field which may be sprayed with different insecticides. Further, the degree of dominance values observed for spinosad presently was between 0 to 1 (Table 1), which signifies incomplete dominance by single gene as opined by Devi *et al.,* (2006). In the lamda cyhalothrin crosses it was found to be completely dominant by a single gene as opined by Wang *et al*. (2009) in the experiment on *Nilaparvata lugenstal*. A resistant strain with dominant allele may develop faster than the strain with recessive trait because, the resistant genotype might have better chance of surviving insecticide treatment, so that this trait will spread faster. Thus, the results obtained in the present study could be useful further to predict the impact of release of insecticide tolerant strains on the subsequent generations of pest species in the target fields.

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