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Inheritance of insecticides tolerance in resistant colonies of *Trichogramma chilonis* Ishii (Hymenoptera: Trichogrammatidae)

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Abstract

Resistant (R) strains of the egg parasitoid *Trichogramma chilonis* Ishii were developed against the three new insecticides *viz.*, indoxacarb, spinosad and tebufenozide through 40 cycles of selection in the laboratory selection pressures. The resistant factor observed was 8.7, 4.8 and 2.4 folds in resistant strain over susceptible strain of *T. chilonis* for indoxacarb, spinosad and tebufenozide, respectively. Mode of inheritance of insecticides tolerance studied using respective resistant 'R' and susceptible 'S' strains of *T. chilonis*. The degree of dominance (D) of indoxacarb tolerance was incomplete recessive for tolerant females and semi dominant for tolerant males. For spinosad, dominance levels were semi dominant for resistant females and incomplete recessive for resistant males. For tebufenozide, resistance was governed by semi dominant gene for resistant females and incomplete recessive gene for resistant males. The backcrossing of F_1 progeny with tolerant parent indicated that strong resistance gene was coded by the coordination of genes already present in indoxacarb male genotype, for both spinosad and tebufenozide R female.

Key words: Genetics of insecticide tolerance, indoxacarb, spinosad, tebufenozide resistance, *Trichogramma chilonis* strain

Introduction

The egg parasitoids of the genus *Trichogramma* are most widely used for biological control of lepidopterous pests in more than 30 countries covering a total area of 3.2 million ha [1]. In India, trichogrammatids are used widely for suppression of insect pests on sugarcane, rice, cotton, vegetables crops, maize etc. [2]. New insecticides *viz.*, indoxacarb (oxadiazine group), spinosad (derived from bacteria - *Sacharopolyspora spinosa*) and tebufenozide (insect growth regulator) are found to be highly promising against a wide range of insects and chronic dosages have a chemosterilizing effect by disrupting both oogenesis and spermatogenesis particularly in IGR-tebufenozide [3-5].

Selection of parasitoids for resistance to pesticides was recognized as a potent method for enhancing their performance. Since the resistant parasitoids released into field will breed with susceptible parasitoids, knowing the extent to which these mating will affect the resistant genotype of the resulting hybrid parasitoid could assist in the release and managing strategies of the resistant strain. Genetic improvement of natural enemies has produced pesticide resistant strains for at least 15 species of parasitoids and predators of insects and mites [6, 7] and has enhanced the efficacy of natural enemies. In our laboratory, resistance to newer insecticides *viz.*, indoxacarb, spinosad and tebufenozide in *Trichogramma chilonis* Ishii was developed through artificial selection and studies were conducted to analyse the mode of inheritance of resistance. The data would be particularly of interest to those who utilise these 'R' strain into the crop ecosystem.

Materials and methods

Strain selection and maintenance: The two strains of *T. chilonis* used for testing the mode of inheritance of pesticides have been selected and reared in the laboratory [8]. The culture of parasitoids maintained on *Corcyra cephalonica* Stainton eggs in the laboratory for the past 20 years was considered as susceptible strain ('S'-strain). The resistant strain ('R'-strain) was selected after exposing 'S' strain in the laboratory for tolerance to indoxacarb, spinosad and tebufenozide simultaneously from sub lethal to field recommended dosages on incremental increase basis. It took 40 generations of exposure to develop 'R' strain. The initial dose was determined based on LC_{50} value for each insecticide. Both strains were maintained at 26 ± 1°C and 65 ± 5 % relative humidity.

Ensuring homozygosity of parental strains: A program of interbreeding and selection was done to minimise heterozygosity at all resistance loci before commencement of genetic analysis. To facilitate crosses and selection, the parasitised eggs of *C. cephalonica* by 'S'-strain and 'R'-strain were sexed after emergence at the adult stage. Each parasitised egg was kept individually in the glass vial (5 × 2cm) for emergence. After emergence 'S'-strain and 'R'-strain were used for the study.

The insecticide solution was prepared in serial dilution by taking dosages double than recommended dosages and reducing dilution by $^1/_2$. For indoxacarb concentrations tested were 145, 72.5, 36.25, 18.125, 9.062, 4.531, 2.265 ppm and water spray, for spinosad concentrations tested were 450, 225, 112.5, 56.25, 28.125, 14.062, 7.03 ppm and water spray and for tebufenozide 280, 140, 70, 35, 17.5, 8.75, 4.375 and water spray. Modified glass vial (20 × 2 cm) with both sides open was used as a testing unit. Required insecticides were sprayed with the help of atomizer. The sprayed glass tubes were dried in the shade. After drying, one end of vial was covered with double-layered black cloth and was fastened with rubber bands. From other side about 100 adult parasitoids were released in each vial. Mortality was recorded after 6 hours of constant exposure. The data obtained on mortality were subjected to Probit analysis by statistical program SPSS version 8.0 to calculate LC_{50} and fiducial limits. Chi-square statistics were calculated to determine goodness-of-fit of the log dose/probit mortality regression lines and for likelihood test as per procedure laid by [9].

Mode of inheritance: 'R' and 'S' strains were crossed and F_1 progeny was back crossed to determine mode of inheritance of resistance as illustrated in Table 1. The mortality recorded was as described above in both side open glass tubes. The data obtained on mortality were subjected to probit analysis (Statistical program SPSS version 8.0, SPSS Inc., Chicago, Illinois 60606, USA) and LC_{50}, fiducial limits, slope and χ^2 values were calculated. The degree of dominance (D) was estimated for the reciprocal F_1 crosses and for back crosses using the formula

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

where X_1 = logarithm of the LC_{50} of the resistant parent, X_2 = logarithm of the LCso of the heterozygous F_1 parent from the cross R × S and X_3 = logarithm of the LC_{50} of the susceptible parent [10]. A D value of 1 indicates complete dominance, a D of –1 indicates complete recessiveness and a dominance value of 0 to +1 indicates semi dominant level of resistance. The resistance factor was calculated by dividing LC_{50} of tolerant parent by LC_{50} of susceptible parent.

The null hypothesis of monogenic resistance was tested using two methods. The number of insects responding from the back crosses F_1 × R parents were subjected to modified chi-square analyses with the binomial variance increased, where ever appropriate, by a heterogeneity factor [11]. The heterogeneity factor was determined as the weighted mean of the individual heterogeneity factors from probit analysis of data from two strains. In addition, the data at each dosage were separately analysed using standard 2 × 2 contingency analyses [12].

Results and discussion

The resistant strain of indoxacarb (IR), spinosad (SR) and tebufenozide (TR) had significantly higher LC_{50} values at 378.45 ppm, 657 ppm and 1792 ppm, respectively for the target insecticides as compared with these lot, the unselected susceptible colony LC_{50} values were 43.5, 139.5 and 756.0 ppm, respectively (Table 2). The reciprocal F_1 crosses (S female × RC male and R female × S male) of resistant population produced intermediate LC_{50} values, which were always closer to those for the resistant colony as compared to values for susceptible colony. All the three insecticides had a significant dose-mortality response on the survival of *T. chilonis* adults. The higher the concentration, the lower the survival (Fig. 1). This response slope was significantly less in the 'R' colony than in the 'S' colony. The mortality in 'R' colony for indoxacarb, spinosad and tebufenozide was less than 10.0%, 20.0% and 10.0% respectively at all the concentrations tested. The mortality in 'S' colony always reached more than 50.0% as concentration increased to 50.0% of field recommended concentration in indoxacarb and spinosad. In all reciprocal F_1 crosses and F_1 back crosses, mortality response line was close to R colony mortality line for three insecticides (Fig. 1, Table 2 and 3).

The resistant factor for IR, SR and TR strain was 8.7, 4.7 and 2.4 respectively over 'S' colony. The resistant factor for reciprocal F_1 crosses was 2.5 (IR female × S male) - 5.1 (S female × IR male), 1.9 (S female × SR male) - 3.3 (SR female × S male) and 1.1 (S female × TR male) - 1.7 (TR female × S male) for IR, SR and TR colonies. The responses of both

Table 1. Crosses and backcrosses of various populations of *Trichogramma chilonis*

Cross	Indoxacarb (I)		Spinosad (S)		Tebufenozide (T)	
	R (female)	R (male)	R (female)	R (male)	R(female)	R(male)
F_1	S (female) × IR (male)	IR (male) × S (female)	S (female) × SR (male)	SR (female) × S (male)	S (female) × TR (male)	TR (female) × S (male)
Backcross	F_1 (female) × PR (male)	PR (female) × F_1 (male)	F_1 (female) × PR (male)	PR (female) × F_1 (male)	F_1 (female) × PR (male)	PR (female) × F_1 (male)

S = Susceptible, IR = Indoxacarb resistant, SR = Spinosad resistant strain, TR = Tebufenozide resistant strain, PR = Resistant parent

the R and S colonies were significantly different as were the reciprocal crosses (Table 3). A likelihood ratio test on the reciprocal F_1 crosses indicated good fit with IR male at 3:1 (χ^2 = 2.57), whereas in IR female it was good fit at 1:1 (χ^2 = 5.28) of resistant: susceptible at dose of 145 ppm. In all backcrosses with IR colony, good fit was achieved at 3: 1 (χ^2 for F_1 female × IR male = 6.88, for IR female × F_1 male = 2.55). The resistance therefore appears from paternal effect in indoxacarb. In contrast in SR, good fit in reciprocal crosses was from paternal side 1:1 (χ^2 = 1.60) and 3:1 (χ^2 = 0.6) from maternal side of resistant: susceptible. In all backcrosses, good fit was achieved 3:1 (χ^2 for F_1 female × SR male = 0.78, for SR female × F_1 male = 0.16) ratio of resistant: susceptible at dose of 450 ppm. However in results for TR were not consistent in a likelihood ratio test. In reciprocal crosses good fit with TR male at 1:1 (χ^2 = 1.38), whereas in TR female it was good fit at 3:1 (χ^2 = 2.28) of resistant: susceptible at dose of 280 ppm. However, in all backcrosses, good fit was achieved 1:1 ratio of resistant: susceptible at dose of 280 ppm. The results indicate that resistant source arising out of paternal effect in IR colony and from maternal effect for SR and TR colonies.

The indoxacarb resistance appeared to be incompletely dominant with the degree of dominance (D) equal to 0.51 for R male (S female × IR male) and incompletely recessive for R female (IR female × S male) with D equal to –0.24. The degree of dominance of spinosad resistance was incompletely recessive for SR male (S female × SR male) with D equal to –0.16 and incomplete dominant for SR female (SR female × S male) with D of +0.55. Similarly for tebufenozide resistant colony, D of crosses with TR male was –0.73 and with TR female, it was +0.19. The results indicated that mode of inheritance differed with the resistant colony to particular insecticide. The indoxacarb resistance was incomplete dominant from paternal side, whereas for spinosad and tebufenozide resistance was incomplete dominant from maternal side.

The backcrossing of heterozygous F_1 population with resistant parent population showed greater inheritance of resistance. The degree of dominance value for all three insecticides resistant colony was greater as compared to values for reciprocal F_1 progeny. The observed dominance in resistance to three insecticides in the selected strain of *T. chilonis* will help in the establishment and stability of the selected strain in the field. In general, the lower the number of

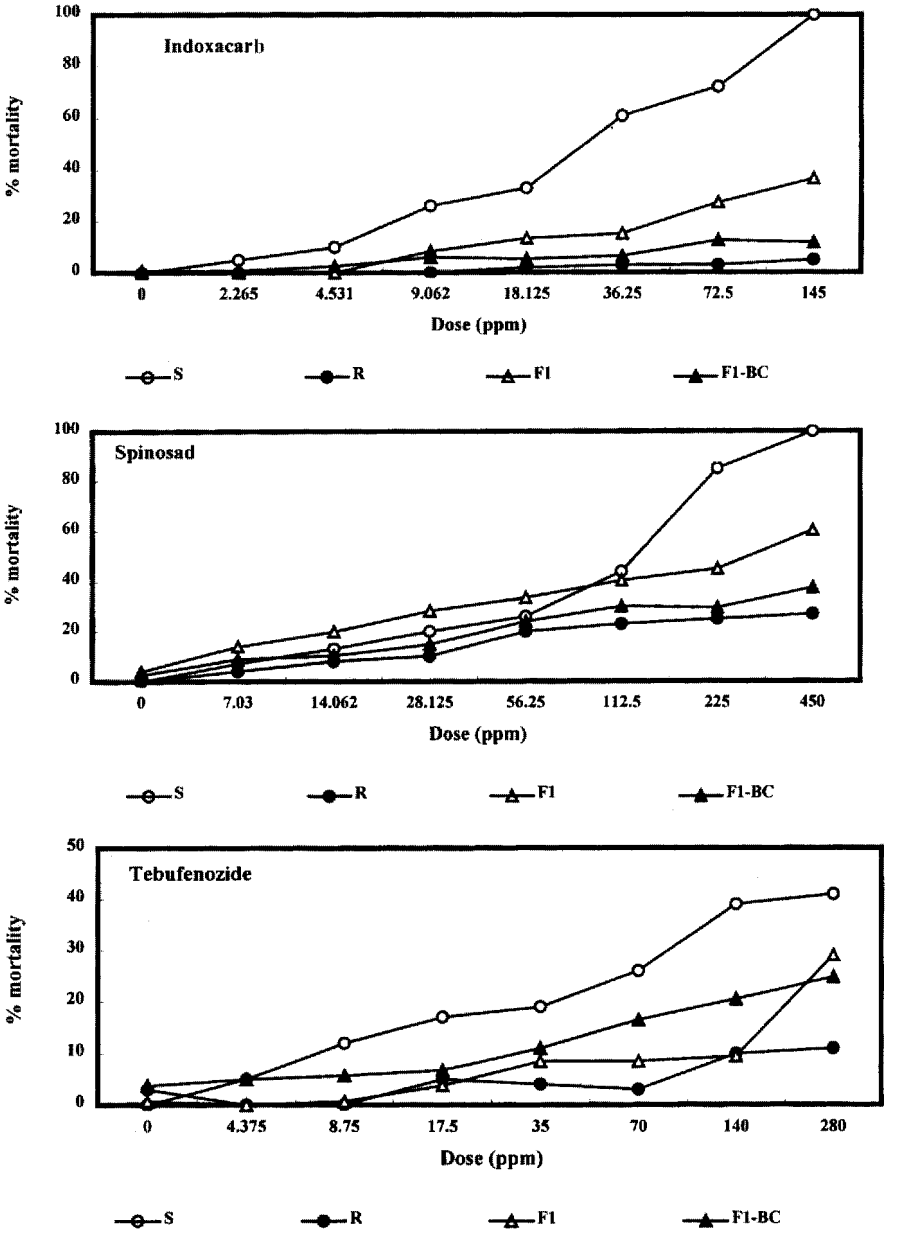

Fig. 1. Mean mortality of *Trichogramma chilonis* to three insecticides (susceptible colony (S), resistant colony (R), F_1 reciprocal cross (F_1) and F_1 backcrosses (F_1-BC) (mortality lines are pooled means of crosses in each category)

Table 2. Response of *Trichogramma chilonis* resistant and susceptible strains and F_1 progeny of their reciprocal crosses to test insecticides

Strain/cross	N	Slope ± SE	LC_{50} (95%FL) (ppm)	Resistant factor LC_{50} R/LC_{50} S	df	χ^2
S - Strain to						
Indoxacarb	800	3.13 ± 0.20	43.5	-	6	34.97***
Spinosad	800	5.03 ± 0.35	139.5	-	6	19.17**
Tebufenozide	800	0.03 ± 0.005	756.0	-	6	54.09***
R - Strain to						
Indoxacarb (IR)	800	0.95 ± 0.28	378.45 (259.55-830.85)	8.70	6	7.52 NS
Spinosad (SR)	800	0.91 ± 0.15	657.00 (382.5-5976.0)	4.70	6	33.40***
Tebufenozide (TR)	800	0.03 ± 0.0001	1792.00 (1111.6-7411.6)	2.37	6	11.05*
S (female) × IR (male)	800	1.75 ± 0.29	221.85 (175.45-387.15)	5.10	6	15.07*
IR (female) × S (male)	800	1.97 ± 0.18	108.75 (69.60-269.70)	2.50	6	65.05***
S (female) × SR (male)	800	2.10 ± 0.21	265.50 (130.5-1710.0)	1.90	6	99.00***
SR (female) × S (male)	800	1.65 ± 0.18	459.00 (337.5-1129.5)	3.29	6	33.92***
S (female) × TR (male)	800	0.06 ± 0.0006	848.40 (464.8-1083.6)	1.12	6	55.85***
TR (female) × S (male)	800	0.06 ± 0.0009	1254.40 (924.0-1870.4)	1.66	6	19.40**

*,**Significant at P = 0.05, 0.05 and P = 0.0001 respectively, NS = Non significant

Table 3. Response of backcrosses of *Trichogramma chilonis* to the test insecticides

Strain	Back cross	F_1 cross source	Slope ± SE	LC_{50} (95%FL)(ppm)	df	χ^2
Indoxacarb	F_1(female) × IR(male)	R(female) × S(male)	1.38 ± 0.28	232.3 (171.1-610.45)	6	26.49***
	F_1(female) × IR(male)	S(female) × R(male)	1.23 ± 0.26	290.0 (185.6-1464.5)	6	15.85**
	IR(female) × F_1(male)	S(female) × R(male)	0.73 ± 0.19	333.5 (198.65-945.4)	6	44.00***
	IR(female) × F_1(male)	R(female) × S(male)	0.98 ± 0.26	374.1 (191.4-703.25)	6	34.56**
Spinosad	F_1(female) × SR(male)	R(female) × S(male)	0.82 ± 0.15	679.5 (252.0-7740.0)	6	56.10***
	F_1(female) × SR(male)	S(female) × R(male)	1.61 ± 0.15	360.0 (211.5-1615.5)	6	81.69***
	SR(female) × F_1(male)	R(female) × S(male)	0.62 ± 0.15	769.5 (414.0-5053.5)	6	27.66***
	SR(female) × F_1(male)	S(female) × R(male)	1.18 ± 0.14	517.5 (297.0-4635.0)	6	55.30***
Tebufenozide	F_1(female) × TR(male)	R(female) × S(male)	0.002 ± 0.0005	1414.0 (907.2-4144.0)	6	12.83*
	F_1(female) × TR(male)	S(female) × R(male)	0.003 ± 0.0006	1338.4 (781.2-2514.4)	6	16.83*
	TR(female) × F_1(male)	R(female) × S(male)	0.006 ± 0.0009	1290.8 (963.2-2340.8)	6	11.21NS
	TR(female) × F_1(male)	S(female) × R(male)	0.007 ± 0.0001	1489.6 (873.6-2197.2)	6	19.23*

*,**Significant at P = 0.05, 0.01 and 0.0001 respectively, NS = Non significant

genes involved and the higher their dominance in backcross, the better is the chances that the resistance will spread and become established.

The single gene conferring incomplete dominance was further confirmed by analysing F_1-BC data using a modified chi square test and a contingency analysis (Table 4). The chi square test was modified to account for the heterogeneous responses of the 'S' and 'R' colonies. The experimental design ensured that the contingency analysis could be conducted using data on the 'S' colony, 'R' colony, F_1 and backcrosses for each particular dosage to that the probability of type one error is reduced [14]. Differences between observed and expected responses of F_1-BC were significant at all dosages in indoxacarb resistant colony and in tebufenozide resistant colonies (Table 4). This type of significant response at all the dosages gives indication of the involvement of single gene [12]. Further, degree of dominance value also was between 0 to 1, which also signifies incompletely dominance by a single gene. The results indicated that resistance in *T. chilonis* to three newer insecticides was conferred by one single incomplete dominant gene with greater dominance from maternal side. These results could be useful in knowing fate of releases of resistant strain in the field and their crossing with susceptible colony.

It has been argued that laboratory selection for insecticide resistance will likely result in a polygenic mode of inheritance, because selection results in small, incremental increases in pesticide resistance over time [13]. However in the present study involvement of large population ≥ 8000 used for selection of resistance did not rule out possibility that a single gene may determine resistance. Difficulty in distinguishing between single and polygenic mode of inheritance data is not uncommon [11, 12]. However, a modified χ^2 test can be used to determine for conclusion of exact mode of inheritance when extra variation is present [11].

In sulfur resistant strain of *Metaseiulus occidentalis*, F_1 progeny with 'R' mothers had greater resistance than those with 'S' mothers [14]. In earlier

Table 4. Chi square and contingency analysis of the response to the test insecticides of the F_1 back cross progeny from the parental resistant strain and susceptible strain

Dose (ppm)	No. tested	Mortality observed	Chi square analysis		Contegency analysis	
			Mortality expected	Modified χ^2	Mortality expected	χ^2
Indoxacarb						
2.285	1000	7	29.98	17.61**	70	7.04*
4.531	1000	20	30.58	3.66*	20	20.4**
9.062	1000	48	31.80	8.25**	48	50.42**
18.125	1000	45	34.46	3.22*	45	10.26**
36.25	1000	53	40.97	3.53*	53	36.74**
72.5	1000	111	59.81	43.81**	111	5.4*
145.0	1000	109	138.22	8.47**	109	0.28
Spinosad						
7.03	800	67	106.32	14.54**	67	2.39
14.062	800	85	108.90	5.24*	85	2.65
28.125	800	129	114.05	1.95	129	16.37**
56.25	800	195	124.94	39.27**	195	0.75
112.5	800	242	149.31	57.53**	242	0.001
225.0	800	234	207.33	3.43**	234	0.07
450.0	800	300	351.08	7.43**	300	0.14
Tebufenozide						
4.375	400	20	29.39	3.00	20	21.05**
8.750	400	23	30.11	1.67	23	24.40**
17.50	400	27	31.59	0.66	27	28.95**
35.0	400	44	34.75	2.45	44	36.87**
70.0	400	66	41.94	13.79*	66	30.00**
140.0	400	82	60.45	7.67**	82	24.52**
280.0	400	99	116.66	2.67	99	18.37**

work also degree of dominance between 0 to +1 were reported to be incompletely dominant [10]. Further, it was shown by back crossing F_1 progeny with resistant parent that D value increased and the likelihood test ratio. This result was similar to observations have been reported for powdery mildew resistance breeding in pea [15]. The genetic basis of pesticide resistance in any natural enemy may be immaterial when releases are made into areas devoid of the natural enemy, but it can be critical if releases are to be made into an area containing susceptible populations [16]. As far as field releases are concerned, females of the 'R' strain presumably would have mated prior to release. If pesticides are applied before releasing the 'R' strain, then there may be substantial reduction in the native parasitoid population such that progeny of the released 'R' strain will have to mate amongst themselves. This will enable to maintain resistance in the field for substantial time. Despite the limitations of laboratory studies in predicting field results, the laboratory selected tolerant strain of *T. chilonis* to newer insecticides appears to be sufficiently resistant and field releases are justified.

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