



Inheritance of Monocrotophos Resistance in Egg Parasitoid *Trichogramma chilonis* (Ishii) (Hymenoptera:Trichogrammatidae)

ONDARA ZABLON KERIMA¹, PATHAPPA NIRANJANA^{1*}, YADAVALLI LALITHA², VINAY KUMAR B.S¹, SUSHIL K. JALALI² and CHANDISH R. BALLAL²

¹Department of PG studies and Research in Biochemistry, Kuvempu University, Jnana Sahyadri, Shankaraghatta, Karnataka –577,451, India.

²National Bureau of Agricultural Insect Resources (ICAR-NBAIR), Post Bag No. 2491, H. A. Farm Post, Bellary Road, Bangalore -560,024, India.

Abstract

Inheritance of monocrotophos resistance was studied in the laboratory population of *T. chilonis*. After 10 cycles of selection pressure under laboratory condition, the resistant strain acquired LC₅₀ value of 0.346 μ L compared to LC₅₀ value of 0.114 μ L in the susceptible strain. Evidence from bioassay of F1 reciprocal hybrid crosses backcrossed with respective resistant 'R' and susceptible 'S' parental strains of *T. chilonis*, aimed to determine mode of inheritance of insecticide tolerance indicated that F1 crosses exhibited a complete dominant response to monocrotophos, with degree of dominance value (D) of 1.62. The resistance factor (Rf) of resistant strain was 3.04 folds and of F1 crosses were 3.675 folds over susceptible strain. Result of this study suggests resistance to the insecticide was probably controlled by a single gene. These results provide the basic information for designing successful management programmes for the control of Lepidopteran pests using resistant strain as a component of IPM.



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Introduction

Egg parasitoids of the genus *Trichogramma* sp. are important natural enemies that have been successfully used in biological control programs for the management of Lepidopteran pests. These pests infest corn, rice, sugarcane, cotton, vegetables, sugar

beets and fruit trees^{22,16}. Approximately 210 species of *Trichogramma* have been identified, among which 30 are documented in India. However, only 12 have been utilized for pest management practices²². Among the *Trichogramma* species, *T. chilonis* is the widely used species in several countries and

CONTACT Pathappa Niranjana ✉ bpniru@gmail.com 📍 Department of PG studies and Research in Biochemistry, Kuvempu University, Jnana Sahyadri, Shankaraghatta, Karnataka, India–577,451, India.

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agro ecosystem, where Lepidopteran pests occur naturally. These parasitoids attack the egg stage of their hosts, preventing them from reaching the larval stage, responsible for crop damage⁶. As a result, they have proved to be promising candidate in integrated pest management (IPM) approaches worldwide.

However, a huge impediment to effective integrated pest management practices is the frequent incompatibility of biological and chemical control measures. Although some classes of pesticide are generally designed to be selectively harmful against one or a few target pest populations, others with broad activity spectra negatively influence other pests and beneficial species, including egg parasitoids. This interference of biological control agents by indiscriminate use of insecticides causes resurgence of more resistant target pests and outbreaks of secondary pests. Intensive toxicological studies have showed undesirable effects of pesticide applications on Trichogrammatids such as reduced parasitism in *T. exigum* Pinto and *T. pretiosum* Riley on *Heliothis zea* Boddie and *Manduca* sp. in pyrethroid treated fields⁵. Intoxication from insecticide application has also resulted in reduced emergence and longevity of the emerged Trichogrammatids^{15,11,27,9,26}.

Development of insecticide resistant natural enemies, which could be introduced in IPM practices, was attempted⁷. The resistant parasitoid released into the field would mate with susceptible parasitoid, and, over subsequent generations resulting to a larger proportion of resistant hybrid that could assist in the release and management strategies of the resistant strain in the field. Subsequently, natural enemies with a proven potential to resist insecticides tend to hinder the manifestation of resistance in pest population, over time¹². By genetic enhancement, about 15 species of parasitoids and predators of insects and mites that are resistant to insecticide applications in the field have been established, with the most classical case being the strains of predatory phytoseiid mites^{3,28}. Employing laboratory selection, azinphosmethyl resistance levels was increased 7.5 fold in the aphid parasitoid, *Trioxys pallidus* Haliday¹² and endosulfan resistance was 15.1 fold in the *T. chilonis*¹⁴. Some natural enemy populations have demonstrated appreciable levels of resistance

to insecticides in the field and can survive field at application rates^{20,2}.

Since resistance is as a result of altered genomic changes in natural populations, a full discernment of the evolution of this phenomenon requires an accurate knowledge of its genetic basis²¹ thus leading to a better estimation of resistance risk in populations. In this study, selection responses of the adult stage of the parasitoid *T. chilonis* Ishii to monocrotophos, using *Corcyra cephalonica*, a major laboratory host, was investigated. The aim this study was to assess the level of tolerance of *T. chilonis* for insecticide application and determine the inheritance of monocrotophos resistance to understand the potential use of *T. chilonis* as a bio-control agent.

Materials and Methods

Rearing of insect

A parasitized card (2×2 cm) of resistant "R" and susceptible "S" eggs of *T. chilonis* was obtained from the National Bureau of Agricultural Insect Resources (NBAIR), Bangalore. The "S" culture parasitoid was maintained in the laboratory for the past five years without exposure to insecticide and the "R" culture parasitoid was collected from the field and selected with (monocrotophos) pesticide in the laboratory for 10 generations. After emergence, the parasitoids were reared and maintained in the laboratory on *Corcyra cephalonica* (Staiton) eggs (killed by ultraviolet radiation) at 26±1 °C, RH 60± 5%, LD 14: 10.

Insecticide

monocrotophos (Milphos 36% EC, Excel Industries, India), an organophosphate insecticide, was used for determining the lethal concentration values (LC₅₀) at varying concentrations of insecticide at five serial dilutions (1/2 dilution). The control treatments were done using water.

Ensuring Homozygosity of Parental Strains

Interbreeding and selection was done to minimize heterozygosity at all resistance loci, before genetic analysis studies were undertaken. To facilitate crosses and selection, the parasitized eggs of *C. cephalonica* 'S'-strain and 'R'-strain were allowed to mate freely after emergence of the adult stage. All parasitized eggs were gently dislodged from

the card and each egg was confined in a glass vial (one egg per vial) for emergence. Following emergence, the adults were mass produced by providing them with *C. cephalonica* eggs. This was done in order to raise enough progeny for treatment with monocrotophos. The insecticide solution was prepared, reducing by ½ starting from the dosage of 1.4µL, in five serial dilutions. monocrotophos was uniformly applied on the inner sides of the cylindrical glass tube (20 × 4 cm) using an atomizer. The tubes were then kept to dry in a shade after which, one end of the dried glass tube was tightly closed with double-layered long black cloth. About 200 adults were released in each glass tube. The mortality was noted after 6 and 24 hours of constant exposure.

The resistant factor was determined by dividing LC₅₀ value of the resistant strain by that of susceptible strain.

Genetic Analysis of Resistant Strain

T. chilonis adults were sorted out based on their sexes. Males and females of both "R" and "S" strains were allowed to cross freely in order to produce hybrid females. The resultant females were provided with *C. cephalonica* eggs to obtain sufficient progeny to be subjected to monocrotophos. The F1 inter-

strain (R♀ × S♂ and R♂ × S♀) reciprocal crosses (Table 1) were exposed to monocrotophos at five serial dilutions (1/2 dilution), with 1.4µL being the highest concentration and untreated control to work out LC₅₀ values. To determine the degree of dominance (D) of the resistant factor, the formula of²³ was employed.

$$D = \frac{2\theta_3 - \theta_2 - \theta_1}{\theta_2 - \theta_1}$$

In which, logθ₁ (LC₅₀) of the susceptible strain, θ₂ = log 10 (LC₅₀) of the resistant strain and θ₃ = log 10 (LC₅₀) of the heterozygous cross 1 (R♀ × S♂). A D value of >1 indicates complete dominance, D value between 0 to < 1 represents incomplete dominance, D value of -1 < shows complete recessive and D value of -1 to 0 is incomplete recessive. To work out the resistance factor, the LC₅₀ values of tolerant strain and F1 cross were divided with that of the susceptible strain.

Mode of inheritance of resistance (Back crosses)

"R" and "S" parental strains were backcrossed with the F1 hybrid to determine mode of inheritance as depicted in Table 1. *C. cephalonica* eggs were

Table 1: F1 crosses and backcrosses of various *Trichogramma chilonis* population

Strain	Monocrotophos
F1 reciprocal crosses	Cross 1 - R♀ × S♂ Cross 2 - R♂ × S♀
Backcrosses	F1 cross 1♀ × RP♂ F1 cross 2♀ × RP♂ F1 cross 1♀ × SP♂ F1 cross 2♀ × SP♂ F1 cross 1♂ × SP♀ F1 cross 2♂ × SP♀ F1 cross 1♂ × RP♀ F1 cross 2♂ × RP♀

provided to the crosses for multiplication to get sufficient progeny, which upon emergence, were exposed to monocrotophos at five serial dilutions (1/2 dilution) from 1.4µL to work out LC₅₀ values. The mortality was recorded after 6 and 24 hours of constant exposure.

Statistical Analyses

All experiments including the LC₅₀ values of the tolerant and susceptible strains, and genetic level of tolerance were repeated three times and performed in triplicate. The data obtained on mortality were subjected to probit analysis, employing a statistical

program SPSS version 15. The data were transformed to log base 10 before probit analysis and antilog of calculated values gave actual LC_{50} and LC_{90} . The fiducial limits, slope, chi-square (X^2) and regression equation were also computed.

Results

Following repeated laboratory selection episodes of *T. chilonis* with monocrotophos, inheritance of insecticide resistance was studied in the bioassay experiment with sequential decrease in dose of

insecticide (1.4 μ L to 0.0875 μ L). The LC_{50} value for resistant strain was 0.346 μ L, while 0.114 μ L was reported for the susceptible strain and the reciprocal F1 crosses (cross 1 R ♀ \times S ♂ and cross 2 R ♂ \times S ♀) produced LC_{50} values of 0.419 μ L and 0.534 μ L, respectively, which were more as compared to values for resistant and susceptible colonies (Table 2). However, of the two F1 reciprocal crosses, (cross 1 R ♀ \times S ♂), was used for the calculation of resistance factor.

Table 2: Toxicity of monocrotophos to the susceptible, resistant and F1 reciprocal hybrid of *T. chilonis*

Strain	LC_{50} (μ L)	95%CL		Slope \pm SE	df	X^2	Regression
		Lower	Upper				
"S"	0.114	0.095	0.132	3.400 \pm 0.246	13	40.23	y = -3.203+ 3.400x
"R"	0.346	0.218	0.545	2.189 \pm 0.106	13	247.98	y = -1.009+ 2.189x
F1	0.419	0.357	0.491	2.944 \pm 0.107	13	84.986	y = -1.111+ 2.944x

The monocrotophos resistance appeared to be complete dominant with degree of dominance (D) value of 1.629. This was verified with the results from LC_{50} values of F-1 reciprocal crosses, which were comparatively greater than the LC_{50} values of resistant and susceptible parents. Thus, it could be inferred from non-overlap fiducial limit test that there was a notable difference between susceptible and resistant and between susceptible and F-1 crosses. The resistance factor (Rf) of resistant strain and F 1 crosses was represented by 3.04 and 3.675 folds, respectively, over susceptible strain.

Genetic analyses of resistant strain which involved backcrossing the F1 hybrids from cross 1 and cross 2 to their homozygous parents yielded a progeny, whose LC_{50} values indicated increase in percentage of tolerance with increased adult survival than individual resistant strain. This was confirmed from the LC_{50} values of backcrosses, which were greater than the LC_{50} values of resistant, susceptible and reciprocal F1 progeny as depicted in (Table 3 and Table 4).

Table 3: Toxicity of monocrotophos to the resistant strain with backcrosses of *T. chilonis*

Strain	LC_{50} (μ L)	95% CL		Slope \pm SE	df	X^2	Regression
		Lower	Upper				
Cross 1							
F1 ♀ \times RP ♂	0.785	0.624	1.037	2.734 \pm 0.134	13	114.89	y = - 0.288+2.734x
F1 ♂ \times RP ♀	0.684	0.593	0.803	2.297 \pm 0.018	13	39.289	y = - 0.379+2.297x
Cross 2							
F1 ♀ \times RP ♂	0.920	0.765	1.161	1.749 \pm 0.098	13	34.723	y = - 0.063+1.749x
F1 ♂ \times RP ♀	0.491	0.397	0.619	1.869 \pm 0.090	13	70.677	y = - 0.577+1.869x

Table 4: Toxicity of monocrotophos to the susceptible strain with backcrosses of *T. chilonis*

Strain	LC ₅₀ (μ L)	95% CL		Slope \pm SE	df	X ²	Regression
		Upper	Lower				
Cross 1							
F1♀xRP♂	0.533	0.382	0.773	1.339 \pm 0.075	13	115.57	y = - 0.336+1.339x
F1♂xRP♀	0.616	0.488	0.806	2.295 \pm 0.100	13	118.012	y = - 0.483+2.295x
Cross 2							
F1♀xRP♂	0.711	0.576	0.915	1.944 \pm 0.088	13	88.328	y = - 0.288+1.944x
F1♂xRP♀	0.522	0.418	0.672	2.211 \pm 0.108	13	74.729	y = - 0.624+2.211x

"S" – Susceptible, "R" – Resistant, LC₅₀ -lethal concentration, Df- degree of freedom, χ^2 - Chi-square, SE- Standard error, CL- Confidence limits of concentration, RP – resistant parent, SP- susceptible parent.

Discussion

The selection of various beneficial insects for resistance to insecticides has been undertaken over the past years, with mixed results. In the resistant strain of *T. japonicum*, another species of Trichogramma, there was a remarkable increase in the LC₅₀ values in response to methamidophos (0.8892 ppm), fenvalerate (8.6511 ppm), and metaphos (0.0592 ppm) insecticides. However, treatment with mipcrin, showed a significant decrease in LC₅₀ (0.1103 ppm) when treated for 36-43 generations²⁹. A 15 -fold increase in *T. chilonis* tolerance to endosulfan after 341 laboratory selection episodes with LC₅₀ values for resistant, susceptible and F1 being 1074.96, 70.91 and 604.96, respectively, was reported¹⁴. In this investigation, *T. chilonis* tolerance to monocrotophos was found to have increased up to 3.04-folds after 10 cycles of selection with insecticide in the laboratory. Repeated laboratory selection of the parasitoid to the insecticide would have contributed to the development of resistance in some parasitoid, which survived due to their distinct genetic makeup. The offspring of these survivors carried the resistance genome, and increased in greater proportion with each subsequent generation of the population.

In *Amblyseius nicholsi* Ehara, resistance to phosmet was as a result of a single semi-dominant gene as reported in¹³. In a generalist pteromalid parasitoid, *Anisopteromalus calandrae*, malathion resistance was inherited as incompletely dominant trait controlled by a single gene¹. Genetic analyses

of backcrosses of endosulfan resistant strains of *T. chilonis* indicated the role of a single semi-dominant gene in inheritance of resistance¹⁴. In this study involving same species, mode of monocrotophos inheritance was complete dominance, which indicated that resistance varied with the resistant colony to particular insecticides. The other mode of inheritance, polygenic, was observed in the laboratory selected resistance to azinophosmethyl in the parasitoid *Trioxys pallidus* Haliday⁴ and predatory mite *Phytoseiulus persimilis* Athias-Heuriot¹⁰.

It has been widely suggested that laboratory selection programme for insecticide resistance will likely result in a polygenic pattern of inheritance due to small incremental increase in pesticide resistance in the selection process over time²¹. However, in the present study, the likelihood of a single gene involvement in the determination of resistance was not discounted. The selection coverage of susceptible population has been identified to determine the choice of a specific mode of resistance, either monogenic or polygenic. While selection within this population inclines towards polygenic resistance, selection outside the population, on the other hand, results in monogenic response that is characterized by single gene mutations²¹. Single gene controlled resistance develops and spreads rapidly when compared to polygenic resistance^{25, 21}. Over time, resistance quickly manifests to new areas through migration of the resistant insect as demonstrated in a study of the organophosphate resistance genes of *C. pipiens*^{19,24}.

This observed dominance in resistance to monocrotophos in the selected strain of *T. chilonis* will help in the establishment and stability of the selected strain in the field, where insecticide with the same mode of action is frequently sprayed to control pests. Thus, this strain can be profitably incorporated into IPM strategies as effective and complement to chemical insecticide. Although conversely, it is important to point out that this study was carried out under laboratory conditions where the parasitoid was subjected to a constant pesticide pressure, under field conditions pesticides might have their negative impact lightened because the biological control agents can benefit from natural shelters or avoid sprayed areas. Furthermore, sunlight degradation plays an important role in the field that also helps to decrease the impact of pesticides on the beneficial arthropods observed in laboratory⁸.

Conclusion

Inheritance of monocrotophos resistance in the laboratory selected strain of *T. chilonis* was studied.

While the resistant strain showed an LC₅₀ value of 0.346 μ L, the susceptible strain displayed an LC₅₀ of 0.114 μ L. Based on the genetic analysis, it was evident that the F1 cross exhibited a complete dominance response to monocrotophos, with degree of dominance (D) of 1.629. The resistant factor for resistant and F1 strains were 3.04 and 3.675 folds over susceptible strain, respectively. These results assert that the resistant strain can be compatible in IPM practices in various crops where insecticide use is higher.

Conflict of interest

No competing interests.

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