Development of endosulfan tolerant strain of an egg parasitoid *Trichogramma chilonis* Ishii (Hymenoptera: Trichogrammatidae)

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A strain of *T. chilonis*, an egg parasitoid of lepidopteran pests tolerant to the most commonly used cyclodiene insecticide – endosulfan was developed in the laboratory. Tolerance to endosulfan was induced by exposing adult parasitoids sequentially from a sub-lethal concentration (0.004%) to the field recommended concentration (0.09%). The strain acquired tolerance to the insecticide after 341 generation of continuous exposure with LC50 values of 1074.96 ppm as compared to LC50 of (70.91 ppm) in susceptible strain. The genetical study showed that F1 crosses exhibited a semi-dominant response to endosulfan with degree of dominance value (D) of 0.58. The resistant factor of tolerant strain was 15.1 folds and of F1 cross were 8.53 folds over susceptible strain. Under net house conditions, the tolerant strain parasitised 56% *Helicoverpa armigera* eggs on potted cotton plants immediately after an insecticide spray, compared to 3% by the susceptible strain. High percentage survival of the immature stages of the tolerant strain proved their ability to withstand the insecticide load. Breakdown of insecticide tolerance in the strain occurred after four generations in absence of insecticide load. Use of the tolerant strain as a component of bio-intensive IPM in various crops where insecticide use is higher is discussed.

**Keywords:** Artificial selection, Endosulfan, Resistance, *Trichogramma chilonis*

The indiscriminate use of insecticides to control crop pests is one of the main causes for secondary pest outbreaks in many crop ecosystems. Use of insecticide tolerant strains of natural enemies offers a way to prevent the development of resistance in the target pests and enhance the potential of the natural enemy in integrated pest management (IPM) programmes. Increase the tolerance by artificial selection in parasitoids of scale insects and predatory mites has been reported.

No insecticide tolerant strains of *Trichogramma* sp. has been reported, even though the parasitoid has been extensively used against several lepidopterous pests in different countries and cropping systems. Scattered information is available on tolerance of *Trichogramma japonicum* Ashmead in rice to fenvalerate and decamethrin. Among the trichogrammatids, *T. chilonis* is most widely used in IPM in India, China, Korea, Taiwan, Japan, Pakistan, Nepal, Reunion Island and as exotic species in Kenya, Spain, South Africa and Australia. The trichogrammatids are susceptible to a broad spectrum of insecticides and reduced parasitism has been reported in *T. exiguum* Pinto & Platner and *T. pretiosum* Riley on *Heliothis zea* Boddie and *Manduca* sp. in plots treated with pyrethroids. Similarly, the drift of insecticide even a mile away and even single application of insecticides in cotton reduced the efficacy of the trichogrammatids.

In India, about 32% of the total cultivated area is under rice, fruits, vegetables and cotton. Cotton crop receives application of more insecticides (55%) followed by paddy (18%) and fruits and vegetables (14%) of the total insecticide usage in India. Greater reliance on synthetic insecticides for the past 20-30 years had altered the ecological niche of bioagents and their potential. In India, natural parasitism of *Helicoverpa armigera* (Hübner) eggs by *Trichogramma chilonis* Ishii in sprayed cotton fields has been reported to range from 0.0-21.7% and parasitoids were recorded only in four out of nine cotton growing states. Considering these, the development of tolerant strain to insecticides thus will be a more apt approach in the management of insect pests of commercial crops like cotton, vegetables and fruits, where the onslaught of insect pests and also the consumption of insecticides are more.

Trichogrammatids are widely used for the suppression of serious borer pests in rice, cotton and...
vegetable crops, particularly against *Scirpophaga incertulas* Walker, *H. armigera*, *Plutella xylostella* Linnaeus etc\(^1\). However, these pests have developed resistance to major groups of insecticides. Many of the insecticides are extremely toxic to several bioagents and this has warranted the development of tolerant strain. For the first time, development of insecticide tolerant strain through artificial selection procedure of the more commonly utilised egg parasitoid *T. chilonis* to the widely used and recommended cyclodiene compound – endosulfan is reported. Commercial utilization of the tolerant strain in different crop ecosystems where insecticides are still used on a very large scale for the control of insect pests was aimed at after the development of the strain.

**Materials and Methods**

*Collection of material and screening*—Four natural populations of *T. chilonis* comprising about 15-20 adults from each location were collected from the parasitised eggs of *H. armigera* infesting cotton crop in different states of India and were reared together in the laboratory on *Corcyra cephalonica* (Stainton) eggs. Endosulfan (Thiodan 35% EC, Excel Industries, India) was used for developing the tolerant strain using an artificial selection procedure.

*Development of endosulfan tolerant strain*—Endosulfan was sprayed with an atomizer to obtain a uniform layer of spray droplets on the inside of the glass tubes (20 × 4 cm) with both ends open. After spraying the vials were dried in shade. One end of the sprayed and dried tube was closed with double-layered long black cloth of (320 µm pore size). About 500 *Trichogramma* adults were allowed to move into the sprayed tube through the open end and after 30 minutes, a card with fresh UV treated 1 cc eggs of *C. cephalonica* (1 cc = 15,000 eggs) was introduced on a card (14 × 2 cm) in the ratio of 50 eggs: 1 female for parasitisation. Adult mortality/survival after 6 hr of release was recorded. The parasitised card was removed from the sprayed tube and kept in a fresh tube for the development of the parasitoids. Per cent parasitism was recorded after five days of exposure. Eggs that turned black were considered parasitised and the number of eggs parasitised in 1 cm\(^2\) area (about 550 eggs) were marked to determine per cent parasitisation.

Initially, endosulfan was sprayed at four concentrations 0.009, 0.018, 0.035 and 0.09% in the tube and after drying the adults were released. As 90% mortality was observed within 1 hr of exposure, the dosage was reduced to 0.004% and successive increases in the concentration applied as 0.004% > 0.009% > 0.018% > 0.026% > 0.035% > 0.044% > 0.053% > 0.061% > 0.07% > 0.09%. At each concentration the set criterion for tolerance was 30 – 50% adult survival after 6 hr of exposure and 90% egg parasitisation were considered for shifting to the next concentration. Continuous laboratory insecticide selection episodes were given at the same concentration till the set parameters were achieved. After achieving tolerance to 0.09% concentration, the tolerant and susceptible strains were exposed to seven serial dilutions (1/2 dilution) of endosulfan starting from 0.09% and untreated control to determine level of tolerance using log dose probit analysis of SPSS programme.

*Genetic analysis of tolerant strain*—Genetic analysis for tolerance was studied by keeping individual parasitised eggs of tolerant (to 0.09% concentration) and susceptible strains for emergence. After emergence males and females of both strains were crossed to obtain hybrid females. Such females were exposed to *C. cephalonica* eggs to obtain sufficient progeny for exposure to endosulfan. Two hundred inter-strain crosses in F\(_1\) were exposed to endosulfan at seven serial dilutions (1/2 dilution) starting from 0.09%. The degree of dominance (D) of the resistant factor was calculated using the formula:

\[
D = \frac{2\theta_3 - \theta_2 - \theta_1}{\theta_2 - \theta_1}
\]

where \(\theta_1 = \log_{10} (LC_{50})\) of the susceptible strain, \(\theta_2 = \log_{10} (LC_{50})\) of the tolerant strain and \(\theta_3 = \log_{10} (LC_{50})\) of the cross \(R \times S\)\(^15\).

The resistant factor was calculated by dividing \(LC_{50}\) value of tolerant strain and \(F_1\) cross with \(LC_{50}\) value of susceptible strain.

*Comparative toxicity of endosulfan to immature stages of tolerant and susceptible strains*—*Corcyra* egg cards were exposed to the tolerant and susceptible strains of *T. chilonis* (@ 100 eggs/female for 24 hr). The parasitised cards of different ages (i.e., 1 day, 5 days and 7 days old) containing the developmental stages of the parasitoids – (egg, larvae and pupae
respectively) were sprayed with the field recommended dose of endosulfan (0.09%). Per cent mortality and adult emergence in the sprayed and unsprayed cards were recorded. Each treatment (stage of parasitoid development in tolerant and susceptible strains) was replicated 10 times at the rate of 10 cards/strain/age interval.

**Tolerance breakdown in the tolerant strain**—The adults of the tolerant strain of *T. chilonis* were exposed to the insecticide after withdrawing the laboratory insecticide selection episodes for 2, 4, 6, 8 and 10 generations. In each exposure, 100 pairs (both males and females) were taken and replicated 10 times. Percentage adult mortality (after 6 hr of exposure) and parasitism were recorded as before.

**Net house trial with tolerant and susceptible strains**—Potted cotton plants were arranged in the two net houses (each measuring 3 × 3 × 4 meters size). In each net house four rows of 4 pots each were maintained. Plants in both the net houses were sprayed at flowering stage with the field recommended dose of endosulfan (0.09%). The plants were inoculated with 12 hr old *H. armigera* eggs @10 eggs/plant after 0, 1, 2, 3, 4 and 5 days of spraying. The eggs were distributed uniformly on the top five leaves. Immediately after inoculating eggs, *T. chilonis* adults of tolerant and susceptible strains were released. After 24 hr, the eggs along with the leaf were collected from the plants and kept in tubes for further development. Per cent parasitism and adult emergence were recorded. The experiment at each treatment (day wise) was replicated 10 times.

**Statistical analysis**—The LC50 values of the tolerant and susceptible strains and genetic level of tolerance were calculated by subjecting the data to log dose probit analysis. The confidence limits, slopes, $\chi^2$ values and regression equation were computed. The comparative percentage toxicity of endosulfan on the immature stages of the tolerant and susceptible strains was statistically analysed by the one-way ANOVA after angular transformation of the data. The toxicity data of the net house trial on the tolerant and susceptible strains was analysed by two-way ANOVA.

**Results**

Development of tolerance to endosulfan was studied with sequential increase in the dose of insecticide (0.004-0.09%). At the lowest concentration (0.004%), the adult survivability was ≤ 10% and parasitism 50% in the initial generation. After continuous exposure for 13 generations survival of 95 and 100% parasitisation was obtained. The sequential increase in dosage resulted in development of a strain with tolerance to the field recommended dose (0.09%) after 341 laboratory insecticide selection episodes with 60% adult survival after 6 hr of exposure and 90% parasitisation (Table 1). Parasitoids took longer time to acquire tolerance at higher dosage. At lower concentrations (0.004-0.018%), parasitoids took < 12-13 generations to develop tolerance, while, at higher concentrations, it took 24 to 90 generations. Exposure of the parasitoids to higher concentration from the lower concentration remitted in low survival and less parasitisation in the subsequent generation. The gradual adaptation to the insecticide resulted in acquiring tolerance to the field recommended concentration. In the susceptible population, over 90% mortality and less than 10% parasitisation was obtained at each concentration compared to insecticide resistant strain of the parasitoid (40% mortality and 90% parasitisation) after successive generations of exposure to insecticide.

The LC$_{50}$ value of the tolerant strain was 1074.96 ppm, for susceptible strain 70.91 ppm and for F1 crosses 604.96 ppm. Genetic analysis of the tolerant strain with backcrosses indicated decrease in percentage of tolerance and adult survival compared to the parent tolerant strain (Table 2). The endosulfan tolerance appeared to be incomplete dominant with the degree of dominance ($D$) equal to 0.58. This supports the results from LC$_{50}$ values of parent and cross were nearer as compared to susceptible LC$_{50}$ value. Based on non-overlap method of fiducial limit test, there was significant difference between susceptible and tolerant strain and between susceptible strain and F1 crosses (Table 2). The resistant factor of tolerant strain was 15.1 folds and of F1 cross were 8.53 folds over susceptible strain.

To assess mortality in immature stages, both the tolerant and susceptible strains were exposed to endosulfan (0.09%). The mortality in the susceptible strain was significantly high in all stages (egg, larval and pupal) when compared to tolerant strain. The adult emergence was significantly less in susceptible strain as compared to tolerant strains. In both strains, the egg stage was more susceptible (50 and 80% mortality in the tolerant and susceptible strains,
respectively) compared to larval and pupal stages (Table 3).

Discontinuous exposure of the tolerant strain to endosulfan after a gap of 2, 4, 6, 8 and 10 generations revealed high mortality after four generations however, parasitism remained high (80.0%). Therefore, the tolerant parasitoids could be multiplied for four generations successfully and thereafter a part of culture has to be subjected successively to laboratory insecticide selection pressure to maintain field tolerance (Table 4).

In net house trials, the tolerant strain parasitised significantly more H. armigera eggs on cotton (56%) than the susceptible strain (3%) immediately after spraying i.e. 0 day after spraying. Mean per cent parasitism of H. armigera eggs (79.8%) and adult emergence (94.3%) (0-5 days after spraying) of the tolerant strain was high and significantly differed with
that of the susceptible strain (38.2 and 64.5% respectively). Adult emergence of tolerant strain was 95% even from 0-day old sprayed plants compared to no emergence from eggs parasitised by susceptible strain (Table 5).

**Discussion**

The selection of resistant population of beneficial organisms (*Trioxys pallidus* (Haliday), *Anisopteromalus calandrae* Howard, *Bracon hebetor* Say etc.) from insecticide treated fields, and their laboratory screening through insecticide selection pressure has been attempted by several workers\(^{16-18}\). Several fold increase in the LC\(_{50}\) values of resistant strains of *T. japonicum* to methamidophos, fenvalerate, and metaphos (0.8892, 8.6511 and 0.0592 ppm, respectively) and decrease in LC\(_{50}\) value of mipcrin (0.1103 ppm) when treated for 36-43 generations\(^{6}\) have been reported. In the present study there was a 15-fold increase in tolerance to endosulfan in *T. chilonis* after 341 laboratory insecticide selection episodes. It is imperative that continued laboratory insecticide selection pressure for several generations had invariably contributed to the physiological adaptation of the parasitoid and had altered the organisms genetic make up to survive and withstand higher doses of insecticide selection pressures.

Maintenance of tolerance is governed by the genetic make up of the organism and the physiological processes both in the host insect and the parasitoid. The nature of resistance and metabolism of the insecticide in the host/parasitoid and the type of parasitoid coupled with other ecological factors seem to play a decisive role in the maintenance or otherwise. Genetic analysis of backcrosses of methidathion resistant strain of *Phytoseiulus persimilis* Athias-Heuriet indicated polygenic inheritance of resistance with lower or higher resistance than parent strains, whereas 10 other substrains (selected during 7 generations) showed significant increase in the resistance\(^{19}\). A single semi-dominant gene controlling the development of phosmet resistance in *Amblyseius nicholsi* Ehara & Lee has been reported\(^{20}\). In the present studies, the inheritance of resistance to endosulfan could have been a single incomplete dominant gene. The indications of inheritance provide for genetic manipulations to have a more effective parasitoid.

The immature stages of the tolerant strain of *T. chilonis* showed significantly higher survival compared to the susceptible strain. The egg stage of the parasitoid was found to be most susceptible as compared to larval and pupal stages. In contrast more

<table>
<thead>
<tr>
<th>Generation gap</th>
<th>Adult mortality (%) (after 6 hr of exposure)</th>
<th>Parasitism (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>40.0</td>
<td>90.0</td>
</tr>
<tr>
<td>2</td>
<td>80.0</td>
<td>95.0</td>
</tr>
<tr>
<td>4</td>
<td>85.0</td>
<td>80.0</td>
</tr>
<tr>
<td>6</td>
<td>90.0</td>
<td>50.0</td>
</tr>
<tr>
<td>8</td>
<td>95.0</td>
<td>25.0</td>
</tr>
<tr>
<td>10</td>
<td>100.0</td>
<td>10.0</td>
</tr>
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</table>

**Table 5—Net house trials of tolerant and susceptible strains of *T. chilonis* on cotton against *H. armigera***

<table>
<thead>
<tr>
<th>Days after spraying</th>
<th>Parasitism by strains (%)</th>
<th>Mean (B) Days</th>
<th>Days after spraying</th>
<th>Adult emergence of strains (%)</th>
<th>Mean (B) Days</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Tolerant</td>
<td>Susceptible</td>
<td></td>
<td>Tolerant</td>
<td>Susceptible</td>
</tr>
<tr>
<td>0</td>
<td>56.0(^{d})</td>
<td>3.0(^{f})</td>
<td>29.5(^{e})</td>
<td>0</td>
<td>95.0(^{a})</td>
</tr>
<tr>
<td>1</td>
<td>78.0(^{b})</td>
<td>18.0(^{f})</td>
<td>48.0(^{d})</td>
<td>1</td>
<td>97.0(^{a})</td>
</tr>
<tr>
<td>2</td>
<td>88.0(^{a})</td>
<td>20.0(^{f})</td>
<td>54.0(^{c})</td>
<td>2</td>
<td>95.0(^{a})</td>
</tr>
<tr>
<td>3</td>
<td>88.0(^{a})</td>
<td>68.0(^{d})</td>
<td>78.0(^{a})</td>
<td>3</td>
<td>94.0(^{a})</td>
</tr>
<tr>
<td>4</td>
<td>86.0(^{a})</td>
<td>58.0(^{d})</td>
<td>72.0(^{b})</td>
<td>4</td>
<td>94.0(^{a})</td>
</tr>
<tr>
<td>5</td>
<td>83.0(^{a})</td>
<td>62.0(^{c})</td>
<td>72.5(^{b})</td>
<td>5</td>
<td>91.0(^{b})</td>
</tr>
</tbody>
</table>

Mean (A) Strains

<table>
<thead>
<tr>
<th>Strain (A factor)</th>
<th>Days (B factor)</th>
<th>Strain × Days (A × B)</th>
</tr>
</thead>
<tbody>
<tr>
<td>SEM</td>
<td>1.0</td>
<td>2.5</td>
</tr>
<tr>
<td>LSD ((P = 0.05))</td>
<td>2.9</td>
<td>7.0</td>
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</tbody>
</table>

<table>
<thead>
<tr>
<th>Strain (A factor)</th>
<th>Days (B factor)</th>
<th>Strain × Days (A × B)</th>
</tr>
</thead>
<tbody>
<tr>
<td>SEM</td>
<td>0.5</td>
<td>1.2</td>
</tr>
<tr>
<td>LSD ((P = 0.05))</td>
<td>1.4</td>
<td>2.5</td>
</tr>
</tbody>
</table>

A factor – Tolerant and susceptible strains

B factor – Days after spraying
resistance at egg stage than pupal or adult stages of *T. japonicum* when treated with insecticides is reported.

The stability of malathion resistance in *Anisopteromalus calandrae* and *Bracon hebetor* for 23 and 21 generations, respectively with no selection pressure was reported. In the present studies, *T. chilonis* maintained tolerance to endosulfan for four generations in absence of laboratory insecticide selection pressure. Introduction, release, establishment and spread of organophosphorous resistant strain of predatory mite, *Metaseiulus occidentalis* Nesbitt in the grapevine orchards in Russia was successful, remained unaffected with insecticide treatments and reduced the pest population. However, sometimes mating incompatibilities and partial mating between a laboratory–reared insecticide resistant and field strains of susceptible natural enemies can delay the establishment of resistant strains and in some cases prevent the establishment of better adapted strains to the local environments.

Evaluation of the tolerant strain in the net house revealed that it could effectively control the pest if released even immediately after the spray of endosulfan and sustained the parasitoid’s activity even after 5 days of spray. Tolerance of the strain to insecticide load immediately after spray is rather more important than later, since metabolic degradation of the insecticide in the host insect occurs naturally after a given period of time, paving way for higher activity of the parasitoid on the host. Further, significant differences in the rate of parasitisation and adult emergence was not observed in the tolerant strain indicating more effectiveness irrespective of the time. Similar observation was made in the malathion resistant strain of *Anisopteromalus calandrae* against *Sitophilus oryzae* (Linnaeus) in wheat.

Insecticide tolerant strains of beneficial organisms (for e.g. *C. carnea*) had been advised to use as an IPM component. The release of insecticide resistant strains of natural enemies can play an effective role in delaying the development of resistance in the pest population. Therefore, the tolerant strain of *T. chilonis* to endosulfan (widely used insecticide), an effective egg parasitoid of several lepidopterous pests can fit in all IPM strategies, particularly in crops of high insecticide consumption (cotton, rice, fruits and vegetables). The technology of tolerance to endosulfan in the egg parasitoid *T. chilonis* was developed for first time in India and was transferred to the industry for large-scale production and field releases on various crops.

**Conclusion**

A strain of *Trichogramma chilonis*, an egg parasitoid of lepidopteran pests tolerant to the most commonly used cyclodiene insecticide-endosulfan (0.09%) was developed in the laboratory. The tolerant strain had LC50 values of 1074.96 ppm as compared to 70.91 ppm of susceptible strain. The genetical study showed that F1 crosses exhibited a semi-dominant response to endosulfan with degree of dominance value (D) of 0.58. The resistant factor of tolerant strain was 15.1 folds and of F1 cross were 8.53 folds over susceptible strain. Under net house conditions, the tolerant strain parasitised 56% of *Helicoverpa armigera* eggs on potted cotton plants immediately after an insecticide spray, compared to 3% by the susceptible strain. This strain can be used as a component of bio-intensive IPM in various crops where insecticide use is higher.

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