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Sudhanshu Bala Nayak

Department of Agricultural
Entomology, Tamil Nadu
Agriculture University (TNAU),
Coimbatore, Tamil Nadu, India

Atanu Seni

Orissa University of Agriculture
and Technology, RRTTS,
Chiplima, Odisha, India

Vinay BK

Division of Nematology,
ICAR-IARI, New Delhi, India

Genetic improvement of egg parasitoid *Trichogramma chilonis* (Hymenoptera: Trichogrammatidae) by artificial selection

Sudhanshu Bala Nayak, Atanu Seni and Vinay BK

Abstract

Genetic improvement of egg parasitoid, *Trichogramma chilonis* highly essential to develop superior strains having insecticide tolerance capacity. A laboratory experiment was conducted in order to evaluate toxicity of some selected insecticides and their effect on parasitism, mortality rate in egg parasitoid *T. chilonis*. Among all the insecticides tested imidacloprid was found the most toxic to *T. chilonis* with LC₅₀ and LC₉₅ value was 0.036 and 0.164 mg a.i./l followed by fenvalerate with LC₅₀ and LC₉₅ value 0.211 and 1.056 mg a.i./l. Based on risk quotient only chlorantraniliprole was found to be harmless to *T. chilonis* while other insecticides were belongs to slightly to moderately toxic category. So there is need to improved potential for pesticide resistance by artificial selection in parasitoids has been shown to be a practical and cost effective method, can fit in all integrated pest management (IPM) strategies.

Keywords: Insecticide resistance, *Trichogramma chilonis*, Artificial selection, Parasitoids, Genetic Improvement

1. Introduction

Trichogramma spp. is the most extensively used parasitoid against several lepidopterous pests in different cropping systems. Among all trichogramma species *T. chilonis* the most commonly used not only in India but also in different country like China, Korea, Taiwan, Nepal, Japan, Spain, South Africa Pakistan, Kenya, and Australia. As we know many of the insecticides are extremely toxic to several biocontrol agents and this has warranted the development of pesticide tolerant strain. For the first time in India Jalali *et al.* (2006) [9] reported the development of insecticide tolerant strain through artificial selection of egg parasitoid *T. chilonis* to endosulfan. The idea of genetic improvement of parasitoids and predators is about 75 years old (Dhaliwal and Arora, 2001) [6]. It can be achieved through artificial selection method, hybridization or recombinant DNA techniques. Biological control agents are artificially selected for resistance to several insecticides (Fournier *et al.* 1987) [8]. Genetic improvement of parasitoids and predators has produced insecticide resistant strains for at least 15 species of natural enemies of insects and mites (Beckendorf and Hoy, 1985) [4] and has enhanced the efficacy of natural enemies. The use of insecticide resistant beneficial organisms can delay the development of resistance in the crop pest species. Petrushov (1987) [12] reported that the introduction of organophosphate tolerant population of the predatory mite *Metaseiulus occidentalis* for control of the grape pest *Eotetranychus pruni* resulted in their field establishment and it remained unaffected by insecticide treatments in Russia. So Jalali *et al.* (2009) [10] developed insecticide and temperature tolerance strain of *T. chilonis* to high temperature (32-38°C), RH 60 % and to three major groups of insecticides i.e. endosulfan (organochlorine), monocrotophos (organophosphate) and fenvalerate (synthetic pyrethroid). Subsequently, a multiple insecticides tolerant strain of *T. chilonis* (MITS-TC) was also developed. There is also report of insecticide tolerant strains have been collected from fields under severe pesticide pressure (Baker and Thorne 1995) [3].

The cross-resistance mechanism between organophosphates, carbamates and pyrethroids in *T. chilonis* could be due to the presence of esterases, glutathione S-transferases and monooxygenases enzymes. Viewing the importance of *T. chilonis* we were interested to investigate whether selection pressure with a particular insecticide could also increase resistance to other insecticides and is the cross-resistance between different insecticides conferred by known resistance mechanisms. That might have important implications in

Correspondence

Sudhanshu Bala Nayak

Department of Agricultural
Entomology, Tamil Nadu
Agriculture University (TNAU),
Coimbatore, Tamil Nadu, India

integrated pest management (IPM), which advocates both chemical and biological control in agroecosystems. The use of pesticide-resistant parasitoids and predators might prevent pest resurgences and secondary pest outbreaks in different agro ecosystems in which chemical control method is a common practice. Keeping current situation in mind there were aimed in development of insecticides resistant strain of *T. chilonis* in laboratory condition.

2. Materials and Methods

2.1 Mass rearing of insect

The parasitoids *T. chilonis* were multiplied on *Corcyra cephalonica* eggs in bio-control laboratory of Tamil Nadu Agricultural University, Coimbatore. The eggs laid by the *Corcyra* moths are collected and sieved to remove the moth scales etc. The pure eggs thus obtained are exposed to ultra-violet light in UV chamber to kill the host embryo but at the same time permit parasitization. A coat of 10% gum arabic is

applied on the cards and the eggs are sprinkled uniformly in a single layer with the aid of a tea strainer. The egg cards are placed into polythene bags of suitable size and the nucleus card of *Trichogramma* are introduced in it. The easiest way to accomplish this is to place a piece of 'Tricho egg card' containing parasitized eggs (i.e. pharate adults) that are ready to yield the adults and to hold them in subdued light for 2-3 days. The emerging parasites readily parasitize the fresh eggs. The parasitoid - host ratio is adjusted accordingly to 1:6 get effective parasitism. The parasitized eggs in the *Tricho Card* turn black in 3 or 4 days and the adult parasitoids emerge in 8 to 10 days from the date of parasitization.

2.2 Insecticides

Insecticides belongs to different mode of action were taken into consideration for the development of insecticide tolerant strain of *T. chilonis* in the laboratory condition. The insecticides formulation used are listed in Table 1.

Table 1: Insecticides selected for safety testing of pesticides in the laboratory

S. No.	Insecticides Formulation and Group	IRAC Mode of Action	Manufacturing company	Trade name
1	Chlorpyrifos 20EC (Organophosphate)	Acetylcholinesterase (AChE) inhibitors	Dow Agro Sciences	Dursban
2	Profenophos 50 EC (Organophosphate)	Acetylcholinesterase (AChE) inhibitors	Syngenta Co Ltd.	Curacron
3	Fenvalerate 20 EC (Synthetic pyrethroids)	Sodium channel modulator	Modern Insecticide Ltd.	Fencor
4	Imidacloprid 17.8% SL (Neonicotinoids)	Nicotinic Acetylcholine receptors	Bayer CropScience	Confidor
5	Chlorantraniliprole 18.5 SC (Diamide)	Ryanodine receptor modulators	E.I. DuPont India Private Limited	Coragen
6	Acephate 75%, SP (Organophosphate)	Acetylcholinesterase (AChE) inhibitors	Rallis India Company	Asataf
7	Cypermethrin 25 EC (Synthetic pyrethroids)	Sodium channel modulator	Syngenta Co Ltd.	Cymbush

2.3 Development of insecticide tolerant strain

Insecticides were sprayed by hand sprayer in order to obtain a uniform layer of spray droplets on the inside of the glass tubes with open end on both sides. After spraying the glass tubes were dried in shade. One end of the glass tubes were closed with double layered long black cloth. About 300 *T. chilonis* adults were allowed to move into the sprayed glass tube through the open end and after 30 minutes, a tricho card with fresh UV treated 1 cc of *Corcyra* eggs were introduced on a card in the ratio of 50 eggs: 1 female for effective parasitization. Adult mortality or survival rate after 6 hour of release was recorded. The parasitized tricho cards were removed from the sprayed glass tube and kept in a fresh tube for the further development of the parasitoids. The Percentage of parasitism was recorded after five days of exposure to insecticides. Eggs that turned black were considered parasitized and number of parasitized eggs in 1cm² area was marked to determine percentage parasitization. For profenophos concentration tested was 1.5 ml/lit spray; for chlorpyrifos 1.0 ml/lit; for chlorantraniliprole 2.0ml/lit; for fenvalerate 0.20 ml/lit spray. Each treatment was replicated five times and compared with a control (water alone). The egg parasitoid *T. chilonis* were exposed four times to each insecticide. Different insecticides were sprayed in order to check percentage parasitization and percentage mortality. Subsequently observations on percentage parasitism, mortality from various treatments were recorded. The data on percent mortality after 24 hours of exposure and per cent parasitism were recorded. The data on mortality and percent parasitism were analysed by one-way ANOVA. For acute toxicity calculation seven different insecticides like fenvalerate, imidacloprid, chlorantraniliprole, acephate, cypermethrin, profenophos and chlorpyrifos were exposed to *T. chilonis* and mortalities were assessed after 24 hours. The mortality values were converted to percentages and

adjusted for control mortalities using Abbott's formula (Abbott, 1925) [1]. The data was subjected to probit analysis (Finney, 1971) [7] after converting the observed mortality into corrected mortality by using Abbott's formula for developing regression equations for dosage mortality responses and to determine the fiducial limits, slope, χ^2 , LC₅₀ and LC₉₅ values. To determine risk of insecticides to natural enemies, risk quotients can also be calculated for the insecticides from the LC₅₀ values at 24 hours after treatment based on the formula given by Preetha *et al.* (2009) [13].

$$\text{Risk quotient} = \frac{\text{Recommended field dose (g a.i./ha)}}{\text{LC}_{50} \text{ of beneficial insect (mg a.i./l)}}$$

Risk quotient	Category
< 50	Harmless
50 to 2,500	Slightly to moderately toxic
> 2,500	Dangerous

3. Results and Discussion

As we know insecticides are still the principal method of controlling pests once the economic threshold has been reached in most of the cropping systems, as they can be relatively cheap and are easy to adopt and apply, fast-acting, and in most instances it is the only method of control of insect pests. Parasitoids and predators are most likelihood to be contacted with the insecticide because of their mobility. So our goal is to maximize pest mortality while minimizing harmful effect of insecticides to natural enemies. The insecticides resistant natural enemies strain could therefore be an alternative option to use them in concurrence with insecticides in different cropping systems. For development of multiple insecticide resistant strain of *T. chilonis* results on percentage parasitism and percentage mortality rates are presented in Table 2.

Table 2: Development of multiple insecticide resistant strain of *T. chilonis*

Insecticide	No. of generations reared	% Parasitism		% Mortality	
		Generation		Generation	
		First	Last	First	Last
Profenophos	5	59.00 (50.21)	88.00 (70.14)	79.00 (63.47)	21.00 (26.94)
Chlorpyrifos	6	45.00 (42.10)	93.00 (75.80)	64.00 (53.33)	17.00 (24.17)
Chlorantraniliprole	7	42.00 (40.32)	95.00 (79.48)	58.00 (49.64)	16.00 (23.41)
Fenvalerate	8	8.60 (16.15)	93.00 (76.32)	90.60 (73.07)	16.00 (22.88)
CD at 5%		6.377	NS	9.224	NS

Data in parentheses is arcsine transformed values.

The median lethal concentrations of different insecticides to *T. chilonis* are presented in Table 3. Among all the insecticides tested imidacloprid was found the most toxic followed by fenvalerate. The order of toxicity to *T. chilonis* based on median lethal concentration (LC₅₀) values was as follows: imidacloprid > fenvalerate > chlorpyrifos > cypermethrin > chlorantraniliprole > profenophos > acephate. Based on risk quotients vauleare presented in Table 4, as compared with other tested insecticides only chlorantraniliprole was found to be harmless to *T. chilonis*. The LC₅₀ and LC₉₅ values were 0.211, 0.036, 2.017, 4.212, 0.854, 2.188, 0.773 and 1.056, 0.164, 61.540, 10.629, 2.990, 16.629, 2.543 mg a.i./l for fenvalerate, imidacloprid, chlorantraniliprole, acephate, cypermethrin, profenophos, chlorpyrifos respectively. Results are in tune with the findings of Preetha *et al.* (2009) [13] who reported that thiamethoxam was showing the highest toxicity among the insecticides tested against *T. chilonis*, with LC₅₀ value of 0.0014 mg a.i./l, followed by imidacloprid (0.0027 mg a.i./l)

and the LC₅₀ values of acephate and endosulfan were 4.4703 and 1.8501 mg a.i./l, exhibiting low toxicity.

Jalali *et al.* (2006) [9] developed *T. chilonis* tolerant strain to endosulfan at 0.09% concentration in the laboratory condition. The LC₅₀ values were 1074.96 ppm and 70.91 ppm in the tolerant strain and susceptible strain respectively, and their genetic study showed that F₁ cross exhibited a semi-dominant response to endosulfan with degree of dominance (D) value of 0.58. For tolerant strain resistant factor 15.1 and for F₁ crosses were 8.53 folds over the susceptible strain. The tolerant strain parasitised 56% of *H. armigera* eggs on potted cotton plants immediately after an insecticide spray, compared to 3% by the susceptible strain under net house condition. Charles *et al.* (2011) [5] also studied the mode of inheritance of insecticide tolerance in *T. chilonis* and reported that LC₅₀ values of endosulfan, spinosad and lambda cyhalothrin tolerant strains were 278.03, 9.84 and 6.23 ppm which is in contrast to the susceptible strains having LC₅₀ values of 106.03, 4.78 and 3.45 ppm respectively.

Table 3: Median lethal concentration of different insecticides to *T. chilonis*

Insecticides	Regression Equation	χ^2	LC ₅₀	Fiducial limits		LC ₉₅	Fiducial limits	
				LL	UL		LL	UL
Fenvalerate	Y= 6.717+2.505X	0.851	0.211	0.146	0.305	1.056	0.334	3.340
Imidacloprid	Y=8.769+2.613X	0.965	0.036	0.026	0.051	0.164	0.058	0.467
Chlorantraniliprole	Y=4.664+1.097X	0.801	2.017	0.807	5.041	61.540	0.349	108.30
Acephate	Y=2.284+4.368X	0.865	4.212	3.365	5.272	10.629	5.723	19.742
Cypermethrin	Y=5.251+3.261X	0.781	0.854	0.641	1.139	2.990	1.190	7.510
Profenophos	Y=4.113+2.566X	0.928	2.188	1.243	3.853	16.629	5.723	19.742
Chlorpyrifos	Y=5.404+3.434X	0.755	0.773	0.578	1.035	2.543	1.098	5.888

Table 4: Risk quotients of insecticides to *T. chilonis*

Insecticides	Recommended dose (g a.i./ha)	LC ₅₀ (mg a.i./l)	Risk quotient	Category
Fenvalerate	60	0.211	284.3602	Slightly to moderately toxic
Imidacloprid	25	0.036	694.4444	Slightly to moderately toxic
Chlorantraniliprole	25	2.017	12.39465	Harmless
Acephate	600	4.212	142.4501	Slightly to moderately toxic
Cypermethrin	50	0.854	58.54801	Slightly to moderately toxic
Profenophos	500	2.188	228.5192	Slightly to moderately toxic
Chlorpyrifos	600	0.773	776.1966	Slightly to moderately toxic

There are many records of pesticide tolerance on natural enemies by many researchers. Xiong *et al.* (1988) [17] reported that field population of *T. japonicum* was found to be tolerant to fenvalerate and decamethrin and also several fold increase in the LC₅₀ values of resistant strains of to methamidophos (0.8892 ppm), fenvalerate (8.6511 ppm), and metaphos (0.0592 ppm) and decrease in LC₅₀ value of mipicrin (0.1103 ppm) when treated for 36-43 generations from the rice

ecosystem. The tolerance of *T. japonicum* to different insecticides revealed that the LC₅₀ of eggs and adults showed 2.49 and 0.94 fold increase respectively. In comparison with field concentrations of insecticide the LC₅₀ value of the resistant strain egg increased by 6.4 times for fenvalerate, 1.62 times for methamidophos 4.03 times for deltamethrin, and 0.82 times for phosphamidon (Xu *et al.* 1986) [18].

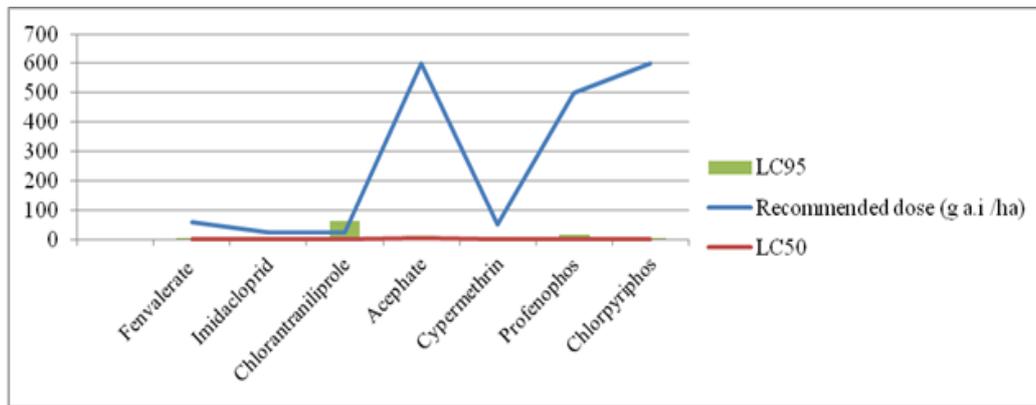


Fig 1: Comparison of LC₅₀ and LC₉₅ values of various insecticides to *T. chilonis* with their field recommended concentrations.

In contrast to the present study, Wang *et al.* (2012) [15-16] reported median lethal concentration LC₅₀ and LC₉₅ values: for imidacloprid were 503.6 and 4399 mg a.i./l; for cypermethrin 104.2 and 716.7 mg a.i./l; for chlorpyrifos 0.058 and 0.48 mg a.i./l; for profenofos 2.26 and 18.09 mg a.i./l in *Trichogramma ostrinae*. Wang *et al.* (2012) [15-16] also reported LC₅₀ and LC₉₅ values of different insecticides to *Trichogramma nubilale* were 311.9 and 2675, 19.48 and 176.8, 0.081 and 0.45, 0.18 and 1.73 mg a.i./l to imidacloprid, cypermethrin, chlorpyrifos, profenofos respectively. Results for chlorpyrifos and profenofos are on par to our findings. Roush and McKenzie (1987) [14] argued that laboratory selection for insecticide resistance strain will likely result in a polygenic mode of inheritance, because selection pressure results in small, incremental increases in insecticide resistance over time. There are some ecotypes of *Trichogramma* are more tolerant to insecticides indicated by Kumar *et al.* (1994) [11]. So selection of parasitoids for resistance to different insecticides now recognised as a potent method for enhancing their performance in different ecosystem.

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