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Detoxifying enzyme profiles in pesticide tolerant strains of *Trichogramma chilonis* Ishii, a hymenopteran parasitoid

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ABSTRACT: Investigation on enzyme assays were carried out for carboxyl esterase (á and â esterase) and glutathione s-transferase in the susceptible and pesticide tolerant *Trichogramma chilonis* Ishii strains such as TCT1, TCT4, TCT5 and TCCb tolerant to endosulfan, spinosad, lamda-cyhalothrin and indoxycarb respectively. The electrophoretic profile of á-esterase enzyme in the susceptible *Trichogramma* strain showed 3 alleles in comparison with tolerant *Trichogramma* strains TcT5, TCT1, TCCb and TCT4 which depicted 5, 4, 3 and 2 alleles respectively. Similarly, â-esterases profile in the susceptible indicated 4 alleles compared to TCT5, TCT1, TCCb and TCT4 strains which showed 6, 5, 3 and 3 alleles respectively. The results of the quantitative analysis showed an increased enzyme activity in the tolerant strains. The increase in á-esterase activity in the tolerant strains TCT1, TCT4 and TCT5 was 2.07, 1.53 and 1.51 times more than the susceptible one and the mean difference was statistically significant and â-esterase activity in the tolerant strains TCT1, TCT4 and TCT5 was 1.41, 1.69 and 1.16 times more than the susceptible one. In the glutathione s-transferase enzyme activity, the mean value of the susceptible strain was 0.029µM and in tolerant strains, TCT1, TCT4 and TCT5 the values were 0.0243, 0.0289 and 0.023µM respectively indicating that the tolerant strains had lesser activity than the susceptible one. Such an increase in enzyme activity indicated the elevated production of detoxifying enzymes such as carboxylesterase to sustain the parasitoid in the field.

KEY WORDS: Carboxylesterase, glutathione s-transferase, electrophoretic profile, alleles

INTRODUCTION

Trichogramma parasitoids are one of the most important groups of biotic agents employed for the control of several lepidopterus pests in the agricultural field. It has been revealed that continuous exposure to insecticides can lead to increased tolerance and development of resistance by the insects (Ganesh *et al.,* 2002). Selection for insecticide resistance is greatly enhanced when an array of insecticides is widely used in agriculture. In this regard, the most important mechanism evolved by insects is the possession of detoxifying enzymes and the modification of the target sites of insecticide through mutation (Hemingway *et al*., 1998). Biochemical studies provide good evidence about the mechanisms involved in insect resistance development. A perusal of literature showed that

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three major enzyme groups namely esterases, glutathione s-transferase (GSTs) and monooxygenases are responsible for metabolically based resistance to organophosphates, organochlorins, carbamates and pyrethroids (Hemingway *et al*., 1998). Esterases are often involved in organophosphate, carbamate and to a lesser extent pyrethroid resistance. Further, GSTs are dimeric multifunctional enzymes that play a role in the detoxification of a large range of xenobiotics (Prapanthadara *et al.,* 1996). GST also give protection against pyrethroid toxicity in insects by sequestering the insecticide (Enayati *et al.,* 2005). The electrophoretic technique provides considerable promise in relating detoxifying enzymes to resistance and as a means of identifying resistant genotypes in vectors and pests. (Humerus *et al.,* 1990). It has also been possible to detect any genetic change arising through alteration in the nucleotide level because enzymes are direct products and any change or variation in the DNA level will be reflected in the proteins (Pasteur and Raymond, 1996). The isozyme polymorphism as evident from gel electrophoresis could be used as biochemical markers in studying the genetics of insecticide resistance in the absence of any visible markers (Chakraborty *et al.,* 1993). Research on the biochemical and genetics of insecticide resistance related to *Trichogramma* are rather limited regarding resistance development. Earlier isozyme studies are restricted to *Trichogramma* systematics (Coa *et al.,* 1986; Lu *et al*., 1988; Miura *et al.,* 1990; Pinto *et al.,* 1992; Pintureau, 1993a, b, 1999; Zhu *et al*., 2002; Summer *et al.,* 2008). In light of the above information, the present study on enzyme assay related to insecticide detoxification was carried out on qualitative analysis of á and â-esterases and quantitative analysis of á, â-esterases and GST in the pesticide susceptible and tolerant strains of *Trichogramma chilonis* Ishii (Hymenoptera: Trichogrammatidae).

MATERIALS AND METHODS

Pesticide susceptible and tolerant *Trichogramma* strains were obtained from the ICAR - National Bureau of Agriculturally Important Insects (NBAII), Bengaluru and maintained for up to three generations in the Department of Zoology, University of Mysore, Mysore, India where the present work was carried out. These strains were reared on *Corcyra cephalonica* eggs. The pesticide susceptible along with four tolerant *Trichogramma* strains namely TCT1, TCT4, TCT5 and TCCb tolerant to endosulfan, spinosad, lamda-cyhalothrin and indoxycarb respectively were employed for the investigations.

Qualitative enzyme assay: á-esterase and â-esterase were analysed to establish the differential isozyme profiles in the susceptible and pesticide-tolerant *Trichogramma* strains by polyacrylamide gel electrophoresis (PAGE). A small dual vertical slab (11x11cm) gel electrophoresis system (Broviga make, Chennai, India) was used. Gels of 0.7mm thickness were cast employing Teflon spacers with separating and stacking gels prepared (with 5% and 3.5% acrylamide respectively). Gel was run at a constant power supply (60V) for nearly four hours at 4°C. Twenty adult *Trichogramma* were homogenized in 25µl of 40 per cent sucrose solution in an eppendorf tube using a pestle. An equal volume (15µl) of supernatant was loaded to each well. Gels were transferred to the Petri dish containing the staining solution. Napthyl acetate was used as substrate and stained with fast blue BB salt. Gels were incubated at 37°C in the dark for 20 minutes.

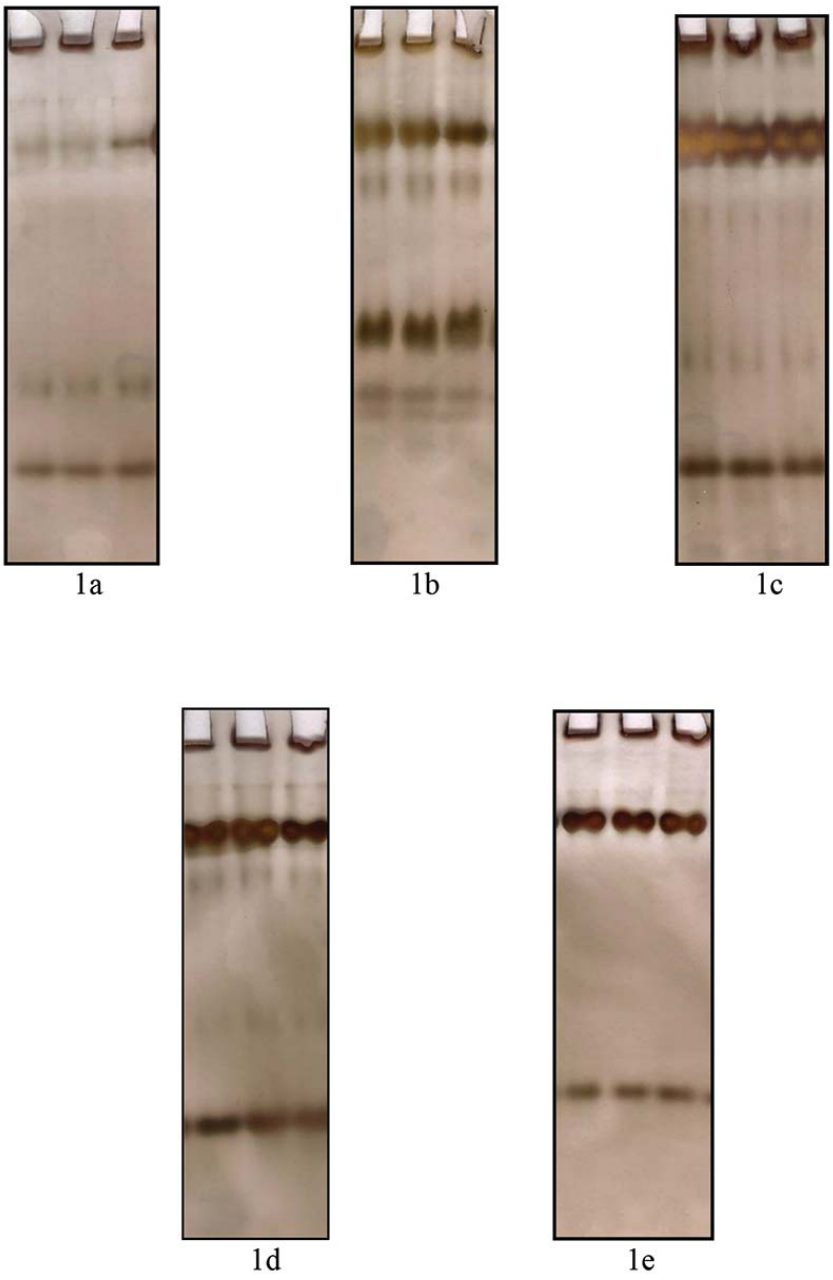
Quantitative enzyme assay: The assay was carried by using the microplate assay method for á-esterase, â- esterase and the spectroscopic method for GST enzyme (Hemingway *et al.,* 1998). Twenty adults *Trichogramma* were homogenised in 200µl of distilled water. Twenty µl of the supernatant was taken and made upto100µl by adding 0.1M KPO_4 buffer (pH 7.2). For establishing esterase activity 100µl of sample was loaded to the wells of the microplate and 100µl of á-naphthyl acetate solution for á-esterase and â-naphthyl acetate for â-esterase were added after 10min incubation in room temperature 100µl of dianisidine - fast blue BB solution was added to each well and kept for 5 min and was read at 620nm for alpha esterase and at 540nm for beta esterase. For GST one ml of sample,1ml of reduced glutathione and 1ml of 1-chloro 2,4-dinitrobenzene

was added and kept for 5 minutes and read at 340nm employing UV spectroscopy. The enzyme activity was reported as µmoles of product formed/minute/mg protein. The paired samples t-test at 0.05 level was employed to compare quantitative values of the enzyme between the susceptible and tolerant *Trichogramma* strains.

RESULTS AND DISCUSSION

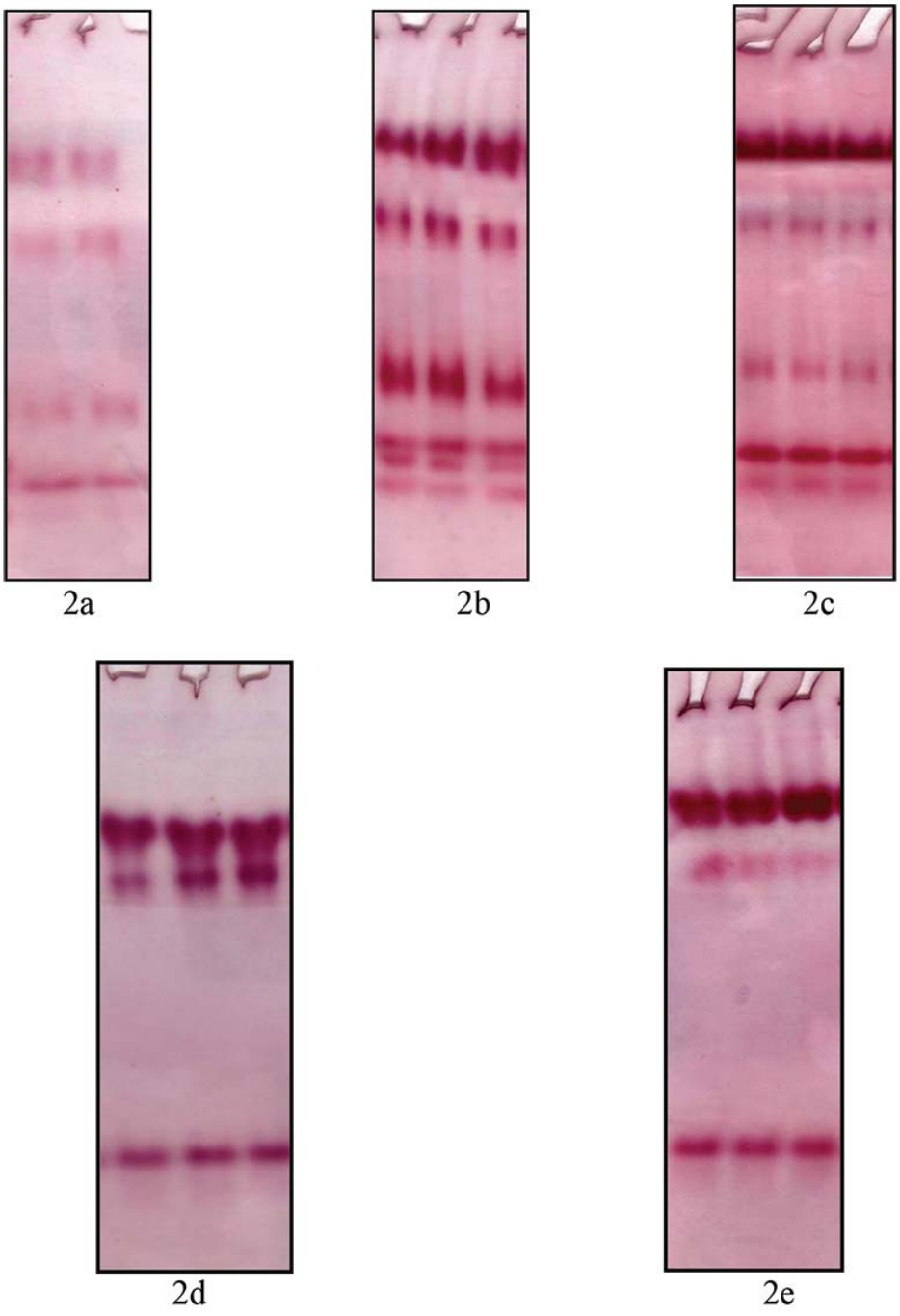
Qualitative enzyme analysis: In the electrophoretic profiles of á-esterase enzyme in the susceptible *Trichogramma* strain (Fig.1a) and in tolerant *Trichogramma* strains TCT5, TCT1, TCCb

Fig. 1 Isozyme profiles of A-esterase in *Trichogramma* strains



Figs. 1a; Susceptible, 1b; lamda cyhalothrin, 1c; endosulfan, d indoxycarb and 1e; spinosad tolerant strains

Fig. 2 Isozyme profiles of B-esterase in *Trichogramma* strains



Figs. 2a; Susceptible, 2b; lamda cyhalothrin, 2c; endosulfan, 2d indoxycarb and 2e; spinosad tolerant strains

Table 1, Allelic variation in the pesticide susceptible and tolerant *Trichogramma chilonis* strains

Enzymes	*Trichogramma chilonis* strains				
	Susceptible	TCT5	TCT1	TCCb	TCT4
No. of alleles	3	5	4	3	2
A – esterase (electromorph)	0.55 - 0.90	0.55 0.65 0.90	0.55 0.65 0.90	0.55 0.65 -	0.60 - -
Common band	1.00 -	1.00 1.03	1.00 -	1.00 -	1.00 -
No. of alleles	4	6	5	3	3
B – esterase (electromorph)	0.55 0.65 0.90 -	0.55 0.65 0.90 0.97	0.55 0.65 0.90 -	0.55 0.65 - -	0.55 0.65 - -
Common band	1.00 -	1.00 1.05	1.00 1.03	1.00 -	1.00 -

Table 2. Differential activity of three detoxifying enzymes in pesticide susceptible and tolerant *Trichogramma chilonis* strains (Mean ± SD)

Enzyme	Susceptible	Strains	Ratio
Á-esterase µmoles napthol produced/min/ mg/protein	0.0153±0.0006	TCT1- 0.0317±0.003*	2.07
		TCT4-0.0235±0.014*	1.53
		TCT5-0.0232±0.003*	1.51
B-esterase µmoles â naptholproduced/min/ mg/protein	0.0305±0.026	TCT1-0.0433±0.004*	1.41
		TCT4-0.0518±0.006*	1.69
		TCT5-0.0261±0.44*	1.16
GST µmoles glutathione produced/ min/mg/protein	0.029±0.0025	TCT1-0.0243±0.0025*	1.19
		TCT4-0.0283±0.003*	1.02
		TCT5-0.0230±0.004*	1.26

* t-value significant at $P<0.05$, n=30, df=29

and TCT4 (Fig.1b, c, d, e respectively), the most common allele is marked as 1.00 and other bands are labelled based on their electrophoretic mobility (Table 1). Two extra alleles in TCT5- lambda-cyhalothrin tolerant (Fig.1b) and one extra allele in TCT1- endosulfan tolerant strains (Fig.1c) were found in comparison with the susceptible strain. There was no extra allele in the TCCb- indoxycarb tolerant strain (Fig.1d) and one allele missing in the TCT4- spinosad tolerant strain (Fig.1e) in comparison with the susceptible strain. Similarly, the isozyme profiles of â-esterase in the susceptible *Trichogramma* strain (Fig. 2a) and tolerant stains TCT5, TCT1, TCCb and TCT4 (Fig. 2b, c, d, e respectively), showed two extra alleles and one extra allele in TCT5 (Fig. 2b) and TCT1 (Fig. 2c) respectively in comparison with susceptible and one allele was missing in TCCb (Fig. 2d) and TCT4 (Fig. 2e) strains.

Quantitative analysis: The mean value of á-esterase activity in susceptible strain was found to be 0.0153µM a-naphthol produced/min/mg protein while it was 0.0317µM in TCT1, 0.0235µM in TCT4 and 0.0232µM in TCT5. The increase in á -esterase activity in the tolerant strain TCT1, TCT4 and TCT5 was 2.07,1.53 and 1.51 times more than the susceptible one and the mean difference was statistically significant (Table 2). Similarly, â-esterase activity in the susceptible strain was 0.0305 µM and in tolerant strains, TCT1, TCT4 and TCT5 activity was found to be 0.0433, 0.0518 and 0.0261 µM respectively. The increase in â-esterase activity in the tolerant strains, TCT1, TCT4 and TCT5 was 1.41, 1.69 and 1.16 times more than the susceptible one. However, in TCT5 the mean value was less than that of the susceptible one which was significantly different. In the GST enzyme activity, the mean value of the susceptible strain was 0.029µM and in tolerant strains TCT1, TCT4 and TCT5 the mean activity values were 0.0243, 0.0289 and 0.023µM respectively which are significantly different. However, the tolerant strains had lesser activity than the susceptible ones.

Studies on insecticide resistance have indicated the specific relevance of esterase to xenobiotic metabolism in several insect species. However, such resistance studies to understand the mechanism in *Trichogramma* are rather limited. The isozyme analysis in *Trichogramma* was carried out to aid in taxonomic identification because of the minute size of the wasp (Coa *et al.,* 1986; Lu *et al*., 1988; Miura *et al.,* 1990; Pintureau, 1993a, b, 1999, Pinto *et al.,* 1992; Zhu *et al.,* 2008; Summer *et al.,* 2008). Esterase isozyme associated with insecticide resistance has been used extensively as a diagnostic tool. For example, elevated esterase banding patterns have been focused on resistant populations of western corn rootworms (Zhou *et al.,* 2002); *Anopheles stephensi* (Ganesh *et al.,* 2002); *Blattella germanica* (Scharf *et al.,* 1997) and *Myzus persicae* (Devonshire *et al.,* 1998). According to Karunarathnae and Hemingway (2001), metabolic resistance to organophosphate compounds in insects is mainly due to qualitative and quantitative differences in the carboxyl esterase. In the present study also qualitative and quantitative changes were characterized in tolerant strains. In the endosulfan (organophosphate) tolerant strain one extra allele and in lambda cyhalothrin (pyrethroid) tolerant strain two extra alleles indicate the elevated activity of this carboxyl esterase compared to the susceptible strains. The result obtained in the present study is in line with that of Ganesh *et al.,* 2002 in *Anopheles stephensi*. There was no extra allele in the indoxycarb tolerant strain and one allele is absent in the spinosad tolerant strain in comparison with the susceptible strain. Further, there was an increase in the intensity of the isozyme bands in indoxycarb tolerant strain. Hence the esterase isozyme may not play any role in the resistance development in the indoxycarb and Spinosad-tolerant strains. However other mechanisms may be involved in the resistance development here. It is also evident from the quantitative results that the activity of carboxyl esterase increased significantly in tolerant strains compared to the control batches. This is in agreement with the results obtained in mosquitoes (Ganesh *et al.,* 2002). However, the increased level of GST enzyme activity in susceptible compared with the tolerant strains is puzzling. Thus, the increased number of esterase enzyme alleles in endosulfan and lambda cyhalothrin tolerant strains

amply proves the onset of the development of resistance in these strains. Maintenance of these pesticide-tolerant *Trichogramma* strains and mass release in the crop fields will help the farmers in containing the pest population. Biological control agents such as *Trichogramma* are perceived to be slow acting in nature and are susceptible to pesticides and other abiotic stresses.

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