EFFECT OF DELTAMETHRIN ON DETOXIFYING ENZYMES IN A FRUIT FLY, Bactrocera cucurbitae (Diptera: Tephritidae)

Deepak Verma, Soni Kumari and Dinesh Kumar
Department of Zoology, CAS, Banaras Hindu University, Varanasi-221 005, India, E-mail: dv959505@gmail.com, dinesh195360@gmail.com, Corresponding Author: Deepak Verma

Abstract: Tephritidae family comprises of phytophagous flies, which includes most destructive pest in the world. Bactrocera cucurbitae is one of the serious pests of horticulture that is spread across a wide range of geographical regions including the Indian subcontinent. It infests 125 plant species, including cucurbits. It damages by laying its eggs inside the host fruit. Eggs hatch into larvae within the fruits and develop by consuming them.

Deltamethrin, a pyrethroid is used for controlling the Bactrocera cucurbitae population. Monoxygenases are involved in the metabolism of many insecticides including pyrethroid. Monoxygenases are phase I while GST are phase II metabolic enzymes. Monoxygenases metabolize a wide range of insecticides by hydroxylation, epoxidation and oxidation. In the present studies, effect of Ld50 dose of deltamethrin on P450, Esterase and GST have been estimated. Results reveal that Monoxygenase level increases continuously after 12 and 24 hr. of treatment. While GST level increases only after 24 hr of deltamethrin treatment. However, Esterase enzymes activity were not altered until 24 hr of treatment. The finding of the present investigation would be discussed in the light of available literature.

Keywords: Glutathione esterase, Bactrocera, Insecticide.

Introduction: Bactrocera cucurbitae is a severe pest of horticulture that is distributed across a wide range of climatic regions including the Indian subcontinent. It infests nearly 125 plant species, preferentially cucurbits [1]. This pest causes damages by puncturing the skin of cucurbits and laying its eggs inside the host fruits. Eggs hatch into larvae within the fruits [2] and develop by consuming them.

Despite the presence of sterile insect release (SIT), mass trapping and mating disruption technologies to control the fruit fly population, insecticides are still proven to be an effective tool [3]. Initially, organ chlorines, organophosphates and carbonates were used, followed by the more recent introduction of pyrethroids, spin sad and other compounds. But repeated application of these insecticides have resulted in insecticide resistance [4], which is a serious problem in agriculture, ending up with the tolerance against insecticides.


Deltamethrin, a pyrethroid is used for controlling the Bactrocera cucurbitae population. In the present studies, we estimated Ld50 dose of deltamethrin and then evaluated its effect on detoxifying enzyme; P450, Esterase and GST.

Materials and Methods

Ld50 Assay: Ld 50 for deltamethrin was determined after topical application (1 µl) of deltamethrin. Polo plus was used for probit
analysis to calculate the lethal dose of insecticide.

**Protein Estimation:** Homogenate was prepared in phosphate buffer (pH 6.8). Homogenate was centrifuged at 10000rpm for 10 minutes. Supernatant was taken out and used for protein estimation by lowry method.

**GST Assay:** Reaction mixtures for assays contain 1.350 ml of 0.1 M phosphate buffer (pH 6.8), 1 mM 1-chloro-2,4-dinitrobenzene (CDNB), 1 mM GSH 75µl, and 20µl of sample. Absorbance were noted down for 5 min at 340 nm due to the formation of S-(2, 4-dinitrophenyl), GSH ($\varepsilon = 9.6$ M$^{-1}$ cm$^{-1}$)\(^\text{[12]}\).

**P450 Assay:** Cytochrome P450 activity was quantified by an indirect measurement of cytochrome P450 by using heme peroxidation \(^\text{[13]}\). 3,3,5,5-tetramethylbenzidine (TMBZ) was used as the substrate. The total reaction volume was 15µl of enzyme solution, 900 µl of 0.625 M potassium phosphate buffer (pH 7.2), 200 µl of TMBZ solution, and 50 µl of hydrogen peroxide (3%). Absorbance was noted down at 630 nm. Standard curve for heme peroxidase activity was prepared using different concentrations of cytochrome C from horse heart. Cytochrome P450 (general oxidase) activity was expressed as equivalent units (EU) of cytochrome P450 per milligram of protein.

**Esterase Assay:** α-naphthyl acetate (0.3 mM) were used as substrate. 20 µL of sample and 1 ml α- naphthyl acetate was in incubated in dark for 20 min at 30°C. After 20 min, 1 ml staining solution was added to it \(^\text{[14]}\). Incubated this mixture for further 20 minutes at room temperature. Absorbance was taken at 590 nm.

**Statistical Analysis:** Data were presented as mean ± SEM (n=3). Statistical significance was analysed by one-way ANOVA followed by post hoc (tukey test), Graph pad 7.

**Results**

Table 1- represent the lethal dose for *Bactrocera cucurbitae*

<table>
<thead>
<tr>
<th>Dose ng/l</th>
<th>Limit</th>
<th>0.50</th>
<th>0.75</th>
</tr>
</thead>
<tbody>
<tr>
<td>LD10 Varanasi</td>
<td>0.11</td>
<td>Lower upper</td>
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</tr>
<tr>
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<td>0.45</td>
<td>Lower upper</td>
<td>0.038</td>
</tr>
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<td>0.189</td>
</tr>
<tr>
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<td>Lower upper</td>
<td>0.610</td>
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<td>5.32</td>
<td>Lower upper</td>
<td>1.648</td>
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<td>15.60</td>
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<td>LD80 Varanasi</td>
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<td>Lower upper</td>
<td>20.830</td>
</tr>
</tbody>
</table>

Fig 1. (A) Showing esterase activity (B) GST activity and (C) represents the p450 activity in *Bactrocera cucurbitae* after treatment of Ld 50 dose of deltamethrin
Discussion

Now a day’s several methods are available for detecting resistance in insects. Initially, laboratory bioassays have been used to detect insecticide resistance on the insect. Subsequently biochemical analysis of detoxifying enzymes were developed that provide more specific information about the resistance potential of insects. In the present studies, the metabolic activity of deltamethrin treated *Bactrocera cucurbitae* was compared with the untreated population of the *Bactrocera cucurbitae*. P450 enzymes are involved in phase I metabolic resistance to deltamethrin. Our results reveal that elevated P450 activity (F = 39.82, P < 0.05) after 12 hr of deltamethrin treatment, may compromise the effective dose of pesticide that reach and binds with their target site. Furthermore, altered GST activity (F = 5.343, P < 0.05) was reported after 24 hr of treatment. GST is an enzyme, which is usually associated with phase II detoxification process. Detoxification by these enzymes is responsible for the cross-resistance of insecticides. Cytochromes P450 induce oxidation or hydroxylation process. It introduces hydrophilic functional groups to the toxin to increase their hydrophilicity. This is phase I detoxification. In phase II, GSTs conjugate the phase I metabolite with hydrophilic compounds such as glutathione. This conjugate is then eliminated out. Most cases of pyrethroid resistance appear to be due to combined activity of P450 and GST. Metabolic enzymes significantly contribute in detoxification of toxin substance in several insects. As most of the insecticides are neurotoxic. Our results showed increased acetylcholinesterase activity in pyrethroid treatment. Based on the above results we may conclude that pyrethroid detoxification in *Bactrocera cucurbitae* is mainly contributed by P450 and GST enzyme as revealed by biochemical analysis. Moreover, it can be suggested that the detoxification system of *Bactrocera cucurbitae* participates in the defence mechanism against insecticide.

Acknowledgements: The financial assistance was provided by UGC, New Delhi, India to Deepak Verma (UGC SRF) is highly acknowledged. This work was also supported by funds from the UGC supported Centre of Advanced Study in Zoology and DST- FIST, Banaras Hindu University, Varanasi, India.

References