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Variation in activity of glutathione S-transferases and cytochrome P450 monooxygenases in tea mosquito bug (*Helopeltis antonii* Signoret Miridae: Hemiptera) after exposure to selected cashew varieties

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Abstract
Laboratory experiments were conducted in order to find out Variation in the activity of two major detoxification enzymes in cashew tea mosquito bug, *Helopeltis antonii* at Department of Agricultural Entomology, Kerala Agricultural University, Thrissur during 2016-2018. The main objective was to study the biochemical basis of the interaction between tea mosquito bug, *Helopeltis antonii* Signoret (Hemiptera: Miridae) and cashew (*Anacardium occidentale*). Tea mosquito bug adults collected at 0, 6, 24, 48 and 72 h after exposure to selected cahew varieties belongs to different susceptibility categories and analysis of detoxifying enzymes like cytochrome p450 monoxygenase and glutathione-s- transferase estimated as per the standard protocol. The results revealed that Defensive enzymes *viz.*, glutathione-s-transferases expressed elevated activity in TMB that fed on less susceptible Raghav (365.262 µmol $min^{-1}mg^{-1}$ protein) and Damodar (501.879 µmol $min^{-1}mg^{-1}$protein) when compared to the highly susceptible varieties. Cytochrome P450 showed highest activity in TMB fed on Damodar (0.372 nmol $min^{-1}mg^{-1}$ protein) and it was having the lowest activity in insect fed on other three varieties. The enhanced levels of detoxification enzymes in insect gives an indication of plasticity of the pest against host plant defense mechanism and same for management of the bug.

Keywords: cashew, cytochrome p450, glutathione-s-trasferase, detoxification enzymes

1. Introduction
The insect-pest problems have been increased with the rapid expansion of cashew crop acreage. More than one fifty species have been recorded in cashew and prominent among which is the tea mosquito bug (TMB), *Helopeltis* spp. (Hemiptera: Miridae). The field screening studies revealed that a few accessions withstand the TMB infestation and they can be grouped in to less susceptible. As the plant has got different adaptations to overcome herbivory, insects attacking host plants have different mechanisms to overcome plant barriers and one of which includes enzymatic detoxification of plant allelochemicals [1]. The major metabolic enzymes detoxifying xenobiotics in insects system include glutathione-S-tranferases (GST) and cytochrome P450 [2]. The primary role of the numerous enzyme systems in insects is the conversion of lipophilic foreign compounds (xenobiotics) into hydrophilic products, thereby enhancing rates of solubilization and excretion [3]. Enhanced detoxification mechanisms could see in insects feeding on plants with higher level of allelochemicals. Induction of insect detoxifying enzyme activity in response to plant allelochemicals provides clear manifestation of biochemical changes occur inside the host plant and insect and has been documented in several studies. The activity of GST enzyme in *Myzus persicae* Sulzer (green peach aphid) was found to be increased upon infestation, in response to secondary metabolites from brassica plants and determined using different host plant species and confirmed using artificial diet with pure allelochemicals added [4]. Cytochrome P450 is another important defensive enzyme having multiplicity and diversity for substrate recognition. It has got tremendous biochemical flexibility in the metabolic profiles of individual organisms. The modes of action of monoxygenase in detoxification mechanism include dealkylation, hydroxylation, deamination, and epoxidation [5].

Therefore, unravelling the detoxification mechanism of bug will help in formulating new management strategies against this severe pest of cashew.

2. Materials and Methods

Collection and maintenance of tea mosquito bug (*Helopeltis antonii* Sign.)

The initial culture of tea mosquito bug was established with collections from Cashew Research Station, Thrissur, Kerala. The insects were collected using wide mouthed test tubes. The collected adults were brought to the laboratory and released in pairs of adult males and female in oviposition cages (60×60×90 cm) for mating and egg laying. Cashew seedlings having tender shoots were provided for egg laying. Nymphs hatched out were reared in separate boxes.

The technique developed by (Sundararaju and John, 1992) [7] was followed for multiplication of TMB.

The first generation adults (female insect) were collected in test tube and prestarved for 3 h. After prestarving each adult insect was released on test varieties and covered with net cloth. Samples were taken at different intervals for the experiment.

Varieties selected for the study

Three months old cashew grafts of four cashew varieties, namely Anagha and Madakkathara-1 which have been reported highly susceptible to TMB and Raghav, Damodar reported as less susceptible, were selected for the experiment. The grafts were maintained in the nursery under ambient conditions.

Detoxification mechanism in tea mosquito bug

TMB adult females were collected before as well as at 6, 24, 48 and 72 h after feeding and analysis detoxifying enzymes like cytochrome P-450 and glutathione -S- transferase were carried out as per the standard protocols.

Glutathione-S-transferase (GST)

Glutathione- S- transferase quantification was carried using the method of (Kao *et al*., 1989) [6]

The supernatant was taken for enzyme assay. One hundred and twenty microliters of 50 mM 2-4 1-Chloro-2,4- Dinitro Benzene (CDNB) and 375 µl of reduced glutathione (GSH) were added to 6.95 ml sodium phosphate buffe (100 mM, pH 6.0). Fifty microliters of enzyme stock were then added. The contents were gently shaken, incubated for 2–3 min at 20 °C and then transferred to a cuvette in the sample cuvette slot of a UV spectrophotometer. Reaction mixture without enzyme was placed in a cuvette in the reference slot. Absorbance at 340 nm was recorded for 5 min with 30 sec intervals. The GST activity was calculated using the formula.

$$\text{CDNB-GSH conjugate µmol mg protein}^{-1}\text{ min}^{-1} = \frac{\Delta \text{ Abs in 5 min} \times 3 \times 1000}{9.6^* \times 5 \times \text{mg of protein}}$$

*9.6 mM/cm – extinction coefficient for CDNB-GSH conjugate.

Cytochrome P450

The cytochrome P450 assay was done according to the method of (Brogdon *et al*., 1997) [8] with slight modifications.

The supernatant was taken for enzyme assay. To the 50µl of prepared enzyme extract, 500 µl of 0.05 % TMBZ solution (10 mg TMBZ dissolved in 5ml absolute methanol mixed with 15ml 0.25nM sodium acetate buffer pH 5), 200 µl potassium phosphate buffer (pH 7.2), and 62.5 µl of 3% hydrogen peroxide were added. The reaction mixture incubated for 30 min. The absorbance was recorded with UV spectrophotometer (Model - Cary-60 UV vis) at 630 nm. The recorded absorbance was converted to end product formation from a standard curve of cytochrome C (0.0025 nM to 0.02 nM) and total activity expressed as n mol equivalent cytochrome P450 mg protein^{-1} min $^{-1}$

3. Results

Cytochrome P450

Six hours after exposure, TMB collected from Damodar showed the highest cytochrome P450 specific activity value of 0.545 nmol/min/mg protein, which was significantly higher than that of bugs exposed to other varieties and also to that of unfed bugs. The cytochrome P450 specific activity values of insect samples from other three varieties showed no significant difference among themselves as also with the corresponding values of samples prior to exposure (Table1).

After 24 h of exposure insect samples from Raghav showed highest value of 0.430 nmol/min/mg protein, followed by Damodar (0.186 nmol/min/mg protein). Bugs from both the above less susceptible varieties had significantly higher enzyme activity as compared to the highly susceptible Anagha (0.047 nmol/min/mg protein), which however was on par with bugs fed on Madakkathara-1 (0.096 nmol/min/mg protein).

There was significant variation in the cytochrome P450 activity of insect samples from different varieties at 48 h after exposure. Insects collected from Damodar had the highest enzyme activity of 0.749 nmol/min/mg protein followed by the highly susceptible Anagha (0.339 nmol/min/mg protein), Madakkatahra-1 (0.122 nmol/min/mg protein) and Raghav (0.083 nmol/min/mg protein), and the last two being on par with each other. While bugs infesting Raghav showed a significant reduction in enzyme activity over that at 24 h. Insect samples from both Damodar and Anagha exhibited significant increase in the enzyme activity over that at 24 h.

Tea mosquito bug fed on Damodar exhibited significantly higher enzyme activity of 0.379 nmol/min/mg protein at 72 h after release as well. Insect samples from Raghav (0.109 nmol/min/mg protein) as well as the highly susceptible Anagha (0.083 nmol/min/mg protein) and Madakkathara-1 (0.074 nmol/min/mg protein) showed comparable enzyme activity. Bugs from the less susceptible varieties showed increased enzyme activity over that at 48 h. while those exposed from highly susceptible varieties had lower enzymatic activity over that at 48 h, however the values were significant only in case of Damodar as well as Madakkathara-1. Without considering time intervals the mean specific activity of cytochrome P450 was observed highest for TMB released on less susceptible variety Damodar (0.372 nmol/mim/mg protein) and observed least for bug released on highly susceptible variety Madakkathara-1 (0.074 nmol/mim/mg protein).

Irrespective of varieties the mean specific activity reached highest value at 48 h following release of TMB (0.323 nmol/mim/mg protein) and lowest mean specific activity was observed for unfed bug (0.004 nmol/mim/mg protein).

Glutathione -S- transferases

The glutathione-S-transferases specific activity in TMB showed no significant variation after 6 h of release in any of the varieties.

As the exposure time reached 24 h there was a rapid and significant increase in GST activity of TMB released on less susceptible Raghav (1128.547 µmol/min/mg protein), whereas all the bugs released on other varieties *viz.*, Damodar, Anagha and Madakkathara-1 showed enzyme activity on par with each other as well as with the corresponding values after 6 h (Table 2).

After 48 h of exposure, peak enzyme activity was observed in TMB released on less susceptible Damodar (1559.604 µmol/min/mg protein), followed by insect samples from highly susceptible Anagha (481.211 µmol/min/mg protein). Bugs from Madakkathara-1 (252.222 µmol/min/mg protein) and Raghav (161.904 µmol/min/mg protein) were got enzyme activity on par with each other. While Damodar, Anagha and Madakkathara-1 showed significantly higher enzyme activity over that after 24 h. TMB from Raghav showed a steep reduction (161.904 µmol/min/mg protein).

The GST activity was found to be decreasing in all the bugs after 72 h of exposure except in case of that released on Madakkathara-1 (398.005 µmol/min/mg protein), which however were on par with Damodar (375.119 µmol/min/mg protein). The reduction from the enzyme activity at 48 h was significant in case of bugs fed on all varieties except Raghav.

Irrespective of time intervals the mean specific activity of GST in TMB recorded the highest value of 501.879 µmol/min/mg protein in bug released on less susceptible variety Damodar. The lowest mean specific activity was observed for TMB released on highly susceptible variety Madakkathara-1 (198.268 µmol/min/mg protein).

Irrespective of the varieties the mean specific activity of GST observed the highest value at 48 h of release (613.735 µmol/min/mg protein) and observed least value at 6 h of release (172.501 µmol/min/mg protein).

4. Discussion

Cytochrome P450

The increased monoxygenase activity in *H. theivora* female fed on *Mikania micrantha* and *Psidium guajava* than on *Cammelia sinensis*. This difference was due to variation in the levels of xenobiotics among these plants. This enzyme has got a wide substrate range and which includes an array of allelochemicals [9]. CytochromeP-450 has got a role in metabolism of wide variety of xenobiotics and secondary metabolites in host plants. This enzyme is involved in transfer of oxygen from molecular oxygen to a substrate, and also they involved in the reduction of other oxygen atom into water. Since these enzymes are involved in both oxidation and reduction reaction they are known as mixed function oxidase [10].

There was increased enzyme activity in TMB released on Damodar following 72 h of release. The phenol content in Damodar also found to be high at 72 h (79.273 mg/g). This finding is in agreement with study conducted by (Feyereisen, 1999) [11]. Where it was observed that, enhanced activity of detoxifying enzyme would help to increase the solubility of plant secondary metabolites and there by aid in the elimination of such toxic metabolites entered inside the body of insect. Another study conducted by (Chandra *et al*.,2016) [12] found that expression levels of cytochrome P450s positively correlate with concentration of gossypol in *Helicoverpa armigera*.

Glutathione-S- transferases (GSTs)

GSTs are a group of multifunctional enzymes, which are involved in conjugation of glutathione and xenobiotic substances for detoxification and protection from oxidative damage [13]. It would catalyse the conjugation of electrophilic compound with reduced glutathione (GSH).

The highest GST activity was recorded in less susceptible Damodar which has got higher secondary metabolites. Another study coinciding with this result has been conducted by Yu (1983) [14], where GST inductions in response to the presence of plant allelochemicals in artificial diets of lepidopteran species *Spodoptera frugiperda* were observed.

The enhanced enzyme activity in TMB revealed its polyphagous nature and the reason for the lack of a completely resistant variety in cashew. Understanding the detoxification mechanism of TMB to variations in secondary metabolites would help to develop alternate pest management strategies.

Further studies with more varieties are needed for the development of biochemical markers and the survival and fitness of TMB reared on each variety should be studied in order to confirm that whether the energy costs for detoxification sacrificed the survival and fecundity of TMB.

In this experiment, the GST activity in TMB was increased after feeding on different cashew varieties, when compared to the enzyme activity before releasing them on their host plants. This result was in conformity with the findings of (Francis *et al*. 2005) [4]. They observed that the activity of GST enzyme in *M. persicae* (green peach aphid) increased in response to secondary metabolites from brassica plants.

Table 1: Variation in glutathione-S-transferase activity (µmol/min/mg protein) of TMB after exposure to selected cashew varieties

Varieties	Mean GST activity (µmol/min/mg protein) after different hours of feeding					Mean
	0 h	6 h	24 h	48 h	72 h	
Less susceptible varieties						
Raghav	211.464	178.297	1128.547	161.904	146.102	365.262
Damodar	211.421	224.105	139.147	1559.604	375.119	501.879
Highly susceptible varieties						
Anagha	211.335	171.522	102.619	481.211	205.267	234.391
Madakathara-1	211.464	116.081	13.569	252.222	398.005	198.268
Mean	211.421	172.501	345.971	613.735	281.123	

CD for varieties : 50.894
CD for period of infestation : 56.901
CD for variety x period of infestation : 113.802

Table 2: Variation in cytochrome P450 activity (nmol/min/mg protein) of TMB after exposure to selected cashew varieties

Varieties	Mean cytochrome P450 activity (nmol/min/mg protein) after different hours of feeding					Mean
	0 h	6 h	24 h	48 h	72 h	
Less susceptible varieties						
Raghav	0.004 (0.064)*	0.091 (0.301)	0.430 (0.638)	0.083 (0.286)	0.109 (0.329)	0.143 (0.324)
Damodar	0.004 (0.064)	0.545 (0.665)	0.186 (0.429)	0.749 (0.856)	0.379 (0.613)	0.372 (0.526)
Highly susceptible varieties						
Anagha	0.004 (0.064)	0.091 (0.301)	0.047 (0.213)	0.339 (0.580)	0.083 (0.286)	0.113 (0.289)
Madakkathara-1	0.004 (0.064)	0.073 (0.270)	0.096 (0.309)	0.122 (0.349)	0.074 (0.271)	0.074 (0.253)
Mean	0.004 (0.064)	0.200 (0.384)	0.190 (0.397)	0.323 (0.518)	0.161 (0.375)	

* √x +0.5 transformed values in parentheses.

CD for varieties : 0.078
CD for period of infestation : 0.087
CD for variety x period of infestation : 0.175

5. Conclusion

The role of insect detoxification enzymes in overcoming plant defence is well established. Analysing defensive enzymes is one of method to study the reason for tolerance in insects. Over production of detoxification enzymes were analysed in this study. Glutathione-S-tranferases (GST) and cytochrome P450 were found to be enhanced upon TMB feeding and showed variation with respect to the susceptibility status of the varieties. Cytochrome P450 activity was highest in TMB fed on less susceptible variety Damodar and observed least in TMB fed on highly susceptible varieties. GST activity was highest in TMB fed on Damodar and least in bug fed on highly susceptible Madakathara-1. The study revealed that, detoxification enzymes play a significant role in mediating interaction between cashew and its herbivore, the tea mosquito bug. The enhanced enzyme activity in TMB revealed its polyphagous nature and the reason for the lack of a completely resistant variety in cashew. Understanding the detoxification mechanism of TMB to variations in secondary metabolites would help to develop alternate pest management start.

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