Journal of Entomology and Zoology Studies

Available online at www.entomoljournal.com

E-ISSN: 2320-7078
P-ISSN: 2349-6800
JEZS 2019; 7(4): 51-56

Received: 25-05-2019
Accepted: 27-06-2019

Nimisha T
Msc. Agricultural Entomology, College of Horticulture Vellanikkara, Thrissur, Kerala, India

Deepthy KB
Assistant Professor, Department of Agricultural Entomology, College of Horticulture Vellanikkara, Thrissur, Kerala, India

Madhu Subramanian
Professor and Head, AICRP on BCCP, College of Horticulture Vellanikkara, Thrissur, Kerala, India

Mani Chellappan
Professor and Head, Department of Agricultural Entomology, College of Horticulture Vellanikkara, Thrissur, Kerala, India

Smitha MS
Assistant Professor, Department of Agricultural Entomology, College of Horticulture Vellanikkara, Thrissur, Kerala, India

Correspondence
Nimisha T
Msc. Agricultural Entomology, College of Horticulture Vellanikkara, Thrissur, Kerala, India

Carboxyl esterase enzyme- biochemical armour in mediating host defence mechanism of tea mosquito bug (*Helopeltis antonii* Signoret Miridae: Hemiptera)

Nimisha T, Deepthy KB, Madhu Subramanian, Mani Chellappan and Smitha MS

Abstract
Tea mosquito bug (TMB*)*, *Helopeltis antonii* Signoret Miridae: Hemiptera) is the major pest of cashew. The present study was conducted in 2018 at College of Horticulture, Department of Agricultural Entomology, Kerala Agricultural University. Biochemical responses of TMB to different cashew varieties belonging to both less susceptible and highly susceptible groups were analyzed in this experiment. The insect biochemical parameters such as total protein and detoxification enzyme activity (carboxyl esterase) of TMB, infested on selected cashew varieties were analyzed before release (0 h) as well as during different time intervals of feeding. The SDS PAGE profiling of total TMB protein at various time intervals revealed the occurrence of different pattern with notable variation in the appearance of protein bands. Comparative studies on detoxification enzyme levels of TMB revealed that there was a rapid increase in the activity of carboxylesterase in bug released on less susceptible varieties Raghav (258.117 µmol/min/mg protein) and Damodar (208.916 µmol/min/mg protein) when compared to the bugs released on highly susceptible varieties such as Madakkathara-1 and Anagha. These findings imply that exposure of insect towards different levels of secondary metabolites and defense enzymes of host plant will result in variation in activity of detoxification enzyme carboxyl esterase and expression of total insect protein. Results of the study will help to open up new avenues in tea mosquito bug management.

Keywords: *Helopeltis antonii*, carboxyl esterase, SDS-PAGE, protein, detoxification mechanism

Introduction
The tea mosquito bug (TMB) is the most destructive pest of cashew causing 30-50 per cent yield loss. During severe outbreak situation even 100 per cent yield loss was reported. The bug feeds on a wide range of host plants including cocoa, neem, annatto and tea, which is reflective of its ability to overcome a wide range of defence reactions of the hosts. Understanding how tea mosquito bug overcomes plant defence is critical for developing resistant varieties of cashew. Several studies have pointed out that insects greatly depend on detoxification enzymes to overcome xenobiotics. Enzymatic studies conducted by Saha *et al*. (2012) [1] observed that carboxyl esterases (GEs) activity was significantly higher in resistant Terai and Dooars populations of *Helopeltis theivora* as compared to susceptible Darjeeling population. Carboxyl esterases are one of the key components of insects xenobiotic defence system including insecticides [2]. In insects the esterase are associated with pyrethroid and organophosphate detoxification [3]. The elevated activity of esterase in *Bemisia tabaci* Gennadius is reported to be a major resistance mechanism against pyrethroid [4]. According to Li *et al*. (2007) [5], esterase deserves detailed attention because of the ubiquitous nature to confer insect with resistance to a range of chemical compounds. Experiments conducted by Karuppaiah *et al*. (2017) [6] revealed that the topical bioassay with pyrethroid insecticides in *Spodoptera litura* population collected from Varanasi found to be more susceptible when compared to Delhi and Sonepat populations and the highest esterase activity was also high in Delhi and Sonepat populations. Murthy *et al*. (2014) [7] conducted a study on insecticide resistance in *Cotesia vestalis* Haliday, a braconid endolarval parasitoid of the diamondback moth, showed that resistant population with elevated esterase activity. Abamectin-resistant strains of Colorado potato beetle showed significantly higher carboxylesterase activity than in the susceptible

strain [8]. Analysing defensive enzymes is one of the methods to study the reason for tolerance in insects. Variation of detoxification enzyme activity of TMB as well as change in insect protein content in response to plant secondary metabolites were analysed in this study.

Materials and Methods

Rearing of tea mosquito bug (Helopeltis antonii Sign.)

The tea mosquito bugs were collected from Cashew Research Station, Madakkathara, Kerala. Adult bugs were collected using test tubes, which were brought to the laboratory and released in pairs of males and females in cages (60×60×90 cm) for egg laying. Cashew seedlings with new flushes were provided for feeding and oviposition. Nymphs hatched out were collected and reared in separate boxes following standard protocol [9]. First generation adult females were collected in test tubes and prestarved for 3 hour. After the end of prestarvation, each adult insect was allowed to feed individually on to three month old grafts of selected varieties. Insect samples were taken at different time intervals (0, 6, 24, 48 and 72 hours) for studying host pest interaction.

Varieties used for the experiment

Three month old cashew grafts of four varieties were used for the study. The varieties selected included Anagha and Madakkathara-1, reported as highly susceptible to TMB as well as Raghav and Damodar reported as less susceptible to TMB.

Evaluating detoxification mechanism in tea mosquito bug

TMB adult females were collected before as well as at 6, 24, 48 and 72 hours after feeding and total protein and carboxyl esterase analysis were carried out as per the standard protocols [10, 11]. Total protein profiling was also carried out by following SDS PAGE.

Sample preparation

Individual adult female TMB was homogenized using pestle and mortar in 500µl of sodium phosphate buffer (pH 7.4). The homogenate was centrifuged at 10,000 rpm for 20 min at 40C. The supernatant was collected and stored in deep freezer (-200C) as 50 µl aliquots until estimation.

Protein estimation

Total insect protein was quantitatively estimated by following the method of Lowry *et al*. (1951) [10]. From the supernatant prepared 50 µl was taken and 2.5 ml reagent C (Reagent A: 2% sodium carbonate in 0.1N NaOH, Reagent B: 0.5% copper sulphate solution in 1% sodium potassium tartarate solution, Reagent C: Mixture of 50 ml of solution A and 1 ml of solution B, prepared just prior to use) was added. The reaction mixture was incubated for 10 min, following which 250 µl reagent D (Reagent D: Folin-ciocalteu reagent (FCR): The commercial FCR was diluted in 1:1 ratio with distilled water before use) was added and again incubated for 30 min. The readings were taken at 660 nm using spectrophotometer (Model: Carry -60 UV vis). The protein content was calculated from the standard graph prepared by using bovine serum albumin (fractionV) and expressed in mg/ml.

Protein profiling by sodiumdodecylsulfate- poly acrylamide gel electrophoresis (SDS- PAGE)

The total protein analysed by SDS-PAGE by following the standard protocol [12]. The gel concentrations used were 4 per cent stacking gel and 8 per cent resolving gel.

Gel casting

The gel plates were thoroughly cleaned with water and wiped with tissue paper. Master plate (10.5×10.5) and base plate (10.5×10.5) were assembled properly and fixed on casting unit. The leakage was checked by adding water in between the plates using a micro pipette. The water was removed and wiped with tissue paper. Resolving gel was prepared and suddenly poured into one corner of the glass mould to make a gel of 7cm height, without air bubble. For removing bubbles on the surface gently added a layer of water with micropipette. The overlay of water was decanted after polymerization (approximate 25-30 min) and pour stacking gel solution prepared as mentioned above and a well comb was introduced at the top of stacking gel and allowed to polymerize the gel for 30 min.

Sample loading

Sample protein (10µg/10µl) from crude insect homogenate was taken in eppendorf tube and boiled with treatment buffer/dye (5 ml dye for 15 ml sample) at 1000C for 5 min in a water bath. After, the comb was removed the wells were cleaned with double distilled water. The casted gel was mounted on electrophoretic apparatus. The electrophoresis tank buffer was added to buffer chamber of the electrophoretic apparatus. The samples were gently loaded in to each well using a micropipette. The marker protein (4µl) was also added in first well for the reference.

Running the gel

The electrophoretic apparatus was connected to power supply unit. Initially, a voltage of 60 V was applied (for stacking gel) until the dye enters the resolving gel. Then the voltage was increased to 100 V. The unit was allowed to run until the tracking dye reaches bottom of the gel (7cm). Then the power supply was stopped. The plates were separated carefully and the stacking gel removed. Resolving gel was carefully transferred to staining box by rinsing with water.

Silver staining

The gels was immersed in fixing solution and kept under shaking for 1 h. The same step was repeated by changing the solution and fixed for 14 h. The gel was then washed in ethanol (30 %) for 10 sec and repeated two times. Then it was washed with deionized water. The gel was then transferred to the pretreatment solution and taken out within 1 min. It was again washed in deionized water thrice for 30 sec, and impregnated in silver nitrate (0.2%) for 20 min in a shaker, followed by washing twice for 10 sec. It was dipped in developing solution for 5 -10 min until clear band appeared with appropriate intensity. The gel was quickly transferred in to stop solution for 30 min followed by washing twice in deionized water.

Carboxyl esterase assay

Esterase assay was carried out by the method described by van Asperen (1962) [11].

Preparation of α-naphthol standard

Stock solution of α-naphthol (10 mM) was prepared by dissolving α-naphthol 0.03605 g in 25 ml methanol. From this, working standards of varying concentrations (400 µmol, 800 µmol, 1200 µmol, 1600 µmol, 2000 µmol) were prepared by pipetting out different aliquots (10µl, 20 µl, 30 µl, 40 µl, 50 µl and 60 µl) and made up to 1 ml with methanol. To this

one ml of standard, 2 ml of extraction buffer (sodium phosphate buffer pH 7.4) was added. Phosphate buffer alone served as blank. The mixture was incubated at 30 0C for 30 min with constant stirring. Dye solution containing 22.5 mg fast blue RR salt in 2.25 ml distilled water and 5% SDS in distilled water (2:5 v/v) was added (0.05ml). The reaction mixture was incubated at 370C for 10 min for the colour development. The intensity of red colour was read at 600 nm absorbance in spectrophotometer. The calibration curve was prepared with OD values and corresponding concentration of alpha naphthol formed.

Sample preparation

Insect sample (3mg) was homogenized in sodium phosphate buffer (pH 7.4), and centrifuged at 10,000 rpm for 20 min at 40C to remove coarse materials. The supernatant was taken for enzyme assay. To 0.1 ml enzyme extract 1 ml 30 mM α-naphthyl acetate (enzyme substrate) dissolved in acetone (0.028 g α- naphthyl acetate in 5 ml acetone) was added. Other steps of enzyme analysis were carried out.

Results

Protein

The highest mean protein content of 0.895 mg/ml was observed in bug released on the less susceptible variety Raghav and the lowest protein was observed in TMB released on less susceptible Damodar (Table 1). The bugs released on less susceptible Raghav consistently had higher protein content compared to the unfed plants throughout the study except at 24 hour after release. The less susceptible Damodar had 54 per cent reduction in protein content when compared to the protein content of unfed TMB, while insect samples from Anagha registered significant reduction in protein content after 72 hour of exposure.

Protein profiling by SDS PAGE

Analysis of total crude protein from 0-24 hour old adult female TMB exposed to different time intervals (0, 6, 24, 48, and 72 hours) showed the distinct expression of about 3-5 stained protein bands with molecular weight ranging from 17-75 kDa.

The analysis also revealed that variation in level of expression was noticed in different samples at different intervals of exposure. Tea mosquito bug fed on cashew varieties showed distinct protein bands majority ranging from 11-17 kDa, 17-20 kDa, and 25-35 kDa compared to control (0 hour of exposure) which is very evident from the gel image as black coloured thick bands during all the exposure intervals (6, 24, 48, and 72 hours). In TMB fed on Raghav, additional band of 48-63 kDa were expressed after 48 and 72 hours of feeding. In case of TMB fed on Anagha, one additional band of 75-100 kDa was expressed during 72 hour of exposure.

Protein band ranging from 35-48 kDa was found to be over expressed in TMB fed on Madakathara-1 after 6 hour and one additional band of 48-63 kDa was expressed after 72 hour of exposure.

Carboxyl esterase activity

Bugs released on Damodar alone showed six fold increase in CaE activity over that of unfed bugs (278.683 µmol/min/mg protein) (Table 2). Twenty four hour after release, bugs from both Raghav and Damodar, with esterase specific activity of 859.683 and 500.464 µmol/min/mg protein respectively, showed significant increase over corresponding values at 6 hour after release. However Anagha and Madakkathara-1 recorded the lowest enzyme activities (32.674 µmol/min/mg protein and 55.463 µmol/min/mg protein respectively). The bugs exposed to the less susceptible Raghav again recorded the highest esterase activity (189.803 µmol/min/mg protein) at 48 hour after exposure. This was significantly superior to the corresponding enzyme activities of other three varieties, which were on par with each other. Seventy two hours after release, bugs exposed to all varieties except Anagha showed comparable esterase specific activity, and was significantly higher than that of Anagha (43.050 µmol/min/mg protein).

The mean esterase activity recorded highest for TMB released on less susceptible variety Raghav (258.117 µmol/min/mg protein) and observed lowest for TMB released on highly susceptible variety Anagha (53.377 µmol/min/mg protein).

Irrespective of varieties the highest mean enzyme activity observed at 24 hour of TMB release and lowest observed for unfed TMB.

Discussion

Insect protein

There was significant variation in protein content of bugs fed on different cashew varieties. Bugs exposed to the less susceptible Damodar alone showed an increase in protein content after six hours. Protein content showed a significant increase after 24 hour only in case of Anagha.

In spite of the pronounced difference in protein content of exposed bugs over time, there was a clear pattern in the response of both the sets of varieties. The protein content in bugs exposed to less susceptible Damodar and Raghav was lowest after 24 hour before registering an increase at 48 hour. On the other hand bugs released on highly susceptible Madakkathara-1 and Anagha recorded highest protein content after 24 hour, then there was a sharp drop followed by an equally sharp increase in protein content. But it would be premature to ascribe the above variation to any specific cause; it could be that the variations in protein content of bugs on highly susceptible varieties correspond to the feeding bout of tea mosquito bug.

After 6 hour of release insect samples from Raghav showed a significant increase in protein content, this could be due to increased feeding. The highest protein content was observed in bug fed on Anagha after 24 hour and in Madakkthara-1 after 72 hour. This could be due to enhanced detoxification enzyme activity.

Since crude protein of insect included metabolic protein as well as detoxification enzymes the exact role of insect protein towards over coming plant defence mechanisms could not able to reveal from this study.

Protein profiling by SDS PAGE in TMB

The electro phoretic pattern of total TMB protein at different time intervals revealed notable variations in the appearance of bands. In the case of whole body homogenate of single adult unfed female TMB (0-24 hour old), the protein bands of~63 kDa and 35-48 kDa were appeared. When the exposure time proceeded, protein bands ranging from 17-75 kDa appeared. This is in conformity with the study conducted by Ayyangar and Rao (1990) [13], where they observed that S. litura when exposed to azadirachtin showed disappearance of few protein bands and appearance of new bands. The reason behind variation in insect protein expression might be due to influence of plant products on endocrine glands of insect, which will cause changes in level of protein. Certain extra

bands appeared after exposure of insect to host plant. Chen and Levenbook (1996) [14] observed appearance and disappearance of protein bands during insect development. SDS PAGE results of three cereal aphid species suggest that three different protein bands might be corresponding to three subunits. The molecular weight of subunits ranged from 24,000 to 28,500 Da. As GST is made of several subunits it may be able to detoxify several allelochemicals [15]. All these studies are pointing towards the fact that protein profile in insects will vary depending on exposure of insects to certain xenobiotics. More detailed studies with Native PAGE has to be carried out, to identify the exact protein bands corresponding to the detoxification enzymes present in TMB.

Carboxyl esterase (CarE)

There was an overall increase in the CarE activity of studied bugs released on all the four varieties upon post feeding. This might be due to exposure of the insect to secondary metabolites present in the plant sap. Inductions of esterase based detoxification mechanism in presence of allelochemicals have been reported in several insects [16].

Bugs fed on both the less susceptible varieties Damodar and Raghav, showed significant increase in the enzyme activity upon post feeding, with peak values being registered at 24 hour after exposure. The enzymatic activity was much lower in case of bugs exposed to the highly susceptible Madakkathara-1 and Anagha. Peak CarE activity was reached only after 48 hour after exposure. This again is in agreement with the categorization of above varieties. The greatest resistance offered by the less susceptible Damodar and Raghav could be due to higher level of secondary metabolites in the varieties, which led to induced production of detoxifying enzymes by the bugs feeding on the varieties. TMB fed on less susceptible Raghav (859.683 µmol/min/mg protein) and Damodar (500.464 µmol/min/mg), having highest esterase activity at 24 hour of feeding meanwhile the phenolic content of these varieties are high. From this, it could be inferred that the insect utilizes most of its energy for detoxification of secondary metabolites present in plants. This is in agreement with the previous findings of Shah *et al*, (2014) [17], where they found that the general esterase activity of Helopeltis theivora increased when it was reared on secondary metabolite rich Mikania micrantaha Kunth when compared to that reared on tea. Another reason for increased specific activity of CarE is due to the induction of inactive forms present in insect upon exposure to secondary metabolites. Esterase present in insects has got different forms that will get induced in the presence of allelochemicals leading to changes in susceptibility of insect towards insecticides [12].

The mean specific activity of CarE in TMB infested on less susceptible varieties viz., Raghav and Damodar was found to be high when compared to TMB fed on highly susceptible Anagha and Madakathara-1. This could be due to the fact that less susceptible varieties are richer in tannins and phenols. The insect has to produce biochemical armours like detoxification enzymes to overcome plant defence. Any kind of stress viz., allelochemicals or insecticides may result in over production of esterase enzymes. These results are in agreement with the findings of Saha *et al*. (2012) [1], where they reported that enhanced esterase activity in H. theivora exposed to insecticidal spray when compared to bugs collected from non-sprayed area.

Conclusion

The role of insect detoxification enzymes in overcoming plant defence is well established. Analyzing defensive enzymes is one of method to study the reason for tolerance in insects. Over production of detoxification enzymes as well as change in insect protein content in response to plant secondary metabolites (tannin and phenol) were analysed in this study. Carboxylesterase (CarE), was found to be enhanced upon TMB feeding and showed variation with respect to the susceptibility status of the varieties. Even though, there was variation in level of total insect protein, since crude protein of insect included metabolic protein as well as detoxification enzymes, the exact role of insect protein towards over coming plant defence mechanisms could not able to reveal from this study. For that further studies with purification and exact identification of protein bands are required. The enhanced levels of detoxification enzymes in TMB indicate plasticity of the pest against host plant defense and chance of resistance development against synthetic insecticides.

Table 1: Variation in total insect protein of TMB on exposure to selected cashew varieties

	Mean protein content (mg/ml) after different hours of feeding					Mean
	0 h	6 h	24 h	48 h	72 h	
Less susceptible varieties						
Raghav	0.833	1.046	0.152	1.294	1.149	0.895
Damodar	0.833	0.689	0.491	0.621	0.382	0.604
Highly susceptible varieties						
Anagha	0.833	0.784	1.659	0.352	0.075	0.790
Madakathara-1	0.833	0.790	0.836	0.417	0.863	0.748
Mean	0.833	0.827	0.785	0.671	0.617	

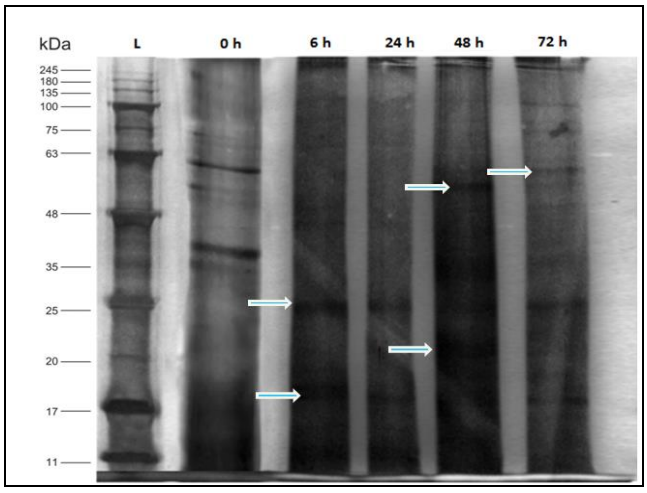
CD for varieties: 0.0933
CD for period of infestation: 0.1044
CD for variety x period of infestation: 0.208

Table 2: Variation in carboxyl esterase specific activity of TMB on exposure to selected cashew varieties

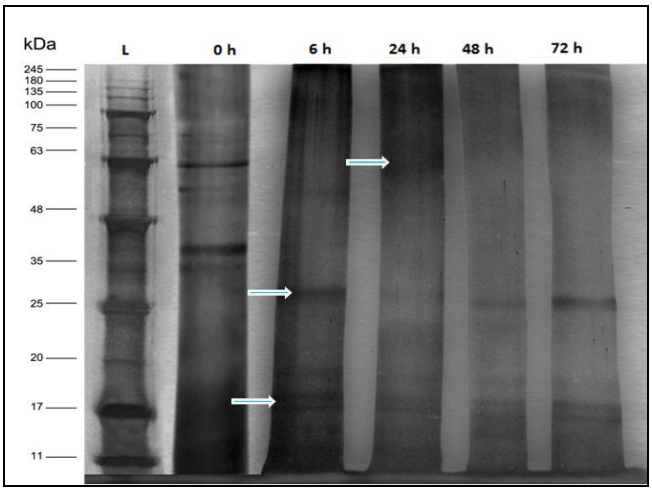
	Mean carboxyl esterase specific activity (µmol/min/mg protein) after different hours of feeding					Mean
	0 h	6 h	24 h	48 h	72 h	
Less susceptible varieties						
Raghav	47.319	60.244	859.683	189.803	133.522	258.117
Damodar	47.319	278.683	500.464	53.920	164.194	208.916
Highly susceptible varieties						
Anagha	47.319	55.178	32.674	88.666	43.050	53.377
Madakathara-1	47.319	55.981	55.463	88.526	141.728	77.804

Mean	47.319	112.521	362.071	105.229	120.624

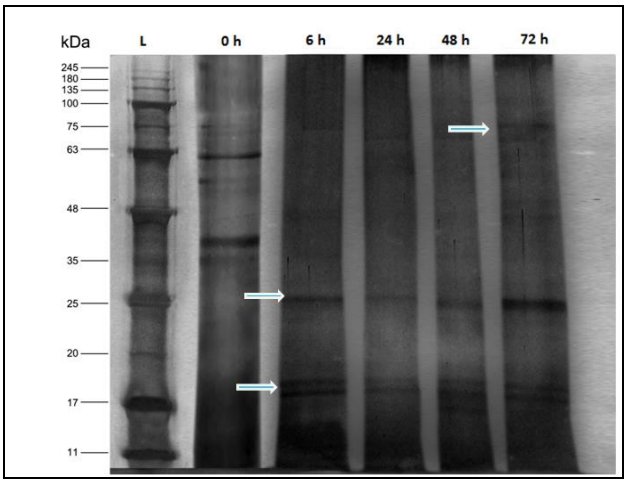
CD for varieties : 40.025
CD for period of infestation : 44.749
CD for variety x period of infestation : 89.498



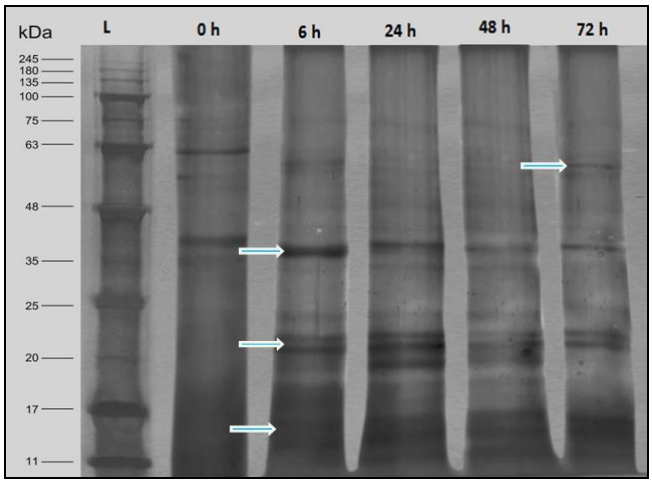
1a. Protein profile of TMB released on Raghav



1b. Protein profile of TMB released on Damodar



1c. Protein profile of TMB released on Anagha



1d. Protein profile of TMB released on Madakkathara-1

Plate 1: SDS PAGE for crude protein of TMB after feeding cashew varieties for different intervals
Lane L: Prestained protein ladder(5µl) Lane 1: control; Lane 2-6: protein isolated at different time intervals (6 h, 24 h, 48 h and 72 h)(10µl)

6. Acknowledgement
Authors are highly grateful to the Kerala Agricultural University for providing necessary facilities and fund during this research.

7. References

1. Saha D, Mukhopadhyay A, Bahadur M. Effect of host plants on fitness traits and detoxifying enzymes activity of *Helopeltis theivora*, a major sucking insect pest of tea. Phytoparasitica 2012; 40(5):433-444.
2. Oakeshott J, Claudianos C, Campbell PM, Newcomb RD, Russell RJ, Gilbert LI. Biochemical genetics and genomics of insect esterases. Comprehensive molecular insect sci. 2005; 5(1):43-50.
3. Liu Y, Zhang H, Qiao C, Lui X, Feng C. Correlation between carboxylesterase alleles and insecticide resistance in *Culex pipiens* complex from China. Parasite Vect. 2011; 4:228-236.
4. Young SJ, Gunning RV, Moores GD. Effects of pretreatment with piperonyl butoxide on pyrethroid efficacy against insecticide resistant *Helicoverpa armigera* (Lepidoptera: Noctuidae) and *Bemisia tabaci* (Sternorrhyncha: Aleyrodidae). Pest Manag. Sci. 2006; 62:114-119.
5. Li X, Schuler MA, Berenbaum MR. Molecular mechanisms of metabolic resistance to synthetic and natural xenobiotics. Annu. Rev. Entomol. 2007; 52:231-253.
6. Karuppaiah V, Srivastava C, Padaria JC, Subramanian S. Quantitative changes of the carboxylesterase associated with pyrethroid susceptibility in *Spodoptera litura* (Lepidoptera: Noctuidae). African Entomol. 2017; 25(1):175-182.
7. Murthy KS, Ramya SL, Venkatesan T, Jalali SK, Verghese A. Biochemical basis of insecticide resistance and determination of esterase enzyme patterns in field collected populations of *Cotesia vestalis* (Haliday) (Hymenoptera: Braconidae) from India. Annu. Biol. Res. 2014; 5(11):7-1.
8. Argentine JA, Clark M, Lin H. Genetics and biochemical mechanisms of abamectin resistance in two isogenic strains of Colorado potato beetle. Pestic. Biochem. Physiol. 1992; 44:191-207.
9. Sundararaju D, John JN. Mass rearing technique for

Helopeltis antonii Signoret (Hemiptera: Miridae)- an important pest of cashew. J Plant. Crops. 1992; 20(1):46-53.
10. Lowry OH, Rosenbrough NJ, Farr AL, Randall RJ. Protein measurement with the folin phenol reagent. J Biol. Chem. 1951; 193:265-275.
11. Van Asperen KA. Study of housefly esterases by means of a sensitive colorimetric method. J Insect Physiol. 1962; 8(4):401-416.
12. Laemmli UK. Denaturing (SDS) discontinuous gel electrophoresis. Nature. 1970; 277:680-685.
13. Ayyangar GSG, Rao PJ. Changes in haemolymph constituents of *Spodoptera litura* (Fabr.) under the influence of azadirachtin. Indian J Entomol. 1990; 52(1):69-83.
14. Chen PS, Levenbook L. Studies on the haemolymph proteins of the blowlfly Pharmia regina-l changes in ontogenetic patterns. J Insect Physiol. 1966; 12:1595-1609
15. Leszczynski B, Matok M, Dixon AFG. Detoxification of cereal plant allelochemicals by aphids: activity and molecular weights of glutathioneS-transferase in three species of cereal aphids. J chemical ecol. 1994; 20(2):387-394.
16. Yang X, Margolies DC, Zhu KY, Buschman LL. Host plant-induced changes in detoxification enzymes and susceptibility to pesticides in the two spotted spider mite (Acari: Tetranychidae). J Econ. Entomol. 2001; 94(2):381-387.
17. Shah S, Yadav RNS, Borua PK. Biochemical defence mechanism in *Camellia sinensis* against *Helopeltis theivora*. Int. J Plant Anim. Environ. Sci. 2014; 4(3):246-253.