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MODULATION IN CERTAIN BIOCHEMICAL CONSTITUENTS OF CASHEW AS INFLUENCED BY TEA MOSQUITO BUG *HELOPELTIS ANTONII* SIGN

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ABSTRACT

The tea mosquito bug (TMB) *Helopeltis antonii* Signoret is amajor constraint in cashew cultivation. Exploring host plant resistance will open up new avenues to manage this. The present study is the on biochemical changes occurring in four cashew varieties belonging to highly susceptible and less susceptible categories towards tea mosquito bug. The results reveal that total leaf protein was found to be more in the less susceptible variety Damodar (0.9925 mg g^{-1}) and the least with the highly susceptible Madakkathara-1 (0.6729 mg g^{-1}). Likewise, the total phenol and tannin were more in the less susceptible Damodar (69.834 mg g-1 and 4.276 mg g-1, respectively) and Raghav (67.207 mg g-1 and 4.420 mg g-1, respectively); with the least values being in the highly susceptible Anagha and Madakkathara-1.The activity of polyphenol oxidase was more in Damodar (0.003158 EU $g^{-1}min^{-1}$) and the least with Anagha (0.001406 EU $g^{-1}min^{-1}$). These observations on the biochemical changes conclude that there is significant variation in the infestation reactions of TMB in the highly susceptible and less susceptible cashew varieties. Also, the defensive molecules such as tannin, phenols and the defensive enzymes like polyphenol oxidase and phenyl alanine lyase were more in the less susceptible varieties Damodar and Raghav.

Key words: *Helopeltisantonii*, cashew, varieties, Damodar, Raghav, protein, tannin, phenol, polyphenol oxidase, phenyl alanine ammonia lyase, susceptibility

Cashew (*Anacardiumoccidentale* L.) is an important plantation crop suffers from low productivity, primarily due to the incidence of pests and diseases. Among the insect pests, tea mosquito bug (TMB), *Helopeltisantonii* Signoret (Hemiptera: Miridae) is the most important. It causes 30-50% yield loss and during outbreak situations even up to 100% loss had been reported (Devasahayam and Nair, 1986). Cashew varieties exhibit wide variation in response to TMB infestation. Hardly any variety has ever been recorded as resistant. However, a few varieties had been reported as capable of withstanding TMB infestation and hence grouped as less susceptible category (Ambika et al., 1979; Sathiamma, 1977). Several studies had been carried out on the variation in susceptibility of cashew varieties towards TMB. However, the biochemical basis of the above variability has not been studied. Understanding the variations in the biochemicals such as phenols, tannins and oxidative enzymes in the host plants indicates these interactions could contribute significantly to early detection of host plant resistance. The present study investigates the biochemical changes in the selected cashew varieties when infested by the TMB.

MATERIALS AND METHODS

Stock culture of TMB was established from the field collected (Cashew Research Station, Thrissur, Kerala) adults. The technique developed by Sundararaju and John (1992) was followed. Three months old cashew grafts of four cashew varieties, namely Anagha and Madakkathara-1 which had been earlier reported highly susceptible as well as Raghav and Damodar reported as less susceptible were selected.

Adult 0-24 hr old female bugs were collected from the laboratory population and starved for 3 hr. Each adult insect was released on to the grafts and were allowed to feed for 0, 6, 24, 48 and 72 hr. Physiologically mature leaves from the middle portion of grafts were taken at specific intervals. This is because the biochemical changes induced by herbivore are systemic in action; hence changes will be uniform in all plant tissues. The leaf sample weighed up to 150 mg, cleaned, wrapped in aluminium foil and freeze dried in liquid nitrogen. The freeze-dried samples were stored at -20^{0}C and were used for analysing variation in phenol, tannin, polyphenol oxidase (PPO) and phenyl alanine ammonia lyase (PAL) following exposure to TMB. Prior to analysis, each sample was ground in sodium phosphate buffer (7 ml, pH 6.5) with pestle and mortar. The homogenate was centrifuged at 10,000 rpm for 20 min at 4^{0}C. The supernatant obtained was used for estimation of leaf

protein, total phenols, tannins, PPO and PAL as per the standard protocols.

Total protein present in the leaf samples were estimated following Lowry et al. (1951). Standard curve of bovine serum albumin (fraction V) was prepared and amount of protein calculated from the graph. Tannin was analysed by vanillin hydrochloride method (Sadasivam and Manickam, 1992). To 1ml supernatant 5 ml of vanillin hydrochloride reagent was added (equal volumes of 8% hydrochloric acid in methanol and 4% vanillin in methanol). Readings were observed in a spectrophotometer (Model-Cary 60 UV vis) at 500 nm after 20 min. Standard graph was prepared with catechin. From the standard graph, the amount of tannin was calculated as per the absorbance values and expressed as catechin equivalents.

The total phenols were determined after Malik and Singh (1980). Folin- Ciocalteau reagent (0.5ml) was added to a test tube containing 0.1ml of sample solution and 2.9 ml distilled water. After heating the mixture for three min, 2 ml of Na_2CO_3 (20 %) was added to the test tube and the absorbance measured at 650 nm using spectrophotometer (Model-Cary 60 UV vis). The standard curve was prepared using known concentrations of catechol. The total phenol contents were calculated from the standard curve and expressed as mg. catechol equivalent of phenol/ g sample.

The PPO activity was analysed following Esterbaner et al. (1977). An aliquot of 0.2 ml was added to a cuvette containing 2.5 ml of 0.1M phosphate buffer (pH 6.5) and 0.3 ml of 0.01 M catechol solution and the readings were recorded using spectrophotometer at 495 nm. The change in absorbance was recorded for every 30 sec. up to 5 min. Enzymatic unit was calculated by using the formula:

Enzymatic unit in the test = $K\times (\Delta x/min)$, where, K is 0.272 for catechol oxidase and Δx is the change in absorbance.

Phenyl alanine ammonia lyase assay was carried out following Paul and Sharma (2005). For the analysis 500 µl of 0.5 M Tris HCl buffer was added followed by 500 µl of the enzyme extract and immediately after that 500 µl of 0.15 M L-phenyl alanine. The mixture was incubated at 37^0C for 60 min. The reaction was stopped by adding 500 µl of 1M trichloro acetic acid and incubated at 40 ^{0}C for 5 min. The absorbance was read in the spectrophotometer at 270 nm. Blank containing buffer and L-phenyl alanine was added after TCA. The rate of the reaction was expressed as mg of trans cinnamic acid formed/ g fresh weight from trans cinnamic acid calibration curve.

The data were subjected to statistical analysis using the statistical packages 'WASP'.1 developed by ICAR-Central Coastal Agricultural Research Institute, Goa.

RESULTS AND DISCUSSION

Adults of 0-24 hr old bugs were allowed to feed for different time intervals on selected cashew varieties and the leaf samples collected were subjected for estimation of total protein, phenol, tannin, PPO and PAL as per the standard protocols (Table1).

All the four varieties recorded decrease in leaf protein content after exposure to TMB, when compared to uninfested plants. Even though, the protein content showed variation during different time intervals, the extent of reduction at the end period of study was more in the highly susceptible Anagha (75.68%) and the least with the less susceptible Raghav (32.17 %). There was no correlation between the susceptibility of varieties and leaf protein content. The less susceptible Raghav and the highly susceptible Madakkathara-1, both registered consistent reduction in leaf protein following feeding. Simultaneously both the less susceptible Damodar and the highly susceptible Anagha revealed increase in protein content after an initial drop in the values. Protein existing as a structural component as well as in several other forms like hormones, enzymes etc. and hence it could be inferred that the leaf protein might have undergone either translocation or conversion to defensive enzymes. More detailed studies are required to identify a firm relation between total protein and TMB infestation.

While analysing secondary metabolites phenol and tannin it was noted that, prior to infestation of TMB, less susceptible varieties Damodar (63.960 mg/g and 6.657 mg/g respectively) and Raghav (61.641 mg/g and 10.335 mg/g respectively) revealed higher values compared to the highly susceptible ones. There was a significant reduction in tannin content of all four varieties after 6 hr of TMB infestation. At the same time phenol content was showing reverse trend, with increased phenol content in all the varieties except Anagha, which showed a reduction of 49%. Tannin content showed wide fluctuation after 48 hr. Damodar with a 4 folds increase over previous value, revealed more tannin content of 6.029 mg/g and Anagha with significant reduction (1.569 mg/g). While in case

Table 1. Effect of TMB infestation on cashew varieties

Varieties	After hours of feeding				
	0	6	24	48	72
Total protein (mg/g)					
Raghav	1.262	0.716	0.683	0.602	0.406
Damodar	1.852	0.694	0.415	0.938	1.063
Madakkathara-1	1.185	0.498	0.604	0.605	0.473
Anagha	1.094	0.503	0.720	1.028	0.828
Total phenol (mg/g)					
Raghav	61.641	72.831	67.223	67.745	66.593
Damodar	63.960	71.014	67.379	67.541	79.273
Madakkathara-1	45.055	68.747	67.709	54.844	67.847
Anagha	42.027	20.578	23.662	31.422	30.435
Tannin (mg/g)					
Raghav	10.335	2.077	5.436	1.033	3.223
Damodar	6.657	1.566	1.403	6.029	5.725
Madakkathara-1	4.975	3.464	0.7844	2.215	3.506
Anagha	4.682	0.887	3.801	1.569	2.820
PPO (EU/g/min)					
Raghav	0.00114	0.0014	0.00242	0.00804	0.00232
Damodar	0.00114	0.00317	0.00298	0.00511	0.00339
Madakkathara-1	0.00141	0.00131	0.00309	0.00367	0.00261
Anagha	0.00036	0.00083	0.00279	0.0018	0.00124
PAL (µmol/g/min)					
Raghav	0.00219	0.00432	0.00317	0.00376	0.00321
Damodar	0.00296	0.00397	0.00396	0.00346	0.00260
Madakkathara-1	0.00215	0.00421	0.00475	0.00430	0.00399
Anagha	0.00243	0.00396	0.00358	0.00411	0.00214

CD (variety x period) for protein: 0.0406; phenol: 6.902; tannin: 0.658; PPO: 0.000507; PAL: 0.000272

of phenol all the four varieties showed consistent increase during the same time interval. Phenols are secondary metabolites that play an important role in imparting resistance against herbivores (Chelliah and Sambandam, 1971). They generally bind with protein, consequently reducing the dietary protein availability to insects or inhibiting the enzyme activity (van Sumere et al., 1975). The present study showed an increase in the phenolics followed by infestation in three out of four varieties evaluated. This is in agreement with an experiment conducted by Kaur et al. (2017). They had reported that the enhanced phenol content in the aphid infested wheat genotypes.

The significantly low phenol content in the highly susceptible Anagha is indicative of the significance of phenolics in mediating cashew TMB interactions. However, the high levels of phenol in equally susceptible Madakkathara-1 suggest that there could be variation in phenol profile and that phenol might not be the most significant biochemical involved in defence. Since all the phenols are having harmful effect towards herbivores more detailed studies with chromatographic techniques are needed.

The two less susceptible varieties had significantly more tannin content than the highly susceptible Anagha

or Madakkathra-1. Similar observations on more tannin content in the less susceptible varieties are known- War et al. (2012) reported that the amount of total phenols and condensed tannin were more in the less susceptible varieties of groundnut against *Helicoverpa armigera*. Post feeding in all the four varieties exhibits regular fluctuations in tannin content, which could be due to higher proportion of hydrolysable tannins which could be translocated to younger tissues which are more vulnerable. Yet the overall tannin content was significantly more in the less susceptible varieties.

The defensive enzymes PPO and PAL had elevated activity in all the four varieties when compared to the uninfested plants. After initial hours of feeding, Damodar gave more value of 0.00317 EU/g/min and followed by Raghav (0.001441 EU/g/min). The highly susceptible Anagha (0.00083 EU/g/min) showed the least PPO activity. Phenyl alanine ammonia lyase, which is also a defensive enzyme in plant showed increased activity during the same hr. of infestation. After 24 hr of exposure to TMB, there was a significant increase in PPO activity in all the varieties except in case of Damodar. During this period, reduction in PAL activity was observed with the varieties Raghav, Damodar and Anagha when compared to previous values. But Madakkathara-1 showed a consistent increase over previous value.

In plant samples taken after exposure to TMB for 48 hr, the maximum PPO specific activity was observed in Raghav (0.00804 EU/g/min); highly susceptible Anagha gave the least (0.0018 EU/g/min). Forty eight hr after the release of TMB, both Raghav (0.00376 µmol/g/min) and Anagha (0.00411 µmol/g/min) showed increased PAL activity over that at 24 hr. Damodar and Madakkathara-1 with PAL values of 0.00346 µmol/g/min and 0.00430 µmol/g/min respectively on the other hand, revealed a reduction in enzyme activity.

On the whole, all the four varieties showed similar response to TMB infestation in terms of PPO. There was an increase in post infestation with peak activity being registered at 72 hr after infestation (except for Anagha). The less susceptible Damodar and Raghav gave comparatively more values of PPO throughout the study period as compared to the highly susceptible Madakkathara-1 or Anagha. PPO catalyse the oxidation of quinones which are toxic to herbivore and microbes (Raj et al., 2006). Helmi and Mohamed (2016) stated that the quinones formed by PPO caused alkylation of essential amino acids and thereby decreased the plant nutritional quality. Quinones also produce oxidative stress in the gut lumen of herbivores. Confirmation of the above role of PPOs and resultant anti-herbivory require further studies.

The PAL activity showed significant increase after infestation. This is in confirmation with the studies conducted by Suganthi et al. (2018) and Blondel et al. (1973); these workers observed increased PAL activity due to biotic as well as abiotic stresses. The reason for increased PAL activity might be due to production of new PAL enzyme as a result of hypersensitive reaction. It was also found that the PAL converted from inactive form to its active form due to stress condition.

PAL is a key enzyme in the phenyl propanoid pathway leading to production of the phenolics namely tannins and lignins. However, more PAL activity translating to increased resistance was not observed in the present study. Several studies had suggested that PAL mediated resistance is not much significant in higher plants like cashew which has got higher level of secondary metabolites (Taiz and Zeiger, 2010). Increased amount of PAL resulted in the synthesis of phenolic compounds as it catalyses the formation of phenolic secondary compounds from phenyl alanine, which is a product of shikimic acid pathway. This pathway will convert simple carbohydrate precursors in to three aromatic amino acids; phenyl alanine, tyrosine, and tryptophan (Herrmann and Weaver, 1999), which are very essential for animal nutrition since animals and insects are unable to synthesize aromatic amino acids (Taiz and Zeiger, 2010). Increased specific activity of PAL in the highly susceptible variety Madakkathara-1 could have contributed to the increased production of the heterocyclic aromatic amino acids that are providing the essential nutrients to feeding by TMB on this variety.

Thus, the present study revealed that the plant secondary metabolites like tannins, total phenols and the defence enzyme PPO have a definite role in imparting resistance to TMB in cashew. This would help to identify resistant varieties in perennial crops especially cashew and resistance screening can be done in the graftling stage itself. 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 energy costs for detoxification sacrificed the survival and feeding condition of the bug.

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