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RESEARCH ARTICLE

BIOCHEMICAL CHANGES INDUCED IN PUNGAM LEAVES BY ERIOPHYID MITE, *ACERIA PONGAMIAE*

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ABSTRACT

The leaf mite, *Aceria pongamiae* Chan. is host specific eriophyid mite infesting pungam leaves. This mite produces foliar galls on the adaxial surface of the lamina which is either epiphyllous or hypophyllous pouch gall; mostly lopsided with a short pedicel and widened body. Galls are mostly solitary but in later stages of growth it becomes dense, masking the entire leaflet and green in colour. Biochemical analysis of the mite infested leaves revealed that there was decrease in moisture content and chlorophyll content. Increase in total sugars and reducing sugars, total free amino acids, phenols, crude protein content and enzyme activities. Among the nutrients, nitrogen was found to increase while phosphorus, potassium, secondary and minor nutrients were found to decreased due to mite feeding.

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INTRODUCTION

India offers a great potential for bio-fuel production in farm sector due to availability of many species of non-edible oil bearing trees. The important non-edible oil species are neem, pungam, mahua and castor. Pungam offers a major scope for bio fuel production and about 10,000 tonnnes of pungam seeds are produced and sold annually across the states of South India (Naik *et al*., 2008). Pungam is also used in ayurvedic and siddha systems of Indian medicine for the treatment of human diseases (Shoba and Thomas, 2001). This economically important tree species is prone to attack by eriophyid mite, *Aceria pongamiae* causing galls on the adaxial surface of the lamina which is either epiphyllous or hypophyllous pouch gall; mostly lopsided with a short pedicel and widened body. Earlier study in plant galls caused by mites has indicated that the growth of gall tissues are associated with the changes in the total sugars, proteins, phenols, IAA and other enzymes (Arya *et al*., 1975). Hence, present studies were conducted to know the biochemical changes in the pungam leaves due to mite feeding.

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MATERIALS AND METHODS

Ten random samples of the healthy and mite infested leaf samples were collected from pungam trees grown at the Botanical gardens of Tamil Nadu Agricultural University, Coimbatore. The initial weight of the samples were recorded and then dried in a hot air oven at 105^0C until a constant weight was obtained and expressed as percentage of moisture content. In the fresh samples chlorophyll a, chlorophyll b and total chlorophyll contents were estimated following the method suggested by Yoshida *et al.* (1971) and expressed in mg g^{-1} fresh weight. Total sugar content was determined by anthrone method (Hedge and Hofreiter, 1962) and expressed in percentage. Reducing sugar was determined following Somogyi (1952) method and expressed in percentage. Total free amino acid was determined following Moore and Stein (1948) method and expressed as mg/g of sample. The method described by Malik and Singh (1980) was followed for the estimation of phenols and expressed as mg /100 g of material. The IAA oxidase activity was estimated following the method described by Gordon and Weber (1951) and expressed as µg of unoxidised auxin g^{-1} hr^{-1}. The method described by (Putter, 1974) was followed for estimation of peroxidase activity and expressed as change in optical density. The samples were dried in a hot air oven at 60°C, powdered in a Willy Mill and utilized for further analysis. The dried samples were subjected to analysis of macro, secondary micro nutrients and crude protein content. Nitrogen content in the leaf sample was estimated by micro-kjeldahl method as per the procedure given by Bremner (1965). This was expressed as percentage on dry weight basis. Total phosphorus content was estimated by triple acid digestion extract using photoelectric colorimeter with blue filter as described by Jackson (1973). The phosphorus content was determined by referring to a standard curve and computed value was expressed in percentage. Total potassium in the leaf sample was estimated from triple acid extract using flame photometer (Jackson, 1973) and the content was expressed in percentage. Protein content of leaf was calculated by multiplying the N content (Humphries, 1956) of leaves with the factor 6.25. Micronutrients *viz*., iron, manganese, copper and zinc were estimated from the triacid extracts using the Atomic Absorption Spectrophotmeter. Percentage analysis and paired 't' test were applied to analyze the data (Gomez and Gomez, 1994).

RESULTS AND DISCUSSION

The moisture content was 54.5 per cent in healthy leaves as against 40.7 per cent in mite affected leaves showing a significant decrease of 25.3 per cent. The total chlorophyll content in healthy leaves and galled leaves were 2.785 mg/g and 1.155 mg/g respectively. The reduction in the total chlorophyll content was significant between healthy leaves and mite infested leaves with a decrease of 58.5 per cent in galled leaves. Chlorophyll a content was 1.780 mg/g and 0.890 mg/g in the healthy and mite infested leaves respectively. The chlorophyll b content was 1.010 mg/g and 0.270 mg/g in healthy and mite infested leaves respectively. Chlorophyll a was on declining trend in mite infested leaves with 50.0 per cent reduction. The loss of chlorophyll b was also higher with 73.3 per cent reduction in galled leaves (Table 1). Decrease in the chlorophyll content might be due to damage of palisade tissue in the galled leaves. Total sugars and reducing sugars showed an increase of 1.995 and 1.160 per cent, respectively in mite infested leaves with while healthy leaves had 1.130 and 0.818 per cent total sugars and reducing sugars, respectively. There was significant increase in total sugars (76.5 per cent) and reducing sugars (41.8 per cent) in galled leaves (Table 2). Similarly, total free amino acid, phenols and crude protein contents also showed an increasing trend in mite infested leaves. The total free amino acid content was 368µg/g in healthy leaves and 874µg/g in galled leaves registering 137.5 percent increase in infested leaves. Phenol content was 599.855 mg/100g in healthy leaves and 967.665 mg/100g, recording significant increase of 61.3 per cent in mite infested leaves. The crude protein content was 7.875 per cent in healthy leaves while it was 12.43 per cent in mite infested leaves which was 57.9 per cent more when compared to healthy leaves (Table 3). The gall act as physiological sinks for supply of nutrients to the mites (Hori, 1992) and presumably increase in total sugars might be due to accumulation of sugars in the galled leaves. Increase in protein content and phenols act as defense in galling insects (Reinbothe *et al*., 1994). Similar changes in the total phenols and total free amino acids in jasmine due to feeding by *Aceria jasmini* Chan was observed by Rajagopal *et al*. (1970). Enzyme assay in mite infested leaves revealed significant increase in the activities of peroxidase and IAA oxidase when compared to healthy leaves (Table 4). The change in absorbance associated with peroxidase activity in healthy leaf sample was 0.792/min/g leaf sample and 1.077 min/g in mite infested sample. The unoxidised auxin associated with IAA oxidase activity was 0.160µg/g/hr in healthy leaves while it was 0.185µg/g/hr in mite infested leaves. Thus an increase of 35.6 and 15.6 per cent activity of peroxidase and IAA oxidase respectively was observed in mite infested leaves. The salivary secretions of mites might increase the auxin levels or perhaps act synergistically with IAA to cause galls which is in close finding of Hori (1992). The nutrient analysis revealed an increase only in the major nutrient, nitrogen, while all other major, secondary and minor nutrients showed a decreasing trend in mite infested leaves compared to healthy leaves (Table 5).

Table 1. Moisture content, chlorophyll a, b and total chlorophyll content of healthy and *Aceria pongamiae* infested pungam leaves

Particulars	Moisture content (%)	Chlorophyll a (mg g^{-1})	Chlorophyll b(mg g^{-1})	Total chlorophyll (mg g^{-1})
Healthy leaves	54.5	1.78	1.01	2.785
Infested leaves	40.7	0.89	0.27	1.155
% increase (or) decrease	-25.3	-50.0	-73.3	-58.5
S.E	2.95	0.093	0.042	0.274
't' value	4.675*	9.572*	17.449*	5.948*

* Significant at 5%

Table 2. Total sugars and reducing sugars of healthy and *Aceria pongamiae* infested pungam leaves

Particulars	Total sugars (%)	Reducing sugars (%)
Healthy leaves	1.13	0.818
Infested leaves	1.995	1.16
% increase (or) decrease	+76.5	+41.8
S.E	0.091	0.049
't' value	9.456*	6.846*

* Significant at 5%

Table 3. Total free amino acid, phenols and crude protein content of healthy and *Aceria pongamiae* infested pungam leaves

Particulars	Total free amino acid (µg/g)	Phenols (mg $100g^{-1}$)	Crude protein (%)
Healthy leaves	368	599.855	7.875
Infested leaves	874	967.665	12.43
% increase (or) decrease	+137.5	+61.3	+57.9
S.E	53.144	62.02	0.679
't' value	9.522*	5.931*	6.712*

* Significant at 5%

Table 4. Peroxidase and IAA oxidase activity of healthy and *Aceria pongamiae* infested pungam leaves

Particulars	Peroxidase (ΔOD min^{-1} g^{-1})	IAA oxidase (µg of unoxidised auxin g^{-1} hr^{-1})
Healthy leaves	0.792	0.160
Infested leaves	1.077	0.185
% increase (or) decrease	+35.6	+15.6
S.E	0.054	0.003
't' value	5.244*	9.320*

* Significant at 5%

Table 5. Macro nutrient content of healthy and *Aceria pongamiae* infested pungam leaves

Particulars	Total nitrogen (%)	Total phosphorus (%)	Total potassium (%)
Healthy leaves	1.26	0.442	2.103
Infested eaves	1.99	0.317	1.028
% increase (or) decrease	+57.9	-28.3	-51.1
S.E	0.108	0.024	0.126
't' value	6.712*	5.137*	8.539*

* Significant at 5%

Table 6. Secondary nutrient content of healthy and *Aceria pongamiae* infested pungam leaves

Particulars	Calcium (%)	Magnesium (%)
Healthy leaves	0.20	0.144
Infested leaves	0.12	0.096
% increase (or) decrease	-40.0	-33.3
S.E	0.006	0.004
't' value	13.256*	11.467*

* Significant at 5%

Table 7. Micro nutrient content of healthy and *Aceria pongamiae* infested pungam leaves

Particulars	Iron (ppm)	Manganese (ppm)	Copper (ppm)	Zinc (ppm)
Healthy leaves	0.70	4.60	1.30	NT
Infested leaves	0.52	1.30	1.00	NT
% increase (or) decrease	-25.7	-71.7	-23.1	-
S.E	0.014	0.176	0.027	-
't' value	12.341*	18.745*	11.162*	-

* Significant at 5%; NT – Not Traceable

The total nitrogen content was 1.26 per cent in healthy leaves as against 1.99 per cent with an increase of 57.9 percent in mite infested leaves. The total phosphorus content was 0.442 per cent in the healthy leaves and 0.317 per cent in the mite infested leaves, with a decrease of 28.3 percent. Potassium content in the healthy and mite affected leaves were 2.103 percent and 1.028 per cent respectively with 51.1 percent reduction due to mite feeding. Depletion of secondary nutrients *viz*., calcium and magnesium was observed due to mite feeding. Calcium content in the healthy leaves was 0.20 percent whereas it was 0.12 per cent in galled leaves, with a decrease of 40.0 per cent in infested leaves.

Magnesium content was 0.144 per cent in healthy leaves and 0.096 per cent in the mite affected leaves with a decrease of 33.3 per cent due to mite feeding (Table 6). The iron, manganese and copper contents were at 0.70 ppm, 4.60 ppm and 1.30 ppm respectively in healthy leaves, whereas it was 0.52 ppm, 1.30 ppm and 1.00 ppm respectively in the mite affected leaves. Zinc was not traceable in the samples studied. Iron, manganese and copper significantly decreased to an extent of 25.7, 71.7 and 23.1 per cent, respectively in galled leaves compared to healthy leaves (Table 7). According to Hartley (1998) stimulus of gall forming insects redirects the leaf resources to the galls which act as nutritive sinks. In conclusion, the changes in biochemical composition of pungam leaves due to mite feeding suggest their role in supply of nutrition to development of mites within the galls and and also aid in defense strategy against herbivory .

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