



Studies on biochemical components, secondary metabolites and photosynthetic pigments in *Mealey bug* infected *Terminalia arjuna*-a primary host plant of Tasar Silkworm *Anthereae mylitta drury*

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Abstract:

Mealey bug (*Maconellicoccus hirsutus*) is a serious pest of *Terminalia arjuna* which is the primary food for Tropical tasar silkworm *Anthereae Mylitta Drury*. It attacks the leaves of the host plant causing considerable damage which alters the leaf quality. An attempt was made to know the changes in the Biochemical components (Total soluble proteins, free amino acids, total reducing sugars, total soluble sugars), secondary metabolites (Starch, Phenols, Phytic acid, Acid detergent fibre) and photosynthetic pigments (total chlorophyll, chlorophyll- a, chlorophyll - b and carotenoids) in *Terminalia* leaves under infestation by Mealey bug. The results revealed that total soluble proteins and total soluble sugars increased by 17.8% and 31.28% while free amino acids and total reducing sugars were decreased by 38% and 64.97%. Total chlorophyll, chlorophyll- a, and chlorophyll- b were reduced significantly as 45.38, 45.95 and 41.94% respectively. The results also show a drastic reduction (44.9%) in the carotenoid content. The experimental results on quantitative estimation of secondary metabolites in mealey bug infestation showed significant increase (45.95%) in ADF and starch (59.5%) whereas phytic acid and phenols were reduced by 18.53 and 46.5% respectively.

Keywords: Biochemical components, secondary metabolites, photosynthetic pigments, Mealy bug, *Terminalia arjuna*.

Introduction:

Terminalia arjuna is the primary host plant of the Tropical Tasar silkworm *Anthereae mylitta drury* in commercial sericulture. Silkworms feed on leaves during their entire larval period and utilize the leaf metabolites for the biosynthesis of silk. It is therefore clear that host plant plays a dominant role in cocoon production as a source of nutrition to the silkworms. However, foliage is prone to depredation by disease

causing organism such as pathogens and pests. Among the several pests known to attack Tasar plants Mealy bug (*Maconellicoccus hirsutus*) has attained a serious status. It has long been recognized that levels of proteins and carbohydrates in plants have significantly influenced by infection and metabolism alters accordingly by the rate of infection [1]. Increase in protein concentration was noted in the various tissues infected with root knot nematodes [2]. An elevated protein levels was also observed in giant cells of okra infected with *M.incognita acrita* [3]. A significant increase in the total sugars and decrease in total reducing sugars of M5 mulberry leaves due to leaf roller infestation and mealy bug infection in hibiscus had been reported [4,5]. Decrease in the secondary metabolites like phenols and phytic acid and increase in starch and ADF (acid detergent fibre) has reported [6, 7] observed a combined loss of total chlorophyll and carotenoids in the thrips - infested mulberry leaves. So the present experiment has taken up to identify the levels of primary, secondary metabolites and photosynthetic pigments in mealybug infected *Terminalia arjuna* a primary host plant of Tasar silk worm *Anthereae mylitta drury*.

Materials and Methods:

The present experiment was conducted in the Department of Biotechnology, Gokaraju Rangaraju Institute of Engineering and Technology Bachupally, Hyderabad, Andhra Pradesh by collecting healthy and Mealy bug infected *Terminalia arjuna* leaves from the field of Central silk board, Chennai, Adilabad, Andhra Pradesh. The leaves were cleaned and then oven dried at 80-90°C for several hours till drying completely. The dried leaves were ground into powder in an electric grinder (Bajaj Mixer grinder). The powdered leaf samples were used to analyze total free

amino acid as ^[8], total proteins ^[9], total soluble sugars and starch ^[10], total reducing

Sugars ^[11]. Fresh *Terminalia arjuna* leaves were utilised to estimate the photosynthetic pigments (total chlorophyll, chlorophyll-a, chlorophyll - b) as Arnon ^[12] and carotenoids ^[13], Phenols ^[14], Phytic acid ^[15] and acid detergent fiber (ADF) ^[16].

Statistical Analysis: Each assay was replicated 3 times. Values were expressed as mean \pm SE of replication and Student's *t*-test was applied to locate significant ($P \leq 0.05$) differences between treated and untreated larvae. Critical differences (CD5%) was analyzed by Tukeys post hoc procedure

Results and Discussion:

Table 1 explains that infected terminalia had shown significant increase (17.8%) in protein content. High protein content correlates with activation of several lines of defense mechanism and release of disease resistant proteins in fungal infected plants ^[17] Increased rate of synthesis of proteins can be correlated with the increase in metabolic activities in infected tissues and addition of viral proteins ^[18]. Mesta plants infected with *yellow vein mosaic* disease have shown increase of disease related proteins because plant pathogens elicit the synthesis of host proteins which prevents the multiplication of pathogens in the host ^[19]. Infected terminalia had shown 38% decrease in its free amino acids. Decrease in the amino acids can be correlated with the increase in protein synthesis. The amount of free amino acids also decreased with time with grown gall infection in sunflower ^[20]. Infected *Terminalia* had shown 31.28% increase in total soluble sugars. The total soluble sugars were increased by 5% and 36% in the Kajali and Kanva-2 mulberry varieties respectively. High carbohydrate content correlates with high infections in plants. Alteration in the sugars may be due to reduction in leaf lamina and malformation of leaves in pest affected plants resulting in less productivity ^[21]. The total reducing sugar was reduced maximum by 64.97% in mealy bug infected terminalia in comparison with the healthy control. A significant reduction in the total reducing sugars in the leaves of K2, S30, S36, S40 and S54 mulberry varieties infested by *M. hirsutus* was also observed

Decrease of total reducing sugars in a highly infected host tissue is due to the excess utilization of sugars by the organisms for their growth and sustainability ^[22]. Decrease in carbohydrate content in the highly infected plant can be correlated with the increase in glucose 6-phosphate dehydrogenase activity and 6-phosphogluconate dehydrogenase in nematode infected soybean ^[23].

Table 1 Effect of Mealy bug infection on biochemical components of *Terminalia arjuna* leaves

Treatment	Total soluble proteins (mg/mg)	Free amino Acids (μ g/g)	Total soluble Sugars (mg/g)	Total reducing Sugars (mg/g)
Infected	132.68 \pm 2.54	6.89 \pm 1.02	2.56 \pm 0.07	43.25 \pm 2.58
Healthy control	112.65 \pm 2.48	11.13 \pm 1.05	1.95 \pm 0.8	123.46 \pm 3.26
CD 5%		0.25	0.12	1.16

CD Critical difference. Each value represents the mean \pm SEM of 3 different observations

Table 2 explains that in comparison with the control infected terminalia had shown a significant increase (59.5%) in the starch content. A significant increase of 27%, 36% and 38% starch content of mealy bug affected Kajali, Kanva-2 and Vietnam mulberry varieties were also reported. The results show that infested terminalia had reduced by 46.5% in its phenolic compounds when compared with the healthy. Significant decrease in total phenolic compounds was also reported in mulberry species attacked by leaf roller. High phenol content in the healthy terminalia leaves is due to high level of resistance as phenols supports the defence mechanism. Phytic acid content found decreased by 18.53% in infested Terminalia. The complexing of phytic acid with nutritionally essential elements and the possibility of interference with proteolytic digestion have been suggested. Thus the reduction in phytic acid content can be correlated with the decrease in proteolytic activity thus an increase in protein content and decrease in amino acids. Acid detergent fibre found increased significantly by 45.95% in infested leaves over

control. Fibre is the ash free material and reduction in the fibre content increases the silkworm crop yield by 30% increase in the feeding ability of silkworm.

Table 2 Effect of mealy bug infection on secondary metabolites of *Terminalia arjuna* leaves

Treatment	Starch (mg/g)	Phenols (mg/g)	Phytic acid (mg/100g)	ADF (%)
Infected	1.85±0.0 6	1.53±0.0 5	1965.25±12.4 5	28.6 8
Healthy control	1.16±0.0 6	2.86±0.0 6	2412.15±9.86	19.6 5
CD5%	0.06	0.04	3.26	0.56

CD Critical difference. Each value represents the mean ± SEM of 3 different observations..

Table 3 explains that total chlorophyll content reduced by 45.38% in the infected leaves when compared with the healthy whereas, chlorophyll a and b got reduced by 45.95% and 41.94%. Significant decrease in the total chlorophyll, chlorophyll – a and chlorophyll – b in the leaves of six mulberry varieties infested by *D. pulverulentalis* was reported^[24]. A reduced amount of total chlorophyll, chlorophyll – a and chlorophyll – b by 32.40, 31.98, 32.87% respectively in the leaf roller infested mulberry plants were also observed. Carotenoids found reduced by 44.9% in mealy bug infestation. The significant decrease of carotenoids in leaf roller infested mulberry variety was due to malformation of leaves. Decrease in chlorophyll and carotenoid contents in infested leaves may be due to loss in chlorophyll synthetase activity.

Table 3 Effect of mealy bug infection on photosynthetic pigments of *Terminalia arjuna* leaves

Treatment	Total Chlorophyll (mg/ml)	Chlorophyll-a (mg/ml)	Chlorophyll-b (mg/ml)	Carotenoids (µg/ml)
Infected	1.18±0.2 5	1.0±0.04	0.18±0.0 2	0.54±0.0 6
Healthy control	2.16±0.3 4	1.85±0.0 8	0.31±0.0 4	0.98±0.0 4
CD5%	0.04	0.06	0.005	0.004

CD Critical difference. Each value represents the mean ± SEM of 3 different observations

Conclusion:

Thus in conclusion, mealy bug infection in *Terminalia arjuna* decreased significantly the components like aminoacids, total reducing sugars, phenols, phytic acid, total chlorophyll-a, chlorophyll-b and carotenoids whereas total soluble proteins, total soluble sugars, starch and ADF (acid detergent fibre) were increased profoundly.

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References:

[1] Kirk, J.T.O. and Allen, R.L. (1965). Dependence of chloroplast pigment synthesis on protein synthesis: Effect of acsiodine biochemistry, biophysics, research communication, Vol 21 p530-532.

[2] Sailaja, D., Lakshmi, V., Venkatapaveen, M., Raghavarao, D., Jagadeesh, S., T. Ravikumar, T., Adithyayadav, M. and Purushothama Chary, T. (2012). Estimation of levels of biochemical components in mealy bug (*Maconellicoccus hirsutus*) infected stem of *Hibiscus rosa sinensis*. International journal of applied biology and pharmaceutical technology, Vol 3(3) pp.410-416.

[3] Lowry, O.H., Rosebrough, N.J., Farr, A.L. and Randa ll, R.J. (1951). Protein measurement with the folin phenol reagent. Journal of biological chemistry, Vol 193 pp.265-275

[4] Neog, K., Das, A., Unni, B.G., Ahmed, G. U and Rajan, R. K. (2011). Studies on secondary metabolites of *Som (Percea bombycina kost)* a primary host plant of muga silkworm (*Anterea assamensis helfer*). International journal of pharma tech research, Vol 3(3) pp.1441-1447.

[5] Sailaja, D., Lakshmi, V., Srilakshmi, P., Chowdhury, A. C.P., Srikanth, C., Sangameshwaran, B.B. and Venkatapaveen, M. (2012). Impact of mealy bug (*Maconellicoccus hirsutus*) infection on biochemical components of

Hibiscus rosa sinensis. International journal of applied biology and pharmaceutical technology, Vol 3(4) pp.233-236.

[6] Owens, R.G. and Specht, H.N. (1966). Biochemical alterations induced in host tissues by root knot nematodes. Boyce Thompson Institute, Vol 23 pp.181-198.

[7] Das, C., Shivanathrao, K.N., Ghosh, P.L., Senguptha, D., Senguptha, K. (1994). Biochemical changes due to thrips infestation in mulberry. National symposium on "Plant science in ninteens", University of Kalyani, APSR, pp. 4-5.

[8] Narayanaswamy, K.C. (2003). Biochemical composition of leafroller infested mulberry leaf Insect environment, Vol 8(4) pp.166-167.

[9] Mahadeva, A. and Nagaveni, V. (2011). Alterations in biochemical components and photosynthetic pigments of mulberry (*Morus Spp.*) attacked by leaf-roller (*Diaphanipulverulentalis*) pest. African journal of biochemical research, Vol 5(14) pp.365- 372.

[10] Yemm, E. W. and Willis, A. J. (1954). The estimation of carbohydrates in plant extracts by anthrone. Biochemistry journal, Vol 57 pp.508-514.

[11] Moore, S. and Stein, W. W. (1948). Amino acid free photometric ninhydrin method for use in the chromatography of amino acid. Journal of biological chemistry, Vol 176 pp.367-388.

[12] Arnon, D. J. (1949). Copper enzymes in isolated chloroplasts, polyphenoloxidase in *Beta vulgaris*. Plant Physiology, Vol 22 pp.1-15.

[13] Litrell, R. H. (1966). Cellular responses of *Hibiscus esculentas* to *Meloidogyne incognita acrita*. Phytopathology, Vol 56 pp.540-544.

[14] Bray, H. G. and Thorpe, W. V. (1954). Analysis of phenolic compound of interest in metabolism. Methods of Biochemistry Analysis, Vol(1) pp.27-52.

[15] Wheeler, E. L. and Ferrell, R. E. (1971). A method for phytic acid determination in wheat and wheat flour. Cereal chemistry, Vol 48 pp.312-316

[16] Wheeler, E. L. and Ferrell, R. E. (1971). A method for phytic acid determination in wheat and wheat flour. Cereal chemistry, Vol 48 pp.312-316.

[17] Hwang, B. K., Ibenthal, W. D. and Heitefuss, R. (1983). Age, rate of growth, carbohydrate, amino acid of spring barley plants in relation to their resistance to powdery

mildew (*Erisiphegraminis f.sp.hordei*). Physiology and plant pathology, Vol 22 pp.1-14.

[18] Charithadevi, M. and Radha, Y. (2012). Induced biochemical changes in CMV infected cucurbit plants. Analysis of biological research, Vol 3(2) pp.863-870

[19] Datta, K., Muthukrishnan, S. and Datta, S. K. (1999). Expression and function of PR protein genes in transgenic plants. In Datta, S. K. (Ed). Pathogenesis related proteins in plants, CRS press, Boca Raton pp.261-291.

[20] Behki, R. M. and Lesley, S. M. (1968). Free amino acid of sunflower hypocotyl tissue during crown gall tumor development. Canadian Journal of botany, Vol 46(3) pp.217-223.

[21] Vansoest, P. J., Robertson, J. D. and Lewis, B. A. (1991). Methods for dietary fibre, neutral detergent fibre and non starch polysaccharides in relation to animal nutrition. Journal of dairy science, Vol 74 , pp.3583-3597.

[22] Shree, M. P. and Umesh, K. N. N. (1989). Biochemical changes in tukra affected exotic mulberry plant. Current science, Vol 58(22) pp.1251-1253.

[23] Horsfall, J. G. and Dimond, A. E. (1957). Interactions of tissue sugar growth substances and disease susceptibility. Z. Pflanzenkr. Pflanzenschutz, Vol 64 pp.415-421.

[24] Miller, G. L. (1972). Use of dinitrosalicylic acid reagent for determination of reducing sugar. Analytical chemistry, Vol 31 pp.426- 428.
