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Original Research

An investigation on the changes in nutrient contents in mealy bugs infested mulberry foliage

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ABSTRACT:

The occurrence of pink mealy bugs (*Maconellicoccus hirsutus* Green) on mulberry (*Morus sp.)* considerably reduces the leaf yield and in turn damages its quality. Hence, an attempt was made to know the changes in biochemical constituents and photosynthetic pigments in mealy bugs infested mulberry foliage. The pest infested mulberry showed a variation in their contents. The nutritive status of the pest infested mulberry leaves may be altered adversely, and feeding such leaves to the silkworm may alter the growth and development of the larvae which inturn affect the quality and quantity of silk production.

Keywords:

Biochemical constituents, mealy bugs, mulberry, photosynthetic pigments.

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INTRODUCTION

Mulberry (*Morus sp.*), the food plant for silkworm *Bombyx mori* L. is of great importance is sericulture industry. The pink mealy bug (*Maconellicoccus hirsutus* Green) is one of the major pests of mulberry, causing severe damage and recurring loss in the leaf yield of about 3000-6000 kg/hectare/year (Kumar *et al.,* 1989). The pest occurs in both irrigated and rain fed gardens causing a characteristic disease – tukra. The feeding behavior (sucking) of mealy bugs results in the curling and crinkling of leaves of apical shoots, swelling and twisting of apical internodes. As a result, the shoots become brittle and the leaves become dark green in colour and deformed. The pest is found to inhabit the folds and knots of the crumpling (Handique and Baruah, 2000). The morphological changes in the tukra affected plant includes arrest of linear growth of the stem, followed by petiole thickening. The lamina of the leaf was found markedly reduced and distorted, ultimately leading to premature leaf fall (Chatterjee and Sarkar, 1993). Immature and mature mealy bugs are found in clusters on the stalks under overlapping leaf-sheath, below the node and spread up and down to the other internodes and buds. The large amount of honey dew secretion by mealy bugs hosts a sooty mould fungus and acts a a vital role in virus transmission (Eid *et al.,* 2011). Cell sap sucking nature of the mealy bugs leads to stunted, yellowing and thin canes of mulberry and ultimately deprived of essential nutrients in the infested plant parts. The present study was taken up to determine the impact of mealy bug's infestation on the biochemical constituents and photosynthetic pigments in the leaves of some popular indigenous mulberry varieties.

MATERIALS AND METHODS

Commercially cultivating six mulberry varieties i.e. M_5, MR_2, Mysore local, S_{36}, S_{54} and V_1 for feeding silkworm were used in the present study. The healthy pink mealy bugs – *Maconellicoccus hirsutus* Green infested leaves were collected from the mulberry gardens situated in and around Bangalore rural (Kanakapura and Ramanagaram taluk) and Tumkur district (Karnataka State, India). The leaves were oven - dried and processed to analyze the free amino acids by Ninhydrin method (Moore and Stein, 1948) and total soluble proteins (Lowry *et al.,* 1951). The reducing sugars were estimated by using DNS (Dinitro-salicylic acid) method (Miller, 1972), while the soluble sugars and starch content were determined by anthrone method (Yemm and Willis, 1954). Folin-ciocalteau was used to determine the total phenols (Bray and Thorpe, 1954). The photosynthetic pigments, chlorophyll and carotenoids were estimated in healthy and mealy bug infested fresh mulberry leaves (Arnon, 1949; Mahadevan and Sridhar, 1986). The results were analysed statistically by applying Student's t -test.

RESULTS AND DISCUSSION

The six biochemical components i.e., free amino acids, total soluble proteins, total soluble sugars, total reducing sugars, starch and total phenols, and photosynthetic pigments *viz*., total chlorophyll, chlorophyll – a, chlorophyll – b, chlorophyll – a/b ratio and carotenoids showed variation in the mealy bugs infested mulberry leaves at all the three maturity levels (tender, medium and coarse) compared to the healthy ones.

Biochemical components (Table – 1)

Free amino acids

The free amino acid contents were decreased in the pest – infested leaves of M_5, MR_2, Mysore local and S_{54} varieties. But, it was significant in the leaves of M_5 variety. The reduction was negligible (0.73 %) in leaves of Mysore local and maximum (14.79 %) in M_5. But, there were no alteration in the free amino acids in the leaves of S_{36} and V_1 variety due to pest attack.

The mulberry leaves are quite rich in amino acid content and therefore satisfy the amino acid requirements

of silkworm (Bose and Majumdar, 1989). In mulberry leaves, the number of amino acids available is twenty. There was an increase in total free amino acids in mealy bugs (*M. hirsutus*) infested mulberry varieties *viz.*, M_5, MR_2, BC_{259}, Tr_4, S_{13} (indigenous), Kosen, Ichinose and Goshoerami (exotic) (Babu *et al.,* 1994).

Total soluble proteins

The total soluble proteins were significantly decreased in the tukra affected leaves of MR_2 variety (2.33 %). The total soluble proteins increased significantly in the leaves of M_5 (2.39 %) and V_1 varieties and non-significantly in the S_{36} (4.26 %) variety. The total soluble proteins were not altered in the leaves of Mysore local and S_{54} varieties due to mealy bugs - infestation.

Shree *et al.* (1989) observed a 10.50 % reduction and 40.00 % increase in the total soluble proteins of tukra affected leaves in Kajli and Kanva-2 mulberry varieties respectively. Shree and Kumar (1989) found that the protein contents were decreased in *M. macroura* and *M. nigra*; the reduction was, however, negligible in *M. nigra*. An increase was observed in the total proteins due to tukra in M_5 and DD varieties (Veeranna, 1997). Damage caused by the insect during infestation altering the metabolic functions and thus, leading to either decline in protein synthesis or mobilization of proteins for repair of the damaged tissues in order to develop resistance to insect bite, may cause the decrease in the crude protein contents. Increase in the protein content may be due to changes in the protein synthesis pattern to overcome the injury and develop resistance (Prasad, 2002; Mahadeva and Nagaveni, 2011).

Total reducing sugars

Alteration was noticed in the total reducing sugars of *M. hirsutus* infested mulberry leaves compared to healthy one. There was a significant reduction in the leaves of M_5, MR_2, Mysore local, S_{36} and V_1 varieties. A maximum (22.22 %) reduction was found in the leaves of Mysore local and minimum (2.56 %) in the leaves of S_{36} variety. There was a negligible (0.78 %) increase in the leaves of S54.

There was a significant increase in the tukra affected *M. macroura*. In *M. cathayana* and *M. nigra*, it was unaltered. Alteration in the reducing sugars may be due to reduction in leaf lamina and malformation of leaves in pest affected plants resulting in less productivity (Shree and Kumar, 1989).

Total soluble sugars

There were changes in the total soluble sugars of mealy bugs infested mulberry leaves. The total soluble sugars reduced significantly in the leaves of M_5 (5.95 %) and non-significantly in V_1 (3.57 %) variety. But, no alteration was noticed in the total soluble sugars of pest attacked leaves of MR_2, Mysore local, S_{36} and S_{54} varieties.

The tukra affected leaves of *M. australis, M. cathayana* and *M. nigra* varieties shows a marginally decreased sugar content. However, there was an increase in *M. macroura* (Shree and Kumar, 1989). Kumar *et al.* (1990) noticed changes in the sugar content of mealy bugs infested leaves of four indigenous (Berhampore, S_{30}. S_{31} and S_{36}) and six exotic (Kosen, *M. multicaulis*, Philippine, Okinawa-2, Tsukasaguwa and Italian) mulberry varieties. There was increased sugar content in Berhampore, Okinawa-2 and Philippine varieties; Whereas, it is decreased in Italian, Kosen, *M. multicaulis,* S_{30}, S_{36} and S_{41} varieties. No difference was observed in Tsukasaguwa. An increase in the soluble sugar content was recorded in the tukra affected leaves of M_5, MR_2, BC_{259}, S_{13}, Kosen and Goshoerami. However, in Tr_4 and Ichinose, it was decreased as noted by Babu *et al.* (1994). Mahadeva and Nagaveni (2011) opinioned similarly.

Starch

The starch content was decreased in the mealy bugs infested leaves of M_5, Mysore local, S_{54} and V_1 varieties. The reduction was minimum (1.72 %) in Mysore local and maximum (5.08 %) in V_1 variety. The

Table 1. Biochemical changes (dry weight) in the mealy bugs - infested mulberry leaves

Mulberry varieties	Free amino acids (mg/g)		Total soluble proteins (mg/mg)		Total reducing sugars (mg/g)		Total soluble sugars (mg/g)		Total starch (mg/g)		Total phenols (mg/g)	
	Healthy	Infested	Healthy	Infested	Healthy	Infested	Healthy	Infested	Healthy	Infested	Healthy	Infested
M_5	17.04	14.52** (-14.79)	163.80	167.70** (+2.39)	82.00	69.60** (-15.12)	1.33	1.25** (-5.95)	0.97	0.96 (-1.83)	1.86	1.80** (-3.23)
MR_2	12.00	10.92 (-9.00)	83.85	81.90** (-2.33)	73.20	70.80** (-3.28)	1.95	1.95 (---)	1.42	1.50 (+5.66)	2.94	3.78 (+28.57)
Mysore local	16.44	16.32 (-0.73)	136.50	136.50 (---)	54.00	42.00 (-22.22)	1.85	1.85 (---)	1.04	1.02 (-1.72)	3.92	3.94 (+0.51)
S_{36}	9.96	9.96 (---)	91.65	95.55 (+4.26)	46.80	45.60** (-2.56)	1.71	1.71 (---)	0.96	0.99 (+2.78)	1.08	1.26* (+16.67)
S_{54}	11.64	11.40 (-2.06)	109.20	109.20 (---)	154.80	156.00 (+0.78)	2.19	2.19 (---)	1.23	1.18 (-4.35)	4.68	5.30** (+13.25)
V_1	15.60	15.60 (---)	142.35	148.20** (+4.11)	44.44	40.80* (-8.11)	2.66	2.57 (-3.57)	1.58	1.50 (-5.08)	2.76	2.84* (+6.09)

** Significant at 1% level; * Significant at 5% level; Values in the brackets ( ) indicate % difference over healthy (+ = more than; - = less than; ---- = not altered).

Table 2. Changes in photosynthetic pigments (mg/g. fresh weight) of mealy bugs infested mulberry leaves

Mulberry varieties	Total chlorophyll (mg/l)		Chlorophyll – a (mg/l)		Chlorophyll – b (mg/l)		Chlorophyll – a/b (mg/l)		Carotenoids (mg/l)	
	Healthy	Infested	Healthy	Infested	Healthy	Infested	Healthy	Infested	Healthy	Infested
M_5	1.62	1.62 (+0.21)	1.36	1.33** (-2.32)	0.25	0.29** (+13.78)	5.37	4.61** (-14.21)	0.82	0.73** (-10.71)
MR_2	2.08	1.51** (-27.37)	1.78	1.23** (-30.946)	0.30	0.28* (-6.28)	5.90	4.35** (-26.27)	1.02	0.65** (-36.23)
Mysore local	1.81	1.29** (-29.16)	1.56	1.07** (-31.51)	0.24	0.22** (11.32)	6.23	4.83** (-22.78)	0.92	0.59** (-35.42)
S_{36}	2.852	2.57 (-9.73)	2.262	1.91 (-15.65)	0.591	0.67 (+12.94)	3.830	2.86 (-25.32)	1.03	1.03 (+0.53)
S_{54}	2.340	0.30** (-1.81)	1.805	1.77** (-1.92)	0.535	0.11** (-80.00)	3.372	3.35 (-0.52)	1.15	1.14* (-0.53)
V_1	1.632	1.29** (-20.692)	1.449	1.09** (-24.46)	0.183	0.20* (+9.08)	7.910	5.48** (-30.76)	0.80	0.68** (-14.91)

** Significant at 1% level; * Significant at 5% level; Values in the brackets ( ) indicate % difference over healthy (+ = more than; - = less than; ---- = not altered).

increase in the starch content was noticed in the leaves of MR_2 (5.66 %) and S_{36} (2.78 %) varieties.

Shree *et al.* (1989) noticed a 27 % and 36 % increase in the starch content of mealy bugs affected Kajali and Kanva-2 mulberry varieties respectively. The starch content was increased in the *M. macroura* and *M. nigra*. The increase was significant in *M. macroura*. There was only a negligible reduction in *M. australis* and *M. cathayana* due to *M. hirsutus* infestation (Shree and Kumar, 1989). It was reported by Mahadeva and Nagaveni (2011).

Total phenols

The pest-infested mulberry showed variation in the phenolic contents. it was decreased significantly in the leaves of M_5 (3.23 %) variety. The phenolic content was increased significantly in the leaves of MR_2, S_{36}, S_{54} and V_1 varieties and non significantly in the Mysore local variety. The increase was negligible (0.51 %) and maximum (28.57 %) in Mysore local and MR_2 varieties respectively.

There were no change in the phenolic content in tender and coarse leaves of M_5 mulberry variety when they were attacked by mealy bugs. The phenolic level was decreased in tukra affected leaves of *M. macroura* and *M. nigra* varieties. However, there was a significant increase in *M. australis* and *M. cathayana* (Shree and Kumar, 1989). Muthegowda *et al.* (1990) reported the pattern of phenol accumulation varied depending upon the positional status of leaves in the tukra affected C_{15} (Conoor series) mulberry variety. There was an initial increase followed by a sudden decrease from the leaf of the 2^{nd} to 10^{th} order. The tukra affected leaves had more phenolics as a result of insect bite. This clearly shows that the pests altered phenolic metabolism in the host leading to biochemical changes. Uritani (1961) proposed that accumulation of phenolics in the host may inactivate the enzyme which inhibits the further advance of the pathogenic organism by limiting its source of nutrients. The most important phenolic compounds implicated in the defense mechanism of plants against pathogens are coumaric acid, phloretin, umberlliferons, caffeic acid, chlorogenic acid and ferulic acid (Agrios, 1969). When such substances are ingested by phytophagous insects along with the food, they get access to natural defense mechanism (Mahadeva and Nagaveni, 2011: Mahadeva and Shree, 2011).

The alteration in the biochemical composition may be because of disturbed host metabolism due to pest damage (Kumar *et al.,* 1990). The altered biochemical constituents lead to imbalance in the nutritional components of mulberry foliage which is not a beneficial character (Mahadeva and Nagaveni, 2011).

Photosynthetic pigments (Table – 2)

The pest infested mulberry leaves of six popular indigenous varieties showed significant changes in the photosynthetic pigments (total chlorophyll, chlorophyll – a, chlorophyll – b, chlorophyll – a/b ratio and carotenoids).

There was a significant decrease in the total chlorophyll content of the foliage of MR_2, Mysore local, S_{36}, S_{54} and V_1 mulberry varieties. The reduction was minimum (9.73 %) and maximum (29.16 %) in the leaves of S_{54} and Mysore local varieties respectively. The total chlorophyll content was increased (0.29 %) non-significantly in the leaves of M_5 variety.

The chlorophyll – a content was significantly decreased in the pest-infested leaves of M_5, MR_2, Mysore local and V_1 and non-significantly in the leaves of S_{36} and S_{54} varieties. It was minimum (1.92 %) in the leaves of S_{54} and high (31.51 %) in the Mysore local varieties. There was a significant reduction in the chlorophyll – b content of tukra affected leaves of MR_2, Mysore local and S_{54} varieties. The decrease was negligible (1.32 %) in the pest infested leaves of Mysore local but high (80.00 %) in the leaves of S_{54} variety. The chlorophyll – b content was increased significantly in M_5, S_{36} and V_1 varieties. The increase was in the range of 9.08 % to 13.78 % in the leaves of V_1 and M_5 varieties

respectively. Depending upon the amount of chlorophyll – a and chlorophyll – b, the chlorophyll – a/b ratio showed variation in *M. hirsutus* infested mulberry leaves. There was a significant decrease in the chlorophyll – a/b ratio in the medium leaves of M_5, MR_2, Mysore local, S_{36}, S_{54} and V_1 varieties. The decrease was minimum (0.52 %) in the leaves of S_{54} but maximum (30.76 %) in the leaves of V_1 variety due to pest - infestation.

The carotenoids content was significantly decreased in the leaves of M_5, MR_2, Mysore local, S_{36}, S_{54} and V_1 varieties. The reduction was minimum (0.53 %) in S_{54} and maximum (36.23 %) in the leaves of MR_2 variety. There was no alteration in the carotenoids content in the tukra infested leaves of S_{36} variety.

The chlorophylls are the essential catalysts of photosynthesis and occur universally as green pigments in all the autotrophic plant tissues. They occur in chloroplasts in relatively large amount. Level of chlorophyll content is one of the criteria for quantifying the photosynthetic rate. Chlorophyll estimates may be required to relate other biochemical changes in the plant tissues (Mahadevan and Sridhar, 1986). There was a decrease (4.00 %) as well as increase (28.00 %) in the chlorophyll content in the mealy bugs infested leaves of Kajali and Kanva – 2 varieties respectively (Shree *et al.,* 1989). There was a significant reduction in the total chlorophyll, chlorophyll – a, chlorophyll – b and chlorophyll – a/b ratio in tukra affected *M. cathayana.* Increase in the total chlorophyll, chlorophyll – a, chlorophyll – b and chlorophyll – a/b ratio were significant in *M. nigra,* marginal in *M. macroura* and negligible in *M. australis*. Diseases affect not only the total chlorophyll content but also alters the ratio between chlorophyll – a and b. The reduction in the pigment(s) and/of laminar area (caused due to feeding nature of insect pests) will result in the decreased photosynthetic efficiency and thereby the productivity. Consequently, the nutritional status of mulberry foliage comes down (Shree and Kumar, 1989; Mahadeva and Nagaveni, 2011; Mahadeva and Shree, 2011) . Total chlorophyll content was increased in Berhampore, Italian, Okinawa-2, Philippine, S_{36} and Tsukasaguwa varieties; whereas, it decreased in Kosen, *M. multicaulis,* Philippine and S_{30}. Negligible difference was observed in the S_{41} variety due to *M. hirsutus* infestation (Veeranna, 1997). There were changes in the photosynthetic pigments (total chlorophyll, chlorophyll – a, chlorophyll – b and carotenoids) in mealy bugs infested eight mulberry varieties *viz*., M_5, MR_2, BC_{259}, Tr_4, S_{13} (indigenous), Kosen, Ichinose and Goshoerami (exotic) (Babu *et al.,* 1994). The levels of total chlorophyll, chlorophyll – a, chlorophyll – b and carotenoids increased strikingly in the tukra leaves. The increase in chlorophyll content was equally contributed by both chlorophyll – a and chlorophyll – b. This increase may be due to the failure of the pathogen to inhibit chlorophyllase, as in okra (Ahmed *et al.,* 1987). In all the mulberry varieties except Kosen and Tr_4, chlorophyll – a/b ratio was lower in tukra leaves compared to that of the control. Thus, the intensity of pest attack, level of damage leads to the variation in photosynthetic pigment in the mulberry leaves.

The alteration in the photosynthetic pigments and biochemical components were observed in many cases where mulberry leaves were infested by various pests such as, jassids (Shree and Mahadeva, 2005), leaf roller (Narayanaswamy, 2003), spiralling whitefly (Chandramohan *et al.,* 2002), thrips (Das *et al.,* 1994) and giant African snails (Kumar, 1997). The Photosynthetic activity adversely affected due to the altered chlorophyll content (Heldt, 1997) and which leads to reduced protein synthesis (Burd and Elliot, 1996). Consequently, the mulberry foliage will be nutritionally inferior. Feeding such low nutritive, pest infested mulberry to silkworms will have an adverse impact on their growth and development, leading to cocoon crop failures (Doureswamy and Chandramohan, 1999; Mahadeva and Shree, 2004; Mahadeva and Shree, 2005). The pest attacked and diseased mulberry leaves

should not be used for silkworm feeding as they are known to affect the commercial characters of cocoons . Necessary arrangements must be made to manage the pests and disease of mulberry plant as it is the only source of food for silkworms.

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