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An Investigation on Changes in Biochemical Contents in Mealy Bugs Infested Mulberry Foliage

Research article

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Abstract

The occurrence of pink mealy bugs (Maconellicoccus hirsutus Green) on mulberry (Morus sp.) considerably reduces the leaf yield and inturn 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, feeding such leaves to the silkworm may alter the growth and development of the larvae in turns affect the quality and quantity silk production.

Keywords: Biochemical constituents; Mealy bugs; Mulberry; Photosynthetic pigments

Introduction

Mulberry (Morus sp.), the only food plant of silkworm Bombyx mori L. is of great importance to 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 [1]. 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 [2]. 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 [3]. 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 vital role in virus transmission [4]. 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

The healthy and mealy bugs infested leaves were collected from six mulberry varieties *i.e.* M_3 , MR_2 , Mysore local, S_{36} , S_{54} and V_1 . The leaves were oven - dried and processed to analyze the biochemical constituents *viz.*, free amino acids [5], total soluble proteins [6], reducing sugars [7], soluble sugars and starch [8], and total phenols [9]. The fresh leaves of healthy and mealy bug infested one were used to estimate the photosynthetic pigments (chlorophyll and carotenoids) [10,11]. Student's t-test were followed for statistical analysis.

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 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 [12]. 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) [13].

Total soluble proteins

The total soluble proteins were significantly decreased in the tukra affected leaves of MR₂ variety (2.33 %). The total soluble proteins increased significantly in the leaves of M₅ (2.39 %) and V₁ varieties and non-significantly in the S₃₆ (4.26 %) variety. The total soluble proteins were not altered in the leaves of Mysore local and S₅₄ varieties due to mealy bugs - infestation.

The protein content in the mulberry leaves shown a major role in silk production by silkworm. The protein was reduced (10.50 %) in Kajli and increased (40.00 %) in mealy bug affected Kanva-2 variety [14]. Similarly, decreased protein content was observed in tukra affected mulberry varieties of *M. macroura* and *M. nigra* by [15]. An increased total protein due to mealy bug infestation in M_5 and DD varieties [16]. The mechanical damage caused by insect during infestation will alter the physiological activity in the host. This altered function may lead to the variation in synthesis or mobilization of proteins to the damaged tissues/area in order to resist insect bite.

This may leads to variation in the total crude protein contents in host [17,18].

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 S_{54} .

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 [15].

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 MR₂, Mysore local, S_{36} and S_{54} varieties.

The Sugar content was decreased marginally in mealy bug infested 3 mulberry varieties (*M. australis, M. cathayana* and *M. nigra*) and noticed increase in sugar in *M. macroura* variety [15]. The four indigenous (Berhampore, S_{30} , S_{31} and S_{36}) and six exotic (Kosen, *M. multicaulis*, Philippine, Okinawa-2, Tsukasaguwa and Italian) mulberry varieties shown a variation in sugar content due to mealy bugs infestation [20]. There was increased sugar content in Berhampore, Okinawa-2 and Philippine varieties. Whereas, it was decreased in Italian, Kosen, *M. multicaulis*, S_{30} , S_{36} and S_{41} varieties. No difference was observed in Tsukasaguwa [20]. An increased soluble sugar was observed in mealy bugs infested leaves of M_{57} , MR_2 , BC_{259} , S_{13} , Kosen, Goshoerami. However, in Tr_4 and Ichinose, it was decreased [13]. Similar variation was also noticed in leaf roller infested mulberry leaves [18].

Table	1: Biochemical	changes	(dry	weight) in	the r	mealy bugs	 infested 	mulberry	leaves.
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Mulberry varieties	Free amino acids (µg/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**	163.80	167.70**	82.00	69.60**	1.33	1.25**	0.97	0.96	1.86	1.80**
	(-14.79)		(+2.39)		(-15.12)		(-5.95)		(-1.83)		(-3.23)	
MR ₂	12.00	10.92	83.85	81.90**	73.20	70.80**	1.95	1.95	1.42	1.50	2.94	3.78
	(-9.00)		(-2.33)		(-3.28)		()		(+5.66)		(+28.57)	
Mysore local	16.44	16.32	136.50	136.50	54.00	42.00	1.85	1.85	1.04	1.02	3.92	3.94
	(-0.73)		()		(-22.22)		()		(-1.72)		(+0.51)	
S ₃₆	9.96	9.96	91.65	95.55	46.80	45.60**	1.71	1.71	0.96	0.99	1.08	1.26*
	()		(+4.26)		(-2.56)		()		(+2.78)		(+16.67)	
S ₅₄	11.64	11.40	109.20	109.20	154.80	156.00	2.19	2.19	1.23	1.18	4.68	5.30**
	(-2.06)		()		(+0.78)		()		(-4.35)		(+13.25)	
V ₁	15.60	15.60	142.35	148.20**	44.44	40.80*	2.66	2.57	1.58	1.50	2.76	2.84*
	()		(+4.11)		(-8.11)		(-3.57)		(-5.08)		(+6.09)	

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

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 increase in the starch content was noticed in the leaves of MR₂(5.66%) and S_{36} (2.78%) varieties.

Increased starch content was observed in the tukra affected mulberry leaves of Kajli and Kanva-2 varieties [14]. There was a variation in the starch content of mealy bugs infested mulberry leaves of *M. macroura*, *M. nigra*, *M. australis* and *M. cathayana* [15]. The variation in starch content was also observed due to leaf roller infestation mulberry leaves [18].

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. Mealy bug infested mulberry varieties (MR_2 , S_{36} , S_{54} , V_1 and Mysore local) shown an increase in total phenols. The increase was negligible (0.51%) in Mysore local and maximum (28.57%) in MR_2 pest attacked mulberry leaves.

There were no changes in the phenolic content in tender and coarse leaves of M_s 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 [15]. The accumulation of phenol depends upon the position of leaves in a mulberry twig in mealy bug infected $\mathrm{C}_{\scriptscriptstyle 15}$ mulberry variety. The insect bite leads to accumulation of more and more phenol in the affected leaves. It may be due to altered phenolic metabolism in response to pest attack [21]. The accumulation of phenolics in the host may inhibit the further advancement of pathogenic organism [22]. 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 [23]. These may acts as a natural defense mechanism by host against the phytophagous insects [18,19].

The variation in biochemical components were observed in other cases like jassids [24], leaf roller [25], spiralling whitefly [26], thrips [27] and giant African snails [28] infested mulberry leaves. The disturbance in the host metabolism may lead to changes in biochemical composition during the mechanical damages caused during pest infestation [19]. The altered biochemical constituents lead to discrepancy in the nutritional components of mulberry foliage which is disadvantageous for the quality silk production [18].

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 pestinfested leaves of M₅, MR₂, Mysore local and V₁ and non-significantly in the leaves of S_{36} and S_{54} varieties. It was minimum (1.92%) in the leaves of S₅₄ and high (31.51%) in the Mysore local varieties. There was a significant reduction in the chlorophyll - b content of tukra affected leaves of MR₂, Mysore local and S₅₄ 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 M5, S36 and V1 varieties. The increase was in the range of 9.08% to 13.78% in the leaves of V, and M_s 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. The tukra affected leaves of $\rm M_{_5},~MR_{_2}$, Mysore local, $\rm S_{_{36}},~S_{_{54}}$ and $\rm V_{_1}$ mulberry varieties shown a significant decrease in the chlorophyll - a/b ratio. The decrease was minimum (0.52 %) and maximum (30.76 %) in the leaves of S_{54} and V_1 mulberry variety respectively due to mealy bugs attack.

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 [29]. 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 [15]. 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 was 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 feeding behavior of the insect pests results in the reduction of pigment(s) and/of laminar area which decreases photosynthetic efficiency and thereby the productivity of the leaves. As a result of it, the nutritional level of mulberry leaves reduces [15], [18] & [20]. Total chlorophyll content was increased in Berhampore, Italian, Okinawa-2, Philippine, S₃₆ and Tsukasaguwa varieties. Whereas, it decreased in Kosen, M. multicaulis, Philippine and S₃₀. Negligible difference was observed in the S_{41} variety due to *M. hirsutus* infestation [16]. There were an changes in the photosynthetic pigments (total chlorophyll, chlorophyll - a, chlorophyll - b and carotenoids) in mealy bugs infested eight mulberry varieties viz., M₅, MR₂, BC₂₅₉, Tr₄, S₁₃ (indigenous), Kosen, Ichinose and Goshoerami (exotic) [13]. The levels of total chlorophyll, chlorophyll - a, chlorophyll - b and carotenoids increased strikingly in the tukra leaves. The increase in chlorophyll content was equally

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Mulberry	I otal chlorophyll		Chlorophyll – a		Chlorop	bhyll – b	Chlorophyll – a/b		Carotenoids	
varieties	Healthy	Infested	Healthy	Infested	Healthy	Infested	Healthy	Infested	Healthy	Infested
M ₅	1.62	1.62	1.36	1.33**	0.25	0.29**	5.37	4.61**	0.82	0.73**
	(+0.21)		(-2.32)		(+13.78)		(-14.21)		(-10.71)	
MR ₂	2.08	1.51**	1.78	1.23**	0.30	0.28*	5.90	4.35**	1.02	0.65**
	(-27.37)		(-30.946)		(-6.28)		(-26.27)		(-36.23)	
Mucoro local	1.81	1.29**	1.56	1.07**	0.24	0.22**	6.23	4.83**	0.92	0.59**
Wysore local	(-29.16)		(-31.51)		(11.32)		(-22.78)		(-35.42)	
e	2.852	2.57	2.262	1.91	0.591	0.67	3.830	2.86	1.03	1.03
3 ₃₆	(-9.73)		(-15.65)		(+12.94)		(-25.32)		(+0.53)	
S ₅₄	2.340	0.30**	1.805	1.77**	0.535	0.11**	3.372	3.35	1.15	1.14*
	(-1.81)		(-1.92)		(-80.00)		(-0.52)		(-0.53)	
V ₁	1.632	1.29**	1.449	1.09**	0.183	0.20*	7.910	5.48**	0.80	0.68**
	(-20.692)		(-24.46)		(+9.08)		(-30.76)		(-14.91)	

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

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

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 [30]. In all the mulberry varieties except Kosen and Tr4, 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 was observed in many cases where mulberry leaves were infested by various pests such as, jassids [24], leaf roller [25] spiralling whitefly [26], thrips [27] and giant African snails [28]. The photosynthetic activities adversely affect due to altered chlorophyll content [31] and which leads to reduced protein synthesis [32]. 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 [33,34]. The diseased or/and pest infested mulberry leaves are nutritionally inferior and not fit to feed the silkworm, because they are known to influence the quality and quantity silk production. 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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