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Nutritional Status and Biochemical Analysis of *Tukra* Infested Mulberry Leaves

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The pink mealybug, *Maconellicoccus hirsutus* is the most serious pest of mulberry causing leaf curl (tukra). Different parameters of effective silkworm rearing on *Tukra* infested leaves, and nutritional parameters were analyzed. The Effective rate of rearing (ERR) was moderate (79.5%), when larvae were fed with *Tukra* infested leaves from brushing to V instars. Similarly, higher larval weight (27.3 g), cocoon weight (16.9 g), shell weight (3.8 g) and shell ratio (22.4) were obtained when silkworms were reared on Tukra infested leaves. Results of biochemical analysis of *Tukra* infested leaves showed chlorophyll a, chlorophyll b and total chlorophyll content were higher (0.8, 0.3 and 1.2 mg) in *Tukra* infested leaves compared to healthy leaves (0.8, 0.4 and 1.1 mg). Similarly, total protein (77.7 mg), total phenol (34.1 mg) and starch (4.9%) were also higher in *Tukra* infested leaves than healthy leaves.

Key words: *Tukra*, Pink mealybug, Biochemical analysis

Mulberry silkworm, *Bombyx mori L.* the source of fabulous silk was domesticated for 5000 years. It is a monophagous feeder on mulberry, Mulberry is attacked by more than 50 arthropod pests (Anonymous, 1975). The pink mealybug, *Maconellicoccus hirsutus* Green is the most serious pest of mulberry. It causes apical shoot malformation popularly known as '*Tukra*'. Severe occurrence was recorded in entire sericulture belt of Tamil Nadu (Baskaran *et al*., 1992). Now it is rated as hard pest to kill because of its sedentary nature, waxy coating over the body and concealed way of living in apical buds. Even though, synthetic chemicals are very effective in checking the pest, they are generally not employed owing to high toxic nature of the compounds sensitive to silkworms. Since, the damage is more on young mulberry leaves an attempt was made on possibility of employing *Tukra* infested leaves in silkworm rearing, through biochemical analysis of infested leaves and its effect on silkworm.

Materials and Methods

An experiment was conducted using mulberry variety (V1) and the silkworm race, PM X CSR 2 in Department of Sericulture, Tamil Nadu Agricultural University, Coimbatore during 2008 - 2009. Each treatment was replicated five times. The rearing was conducted as per the standard recommendations (Kishnaswami *et al*., 1973; Benchamin and Nagaraj, 1987). The details of treatments are as follows.

T_1 – Feeding of healthy leaves upto V instar (check)

T_2 – Feeding of *Tukra* infested leaves up to II instar

T_3 – Feeding of *Tukra* infested leaves up to III instar

T_4 – Feeding of *Tukra* infested leaves during late IV & V instars

T_5– Feeding of *Tukra* infested leaves from brushing to V instar

Economic Parameters such as larval weight, cocoon weight, shell weight, shell ratio, larval duration and ERR were worked out. Similarly, food consumption and conversion tests were done as per the technique suggested by Benchamin (1989).

Biochemical analysis for nutritional parameters

The samples used in the analysis were collected from the top, middle and bottom of *Tukra* infested and healthy leaves of mulberry plants. The chlorophyll a, chlorophyll b and the total chlorophyll content were estimated in the leaf samples by the method suggested by Argon (1946). Other biochemical parameters were analysed according to standard procedures, that include protein (Lowrey *et al.* (1951), starch (Hedge, 1962), reducing sugars (Somogyi, 1952), total sugars (Hedge, 1962) and total phenols (Malik and Singh, 1980). These biochemical components of *Tukra* infested leaves were compared with healthy leaves.

Results and Discussion

Rearing parameters

Larval weight

The mean weight of 10 larvae ranged from 23.2 to 27.3 g in different treatments. A highest of 27.3 g was found in silkworms fed with *Tukra* infested leaves from brushing to V instar. This result might

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be attributed to production of more silk with higher conversion rate of body mass to silk production. It was followed by *Tukra* infested leaves fed during fourth and fifth instars (27.2g), up to second instar (24.9g), and upto third instar (23.4g). Larval weight was 23.2g/ 10 larvae in treatment were larvae received healthy leaves.

Cocoon weight

The mean weight of 10 cocoons ranged from 14.5 to 16.9g in treatments. It was higher (16.9g) in *tukra* infested leaves from brushing to V instar which was followed by *tukra* leaves fed during fourth and fifth instars (16.0g), whereas the cocoon weight was 14.5g in health leaves. However, feeding of *Tukra* leaves up to third and second instar stages recorded cocoon weight of 15.4 and 15.0 g respectively.

Shell Weight

The shell weight varied from 3.1 to 3.8g/ 10 cocoons in treatments. Feeding of *Tukra* infested leaves from brushing to V instar recorded the highest of 3.8 g, which was followed by feeding of *Tukra*

Table 1. Effect of feeding of *Tukra* infested leaves on larval, cocoon and consumption characters

Treatment	Larva and cocoon characters (n=10)						Consumption test (n=10)				
	LW (g)	CW (g)	SW (g)	SR (%)	LD (days)	ERR (%)	Silkworm litter dry weight (g)	Leaf consumption	Conversion ratio to		
								fresh weight (g)	Larval body	Cocoon	
T_{1-} Feeding of healthy leaves up to V instar (check)	23.2_{c}	14.5_{c}	3.1_{d}	21.1_{b}	25.8_{a}	85.8_{b}	24.5_{c}	135.4_{b}	5.8_{a}	9.3_{a}	
	(4.9)	(3.9)	(1.9)	(4.6)	(5.1)	(67.9)	(5.0)	(11.7)	(2.5)	(3.1)	
T_{2} - Feeding of *tukra* infested leaves up to II instar	24.9_{b}	15.0_{bc}	3.1_{d}	20.7_{c}	25.7_{a}	86.5_{b}	21.4_{e}	121.6_{c}	4.8_{c}	8.0_{cd}	
	(5.0)	(3.9)	(1.9)	(4.6)	(5.1)	(68.4)	(4.7)	(11.0)	(2.3)	(2.9)	
T_{3} - Feeding of *tukra* infested leaves up to III instar	23.4_{c}	15.4_{bc}	3.3_{c}	21.5_{abc}	25.7_{a}	89.0_{a}	21.6_{d}	121.2_{c}	5.1_{b}	7.7_{d}	
	(4.9)	(4.0)	(1.9)	(4.7)	(5.1)	(70.6)	(4.7)	(11.0)	(2.4)	(2.9)	
T_{4} - Feeding of *tukra* leaves during IV and V instars	27.2_{a}	16.0_{b}	3.5_{b}	22.3_{a}	23.3_{b}	78.7_{c}	25.0_{b}	141.7_{a}	5.2_{b}	8.8_{b}	
	(5.3)	(4.1)	(2.0)	(4.8)	(4.9)	(62.5)	(5.0)	(11.9)	(2.4)	(3.0)	
T_{5} - Feeding of *tukra* infested leaves from brushing to V instar	27.3_{a}	16.9_{a}	3.8_{a}	22.4_{a}	$24.5a_{b}$	79.5_{c}	25.8_{a}	136.5_{b}	5.0_{bc}	8.1_{c}	
	(5.3)	(4.2)	(2.1)	(4.8)	(5.0)	(63.1)	(5.1)	(11.7)	(2.3)	(2.9)	
CD(P=0.05)	0.99	0.67	0.06	1.10	1.69	0.80	0.41	2.76	0.26	0.36	

LW- Larval Weight; CW- Cocoon weight; SW- Shell weight; SR- Shell ratio; LD – Larval Duration; ERR = Effective Rate of Rearing(%)
Figures in the parentheses are x+0.5 transformed values
*Figures in the parenthesis are arc sine transformed values
In a column mean followed by a common letter are significantly different by DMRT (P=0.05)

infested leaves during fourth and fifth instars (3.5g). Feeding of Tukra infested leaves up to second instar and third instar registered 3.1 and 3.3 g respectively. Healthy leaf feeding recorded 3.1 g/ 10 cocoons.

Shell ratio

Shell ratio was higher (22.4%) in larva fed with *tukra* infested leaves from brushing to V instar which was followed by feeding of *tukra* infested leaves during fourth and fifth instars (22.3%). In other treatments it ranged from 20.7 to 21.5. The minimum ratio was obtained in, *tukra* infested leaves fed upto second instar (20.7%).

Larval duration (Days)

Shorter larval duration of 23.3 days was recorded in larvae fed with *Tukra* infested leaves during fourth and fifth instars followed by 24.5, 25.7 and 25.7 days when fed with *Tukra* infested leaves from brushing to V instar, from first to third instars and up to second instar respectively, whereas the larval duration was 25.8 days in larvae reared on healthy leaves. A reduction of 2.4 days was recorded when fed larvae were fed with *Tukra* infested leaves from brushing to V instar than the larvae fed with healthy leaves.

Thus, feeding the larvae from brushing to V instar with *Tukra* infested leaves induced change in economic parameters of larvae. Feeding silkworm larvae with *Tukra* infested leaves at chawki stage caused increase in the larval weight of 23.4 to 24.9 g. The late age larvae feeding on *Tukra* infested leaves showed significant reduction in larval period of 23.3 days compared with check (25.8 days). The cocoon character also proved higher in larvae fed with *Tukra* infested leaves over check.

Effective rate of rearing (ERR)

The highest percentage of ERR of 89.0 was recorded , when larvae were fed with *Tukra* infested leaves up to third instar and followed by feeding *Tukra* infested leaf up to second instar (86.5%) and check (85.8%). Feeding *Tukra* infested leaves from brushing to V instar recorded 79.5 per cent ERR whereas, feeding of IV & V instars with Tukra infested leaves resulted in 78.7 per cent of ERR. Feeding silkworm with *Tukra* infested leaves during various instars was found to improve larval and cocoon characters, and consumption indices. This is in conformity with the studies made by Thangamani and Vivekanandan (1983) who reported no significant difference in larval instars fed with *Tukra* infested leaves and healthy leaves fed larva. The larval duration, cocoon weight, shell weight, silk ratio and ERR did not register apparent difference between the treatments.

Silkworm litter (dry weight)

The silkworm litter dry weight of 10 larvae was higher in feeding of *Tukra* infested leaves from brushing to V instar (25.8g) followed by feeding of *Tukra* infested leaves during fourth and fifth instars with 25.0 g and check with 24.5 g. Feeding of *Tukra*

Table 2. Chlorophyll content of *Tukra* infested and healthy leaves

Parameter	Nature of Leaves Top		Middle	Bottom
Chlorophyll a#(mg/g of leaf)	Tukra	0.9ns	0.8*	0.8*
	Healthy	0.9ns	0.8*	0.7*
t value		0.2096	0.0562	0.0003
Chlorophyll b#(mg/g of leaf)	Tukra	0.4*	0.3*	0.3*
	Healthy	0.8*	0.3*	0.2*
t value		0.0003	0.0002	0.0235
Total Chlorophyll#(mg/g of leaf)	Tukra	1.2*	1.2*	1.1*
	Healthy	1.1*	1.1*	1.0*
t value		0.0056	0.0001	0.0003

#Mean of five replications. ns – Not significant
In a horizontal row means of the corresponding indices
*are significantly different at P=0.05 with dependent sample t-test

infested leaves up to second instar recorded and up to third instar recorded litter weight of 21.4 and 21.6 g respectively.

Leaf consumption (Fresh weight)

The leaf consumption of 10 larvae was more (141.7g) in feeding of *Tukra* infested leaves during fourth and fifth instars which was followed by feeding of *Tukra* infested leaves from brushing to V instar with 136.5 g, and check with 135.4 g. Feeding of *Tukra* infested leaves up to second and third instar stage silk worms recorded a leaf consumption of 121.6 and 121.2g respectively. This is beneficial in endemic areas where there is minimal possibility of pink mealybug management.

Table 3. Biochemical composition of *Tukra* infested and healthy leaves

Parameters	Leaves	Top	Middle	Bottom
Total protein#(mg/g of leaf)	Tukra	75.2*	76.9*	80.9*
	Healthy	69.0*	68.2*	71.5*
	t value	0.0001	0.0001	0.0001
Total phenols#(mg/g of leaf)	Tukra	36.9ns	34.5ns	30.9ns
	Healthy	34.7ns	32.2 ns	30.9ns
	t value	0.2331	0.2003	0.0783
Total sugars#(mg/g of leaf)	Tukra	20.1*	17.2*	20.2*
	Healthy	20.8*	19.6*	21.4*
	t value	0.0510	0.0001	0.0001

#Mean of five replications. ns – Not significant
In a horizontal row means of the corresponding indices * are significantly different at P=0.05 with dependent sample t-test

Conversion ratios

The minimum food to body mass conversion ratio of 4.8 was found in feeding of *Tukra* infested leaves up to second instar which was followed by feeding of *Tukra* infested leaves from brushing to fifth instar (5.0), up to third instar with (5.1) and during fourth and fifth instars (5.2) as against 5.8 in check.

The maximum food to cocoon conversion ratio was recorded in check fed with fresh leaves (9.3) which was followed by feeding of *Tukra* infested leaves during fourth and fifth instars (8.8), from brushing to V instar (8.1). Conversion ratio was 8.0 and 7.7 in *Tukra* infested leaves fed up to second instar and third instar respectively.

Biochemical analysis

Chlorophyll (mg/g of leaf)

Tukra infested leaves recorded chlorophyll a, chlorophyll b and total chlorophyll contents of 0.8, 0.3 and 1.2 mg as against 0.8, 0.4 and 1.1 mg in healthy leaves respectively. The total chlorophyll content was higher in top leaves of both Tukra infested (1.2 mg) and healthy leaves (1.1 mg). In middle and bottom of healthy leaves it was 1.1 and 1.0 respectively.

Total Proteins (mg/g of leaf)

The total protein content was higher (77.7 mg) in Tukra infested leaf as against 69.6 mg in healthy leaf. In Tukra infested plant, the total protein content was more in bottom leaves (80.9 mg), and it was 76. 9 and 75.2 mg in middle and top leaves respectively. In healthy plant, it was more in bottom leaves (71.5 mg) followed by middle (68.2 mg) and top leaves (69.0). Collaborative evidence of increased chlorophyll, protein and phenol content was in *Tukra* infested leaves documented earlier by Muthe Gowda *et al.* (1990), Ramansureshbabu *et al*. (1994) and Nagaraja and Somasundaram (1990).

Total Phenols (mg/g of leaf)

Total phenols were higher (34.1 mg) in *Tukra* infested leaves than healthy leaves (32.6mg). This is in conformity with results of Coumararadja (1985) who reported increase in phenol content due to mealybug attack in rice. The top, middle and bottom leaves of Tukra infested plants had 36.9 mg, 34.5 mg and 30.9 mg of phenols respectively. In healthy plant, it was 34.7, 32.2 and 30.9 mg respectively.

Table 4. Biochemical composition of *Tukra* infested and healthy leaves

Parameters	Nature of Leaves Top		Middle	Bottom
Total reducing sugars#	Tukra	18.5ns	15.6*	18.2*
(mg/g of leaf)	Healthy	18.6 ns	17.3*	18.4*
t value		0.3954	0.0002	0.1121
Total non reducing sugars#	Tukra	0.9*	2.4*	1.9*
(mg/g of leaf)	Healthy	2.2*	2.6*	2.6*
t value		0.0021	0.0065	0.0002
Total starch (%)#	Tukra	5.4*	4.6*	3.5*
	Healthy	5.5*	3.6*	5.6*
t value		0.0594	0.0001	0.0001

#Mean of five replications. ns – Not significant
In a horizontal row means of the corresponding indices * are significantly different at P=0.05 with dependent sample t-test

Total Starch (%)

The higher starch content of 4.9% was recorded in healthy leaves than in *Tukra* infested leaves (4.5%). This is in consonance with the findings of Ramasureshbabu *et al.* (1994) who reported reduction in starch content in *Tukra* leaves. Starch content progressively decreased as age of the leaf advanced. *Tukra* infested leaves contained 5.4, 4.6 and 3.5 per cent total starch in top, middle and bottom leaves respectively. In healthy leaves, it was 5.5, 3.6 and 5.6 per cent respectively.

Total Sugars (mg/g of leaf)

The total sugar registered higher level (20.6 mg) in healthy leaves than in *Tukra* infested leaves (19.2 mg). The total sugar content in top, middle and bottom leaves was 20.1, 17.2, and 20.2 mg in *Tukra* infested leaves. Ramasureshbabu *et al.* (1994) found no significant change in sugar content in *Tukra* infested leaves compared of healthy leaves.

Total reducing sugars (mg/g of leaf)

The reducing sugar content was higher (18.1 mg/ g) in healthy plant leaf, whereas, it was lesser in *Tukra* infested leaves (17.4 mg/ g). Top, middle and bottom leaves of *Tukra* infested leaf had 18.5, 15. 6 and 18.2 mg of total reducing sugars respectively.

Non- reducing sugars (mg/g of leaf)

The non reducing sugar content was also higher (2.5 mg) in healthy leaves against 1.7 mg in *Tukra* infested leaves. The top, middle and bottom leaves of *Tukra* infested plant recorded 0.9, 2.4 and 1.9 mg respectively. Whereas, in healthy plants, top, middle and bottom leaves had 2.2, 2.6 and 2.6 mg/g of non reducing sugar content respectively.

The above study showed that feeding of silkworm larvae with *Tukra* infested leaves from brushing to V instar stage, significantly increased various larval and cocoon characters. Biochemical characters of *Tukra* infested leaves were also on par with healthy leaves. Hence, *tukra* infested leaves can be considered in silkworm rearing as a supplement to larval feed.

References

Anonymous, 1975. Text Book of Tropical Sericulture. Japanese Volunteer Corporation, Tokyo, Japan. pp.550 - 552.

Argon, D. 1946. Copper enzyme in isolated chloroplast polyphenol oxidase in *Beta vulgaris. Plant Physiol*., **24**: 1-15.

Baskaran, P., Ramanujam, K., Muralikumaran, C. and Radhakrishanan, N.V. 1992. Incidence and severity of mealybug – Associated mulberry leaf curl (Tukra) in Tamil Nadu. In : National Conference on Mulberry Sericulture Research Abstract. 1992-93. Central Sericultural Research and Training Institute, Mysore, pp.49-50.

Benchamin, K.V. and Nagaraj, C.S. 1987. Appropriate Sericulture Techniques. In: Jolly, M.S. (ed.) Chapter 4. *ICTRETS*, Mysore, India, pp.63-106.

Benchamin, K.V. 1989. Bioassay techniques for evaluation of mulberry leaf quality. In: *Genetic Resources of Mulberry and Utilization*. Central Sericultural Research and Training Institute, Mysore, pp.224-226.

Coumararadja, M. 1985. Studies on the biology and control of rice mealybug *Brevennia rehi* Lindinger (Pseudococcidae**:** Homoptera). M.Sc. (Ag.) Thesis, Tamil Nadu Agric. Univ., Coimbatore, p.151.

Hedge, J.E. 1962. In: Carbohydrate Chemistry (Whisler and Miller eds.) Academic Press, New York, pp.40-42.

Krishnaswami, S., Narasimahanna, M.N., Suryanarayan, S.K. and Kamaraj. 1973. Manual on Sericulture-2, Silkworm Rearing, F.A.O. *Agric. Services Bull.,* **15**: 54-88.

Lowrey, O.H., Rosebrough, N.J., Farr, A.L. and Randall, R.J. 1951. Protein measurement with folin phenol reagent. *J. Biol. Chem.,* **193**: 268-275.

Malik, C.P. and Singh, M.P. 1980. Plant Enzymological and Histoenzymology - A Test Manual. Kalyani Publishers, New Delhi, p.286

Muthegowda, M. Santhkumari, N.N. Umeshkumar and Boraiah,G. 1990. Changes in total phenolics of tukra-affected mulberry plants. *Sericologia,* **30:** 263-266.

Nagaraja and Somasundaram, P. 1990. Higher protein content in tukra affected chawki leaves and its interesting effect on silkworm rearing. *Indian Silk,* **29:** 35-37.

Ramasureshbabu, Davi Dorcus and Munisamy Vivekanandan. 1994. Changes in morpho-physiology, water relations and nutrients in *Tukra* diseased leaves of a few mulberry varieties. *J. Seric. Sci. Jpn,* **63:** 183-188.

Somogyi, M. 1952. Note on sugar determination. *J. Biol. Chem.,* **95**: 9-23.

Thangamani, R. and Vivekanadan, M. 1983. Effective utilization of *tukra* diseased mulberry leaves as silkworm *Bombyx mori* L. feed. *Sericologia,* **23**: 211-223.

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