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EFFECTS OF MANGROVE PLANT EXTRACTS AGAINST THE PINK MEALY BUG (*MACONELLICOCCUS HIRSUTUS GREEN)* ON AMINO ACIDS AND ENZYME ACTIVITY IN SILKWORM, *BOMBYX MORI* L.

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ABSTRACT
Mulberry, *Morus alba*, (L.) Leaves are the predominant food source for silkworm, *Bombyx mori* rearing. Pink mealy bug infests the mulberry plants and cause Tukra diseases that leads to qualitative loss of mulberry plantation. Hence a preliminary study was carried out using plant extracts as natural botanicals origin by spraying tukra infested mulberry leaves. The mangrove plant extract sprayed to tukra infested mulberry leaves fed to the silkworms and its impact on protease and transaminases activity in tissues like fat bodies and haemolymph was studied. For the study, healthy leaves (Control) and plant extracts viz., *Ceriops decandra and Suaeda nudiflora* were sprayed to tukra infested V1 mulberry variety and fed to Silkworm (PMxNB4D2 Bivoltine hybrid). Protease and ALAT activity gradually increased, this increase however was significant (P>0.05). AAT there was a gradual decrease from day 4 to day 6, this decrease was however non-significant. Foliar spray of the extracts hold greater promise for control of tukra infested mulberry leaves and did not affect enzyme activity in silkworms. This can sturdily suggest that the natural plant extract sprayed with infested mulberry leaves can be effectively utilized for the silkworm rearing instead of pesticides, insecticides for mulberry sericulturistes.

KEYWORDS: Mealy bug, Plant extracts amino acids.

INTRODUCTION
The silkworm, *Bombyx mori* L. is an important economic insect and also a tool to convert leaf protein into silk. The industrial and commercial use of silk, the historical and economic importance of production and its application in all over the world finely contributed to the silkworm promotion as a powerful laboratory model for the basic research in biology (Ramesh- Babu *et al*., 2009). Due to unfavorable conditions in the environment the pests, insects, bacteria and fungus plays an important role in agriculture, causing a problem to the farmers. As the farmers are using various pesticides and insecticides to control the diseases in agriculture, but the pests are resistive to that pesticides and multiplying the bugs in the plants and decreasing the productivity. Mulberry foliage is also vulnerable to various pathogens and pests and the pests not only reduce the yield but also alter the biochemical components in mulberry leaves which are obviously nutritionally inferior, it leads to crop failure. This focuses on major pest *i.e*., of pink mealy bug, *Maconellicoccus hirsutus (Green)* attack the mulberry plantation, but the exact molecular level interaction is yet to understand and involvement of virus was ruled out. According to the reported literature, early diagnosis methods for the tukra incidences in mulberry (SugnanaKumari *et al*., 2003) and various studies on bio-control of the mealy bug by beetle, *Cryptolaemus montrouzieri* of an exotic enemy as a part of pest management program (Kishore *et al*., 1995; Chakraborthy *et al*., 1999; Katiyar *et al*., 2000; Masilamani *et al*., 2003 Manjunath *et al*., 2003).

Medicinal plants are the richest bio-resources of folk medicines and traditional systems of medicine; and food supplements, nutraceuticals, pharmaceuticals and chemical entities for synthetic drugs. The use of plants or their extracts for the treatment of human disease predates the earliest stages of recorded civilization, dating back at least to Neanderthal period. Pathogenic bacteria have developed resistance against existing antibiotics due to indiscriminate use of antimicrobial drugs to treat the infectious diseases and also more toxic for human being during long term thera

The intermediary metabolism of amino acids is the deamination, which is catalysed by deaminases. The resulting ketoacids may act as acceptors for the transfer of amino groups from other aminoacids, serve as substrates for fat and carbohydrate synthesis or join the main channel of oxidation via the tricarboxylic acid

cycle (Chen, 1966). Of all transaminases, aspartate and α - ketoglutaric acid to oxaloacetic acid and glutamic acid, while alanine amino transaminase (ALAT) catalyses the inter conversion of alanine and α - ketoglutaric to pyruvic acid and glutamic acid (Goldstern and New holme, 1980; Martin *et al.*, 1983). These enzymes are widely present in all organisms and function as link between protein and carbohydrate metabolisms and the net outcome is incorporation of ketoacids into the TCA cycle. The enzyme glutamate dehydrogenase (GDH) also plays a significant role in the catabolism of aminoacids. GDH not only channels the nitrogen from glutamate to ammonia but also catalyses the amination of α - ketoglutarate by using free ammonia (Harper, 1986). Transaminases are present both in the cytosol and mitochondrial fraction whereas GDH in mitochondria. There is much evidence for the shifts in the activities of these enzymes to a variety of environmental and physiological conditions (Bursell, 1963; Knox and Greengard, 1965; Bonitenko, 1974).

MATERIALS AND METHODS

Maintenance of Silkworms

For the present investigation, the popular south Indian cross breeds (CB) silkworms PMxNB4D2 of Bivoltine breeds of Mulberry silkworms variety, *Bombyx mori* (L) was used as test materials. The disease free laying (DFLS,) of this cross breed were produced under field conditions and brought to the laboratory.

Maintenance of botanical Sprayed tukra infested mulberry leaves

Mulberry crop was maintained by following standard agronomic practices. Treatments were imposed on 15th day of pruning in each plot, five plants were randomly selected and the population of pink mealy bug was counted. In each plant, population was counted on three leaves (top, middle and bottom). The total number leaves per plant were also counted and the population was expressed as number per leaf. Observations were made just before spraying (pre-treatment count), 3, 5 and 7 days after spraying. The following plant extracts with naturally existing insecticidal properties were selected for preparation of aqueous plant extracts *Ceriops decandra and Suaeda nudiflora.*

Preparation of the plant extracts

Ceriops decandra and Suaeda nudiflora. Ding Hou plant was collected from Koringa Mangrove forest, near Kakinada, Andhra Pradesh, India (Fig. 1). Leaves of this plant were thoroughly washed and dried in shade. The dried plant material was made into a coarse powder by means of electrical grinder. The dried powdered plant material was extracted in different solvents viz., Hexane, Benzene Chloroform, Ethyl acetate, Acetone and Methanol. The resulted extracts were filtered and then concentrated on a rotoevaporator for solvents elimination and the crude extracts were preserved in sterile, air tight containers for further analysis.

Enzymatic studies in Silkworm fed with botanical-Sprayed Mulberry leaves

A bioassay was conducted to find out the effect of feeding healthy and botanical-Sprayed leaves on silkworm hybrid, PMxNB4D2. Leaves were collected from plots from 0, 2, 5, 7, 10, 15 and 20 days after spray and were fed to fifth instar silkworm. The haemolymph was drawn out from the larvae by puncturing the proleg. The haemolymph was collected in small ice cooled test tubes rinsed with phenylthiourea solution (1% w/v). Dissection of fat bodies was made in cold condition (40C) after making a longitudinal mid – ventral incision along the entire body length and carefully pinning back the cuticle. The fat bodies, free from adhering connective tissues, were carefully taken with the help of forceps and washed with physiological saline (0.9% Nacl). The excess water was removed with the filter paper. The required weight of the tissue was weighed nearest to 0.1mg and used for biochemical analysis. Protease activity was determined by Davis and smith (1955). Activities of alanine and aspertate aminotransferases in the tissue were estimated using the method of Reitman and Frankel (1957).

STATISTICAL ANALYSIS

All the results obtained in this investigation were subjected to statistical analysis. The standard deviation was calculated and ‘t’ values were derived between the control and experimental. The levels of significance were noted from the standard‘t’ values and represented in the respective histogram.

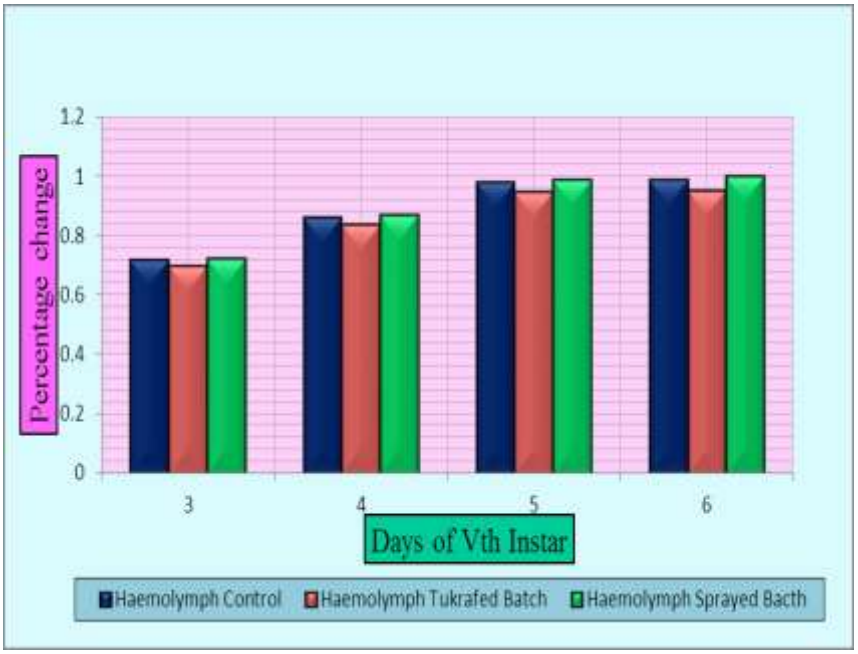
RESULTS

The Protease level in the haemolymph and fat bodies fed batch with botanical sprayed infested mulberry leaves gradually increased at all the days relative to respective haemolymph controls 0.722, 0.863, 0.982,0.989 fat bodies0.451, 0.492, 0.538, 0.563, (Table1) and ALAT levels increased gradually relative to respective controls haemolymph0.113, 0.121, 0.136, 0.151 and fatbodies 0.421, 0.653, 0.721, 0.743, (Table2). The AAT activity level gradually decreased at day6 relative to controls haemolymph 0.124, 0.131, 0.138, 0.156 and fat bodies 0.625, 0.641, 0.716, 0.886, (Table3).

Table: 1. Estimation of protease in haemolymph (mg/100ml) and fat bodies (mg/gm wet wt.) The hybrid race of multi X bivoltine (PMxNB4D2) of silkworm, *Bombyx mori* when fed with tukra and botanical extract sprayed mulberry leaves at days of Vth instar larvae. Each value is a mean of six replicants and Percent change over control is given in parenthesis.

	Name of the tissue		Days of Vth instar			
			3	4	5	6
Race/breed	Haemolymph	Control	0.722	0.863	0.982	0.989
		S.D±	0.027	0.031	0.035	0.033
		Sprayedbatch	0.726	0.0.870	0.990	0.999
		S.D ±	0.091	0.032	0.039	0.031
		%	0.550	0.694	0.720	0.521
		't' test	N.S	N.S	N.S	N.S
		Tukrafedbatch	0.701	0.837	0.946	0.951
		S.D ±	0.091	0.025	0.027	0.036
		%	-0.550	0.460	0.400	0.200
		't' test	N.S	N.S	N.S	N.S
PMxNB4D2	fatbodies	Control	0.451	0.492	0.538	0.563
		S.D ±	0.018	0.019	0.017	0.023
		Sprayedbatch	0.456	0.498	0.550	0.579
		S.D ±	0.015	0.013	0.027	0.019
		%	1.100	0.420	0.940	1.249
		't' test	N.S	N.s	N.S	N.S
		Tukrafedbatch	0.413	0.446	0.510	0.536
		S.D ±	0.017	0.016	0.015	0.014
		%	0.460	0.810	0.400	0.530
		't' test	N.s	N.S	N.S	N.S

S.D±: standard deviation
P=level of significance
N.S=Non significant



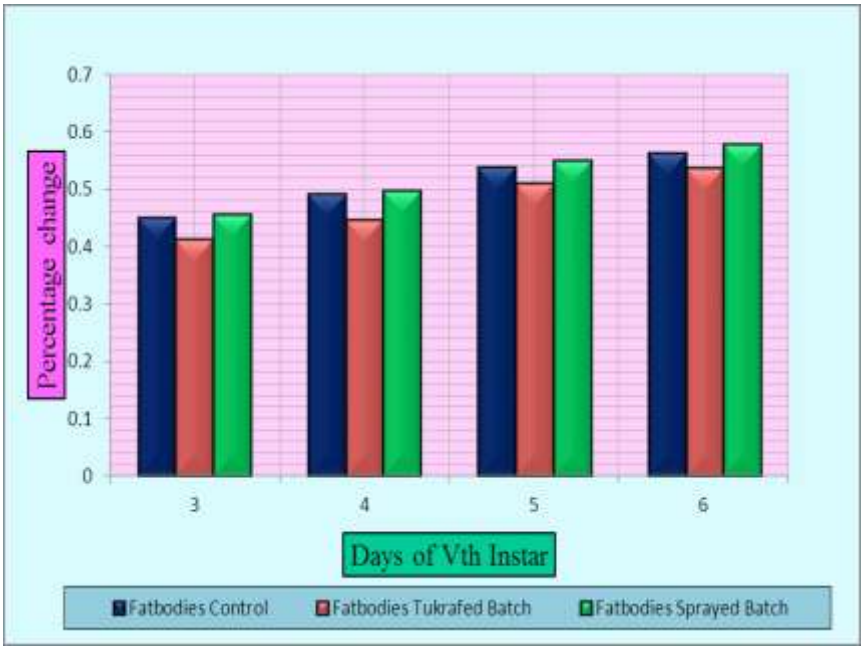
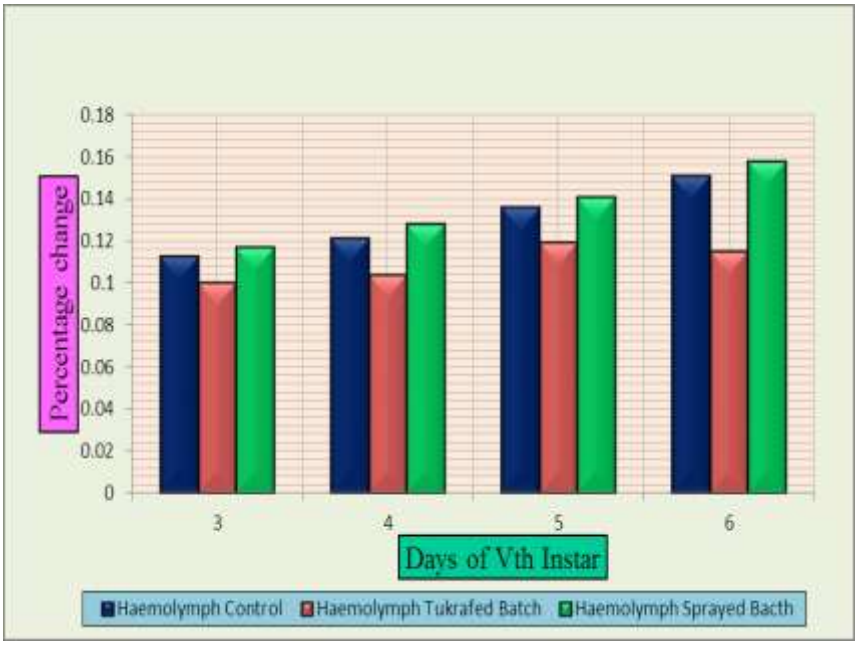


Fig: 1 Percent change over control in protease of haemolymph and fatbodies of PMxNB4D2 hybrid silkworm at different days of Vth instar larvae fed with tukra and botanical extract sprayed mulberry leaves.

Table: 2. Estimation of ALAT in haemolymph (mg/100ml) and fat bodies (mg/gm wet wt.)The hybrid race of multi X bivoltine (PMxNB4D2) of silkworm, *Bombyx mori* when fed with tukra and botanical extract sprayed mulberry leaves at days of Vth instar larvae. Each value is a mean of six replicants and Percent change over control is given in parenthesis.

	Name of the tissue		Days of Vth instar			
			3	4	5	6
Race/breed	Haemolymph	Control S.D±	0.113 0.0046	0.121 0.0041	0.136 0.0039	0.151 0.0035
		Sprayedbatch S.D % 't' test	0.117 0.0062 3.530 N.S	0.128 0.0071 5.760 N.S	0.141 0.0058 3.960 N.S	0.158 0.0065 5.630 N.S
		Tukrafedbatch S.D % 't' test	0.100 0.0052 1.760 N.S	0.104 0.0055 2.470 N.S	0.119 0.0045 2.240 N.S	0.115 0.0039 2.640 N.S
PMxNB4D2	fatbodies	Control S.D	0.421 0.0160	0.653 0.0170	0.721 0.0150	0.743 0.0190
		Sprayedbatch S.D % 't' test	0.436 0.0270 1.180 N.S	0.668 0.0310 0.790 N.S	0.738 0.0270 0.970 N.S	0.759 0.0290 0.800 N.S
		Tukrafedbatch S.D % 't' test	0.403 0.0150 0.470 N.S	0.626 0.0190 0.450 N.S	0.714 0.0210 0.430 N.S	0.726 0.0250 0.400 N.S

S.D±: standard deviation
P=level of significance
N.S=Non significant



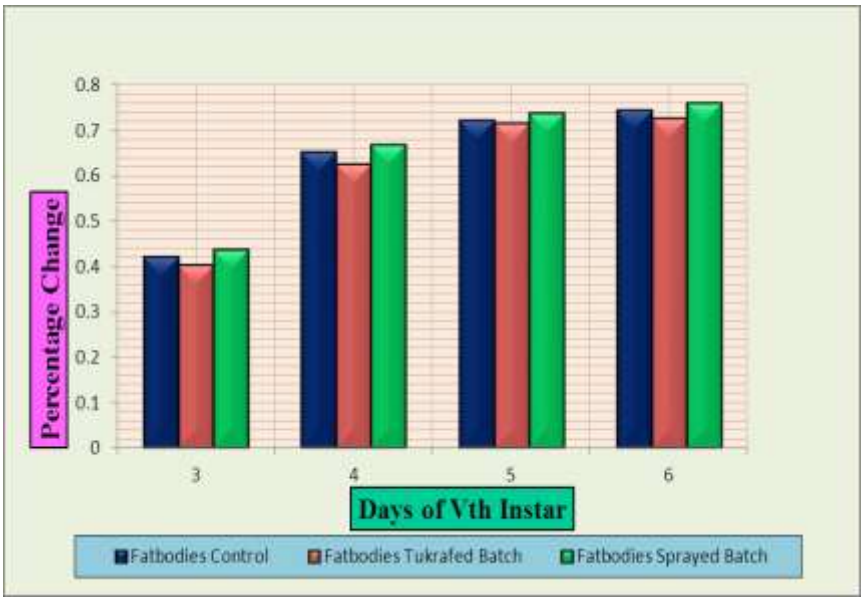


Fig: 2 Percent change over control in ALAT of haemolymph and fatbodies of PMxNB4D2 hybrid silkworm at different days of Vth instar larvae fed with tukra and botanical extract sprayed mulberry leaves.

Table: 3. Estimation of AAT in haemolymph (mg/100ml) and fat bodies (mg/gm wet wt.)The hybrid race of multi X bivoltine (PMxNB4D2) of silkworm, *Bombyx mori* when fed with tukra and botanical extract sprayed mulberry leaves at days of Vth instar larvae. Each value is a mean of six replicants and Percent change over control is given in parenthesis.

Race/breed	Name of the tissue		Days of Vth instar			
			3	**4**	**5**	**6**
PMxNB4D2	Haemolymph	Control	0.124	0.131	0.138	0.156
		S.D±	0.0052	0.0062	0.0059	0.0048
		Sprayedbatch	0.123	0.132	0.149	0.159
		S.D	0.0048	0.0062	0.0059	0.0049
		%	2.410	3.052	2.109	2.169
		't' test	N.S	N.S	N.S	N.S
		Tukrafedbatch	0.131	0.157	0.164	0.181
		S.D	0.0046	0.0057	0.0046	0.0039
		%	2.398	3.042	2.090	2.152
		't' test	N.S	N.S	N.S	N.S

	Fat bodies	Control	0.625	0.641	0.716	0.886
		S.D	0.0230	0.0310	0.0410	0.0480
		Sprayedbatch	0.623	0.639	0.716	0.886
		S.D	0.0227	0.0308	0.0519	0.0502
		%	0.650	0.310	0.562	0.796
		't' test	N.S	N.S	N.S	N.S
		Tukrafedbatch	0.0.631	0.675	0.731	0.908
		S.D	0.0225	0.0302	0.0507	0.0497
		%	0.644	0.304	0.556	0.786
		't' test	N.S	N.S	N.S	N.S

S.D±: standard deviation
P=level of significance
N.S=Non significant

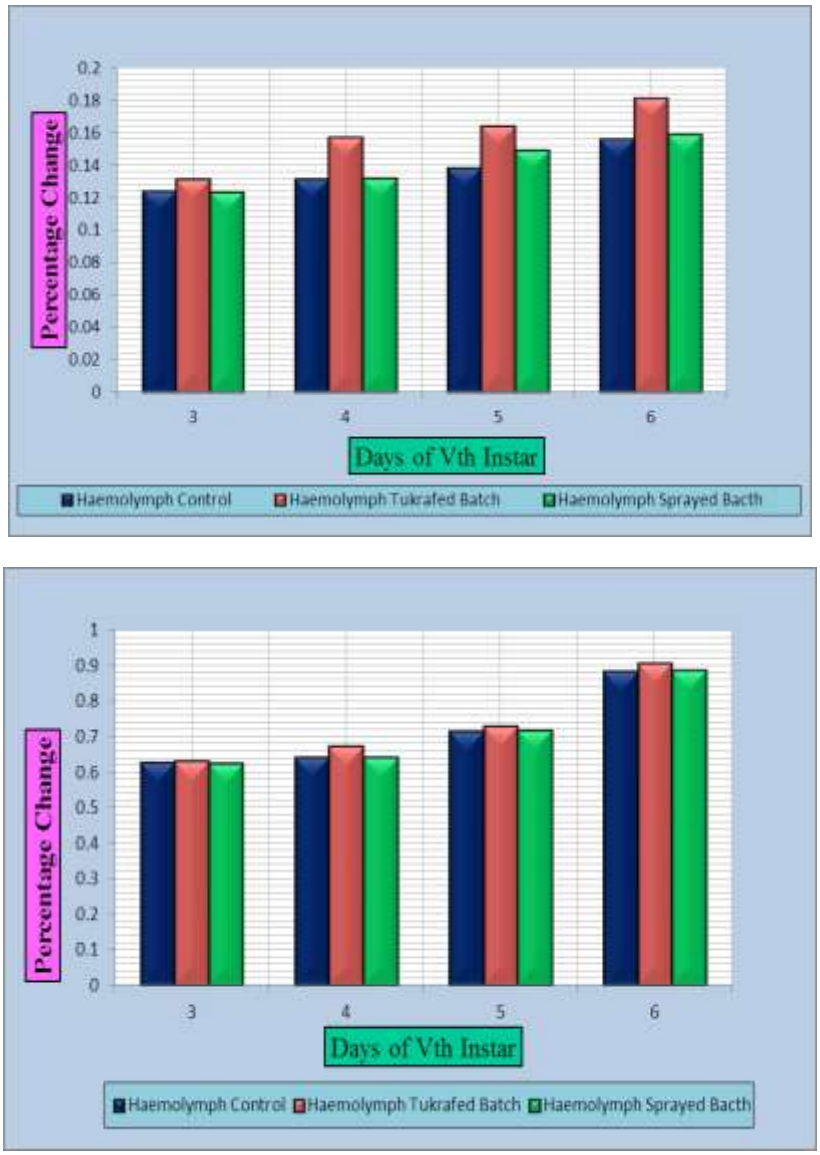


Fig: 3 Percent change over control in AAT of haemolymph and fatbodies of PMxNB4D2 hybrid silkworm at different days of Vth instar larvae fed with tukra and botanical extract sprayed mulberry leaves.

DISCUSSION

The tukra infected mulberry leaves with symptoms of minute mealy bugs, infected mulberry garden, curling of apical leaves (Kumar *et al*., 1997) and the beetles feeds on plant sap and decreases the leaf protein and moisture contents by 17.8 and 3.57%, respectively. The mulberry infested with *M.hirsutus* (green) is a major pest of mulberry in southern parts of India and has become regular pest of mulberry in Andhra Pradesh and Tamil Nadu and other southern states especially during warmer. It has been reported that most of the mulberry varieties were susceptible for the mealy bug, *M. hirsutus* (green)

attack (Muralikumaran and Baskaran, 1992 Mukhopadhy *et.al*, 2006). Leaf curling with mealy bug is the symptoms of tukra infested mulberry and to find out whether spray of the aqueous plant extracts of *Ceriops decandra and Suaeda nudiflora* extract were sprayed to tukra infested V1 mulberry variety and fed to Silkworm (PMxNB4D2 Bivoltine hybrid). As the control of mealy bug, application of chemical pesticides are not advised since they harm the silkworms and recently non chemicals avenues like botanicals acted as an efficient alternative for the pesticides in mulberry garden. Mukhopadhyay *et al.* 2008; 2009) reported that when silkworms fed with botanical sprayed of infested mulberry leaves after observing the waiting period and feeding to silkworms there was no impact on the economic parameters of cocoons. Trehalose is a non-reducing disaccharide, the principal haemolymph sugar, is maintained at a steady state in insects through homeostatic regulation at all stages of the life cycle (Wyatt, 1967).

Protein metabolism is considered as most important in the silkworm physiology because of its vital role in the determination of chemical characteristics of silk proteins (Shigematesu, 1960b). In the study, significant variations were observed in the protease activity, ALAT, AAT, metabolisms of the fifth instar larva of silkworm i.e., (CSR2) on feeding of tukra affected leaves. Vamseedhar *et al*., (2000) reported the impact of mealy bug infestation of mulberry (malformed, curled leaves) was assessed for their nutritive value and relative ability to support the growth and development of *Bombyx mori* of this cross breedCSR2. Results showed that tukra leaves sprayed with natural extracts had better nutritive value with increased proteins and amino acids. The yield of mulberry leaves is reduced in mealy bug affected plants depending on the intensity of infestation (Veeranna, 1997). The utility of natural plant extract insecticides in mulberry ecosystem is determined not only the efficacy of the chemicals against target pests but as well as by the safety to silkworm (Etebari and Bizhannia 2006; Kannan, R. and Sathyaseelan. 2009 Muthuswami *et al.*, 2010).

Protease activity the of haemolymph and fat bodies of PMxNB4D2 hybrid silkworm is accompanied by an insignificant elevation in protease activity (Table1). These results also suggest less proteolytic activity and enhanced protein synthetic potentials. The breakdown of proteins could indicate the domination of proteolysis over synthesis under enhanced proteolytic activity (Harper *et al.*, 1979). The alanine and aspartate amino transferases which function as a startgic link between the carbohydrate and protein metabolism are known to be altered under several pathological and physiological conditions. In the present study, a steady increase in the activities of ALAT in the haemolymph and fatbodies of the silkworm *Bombyx mori* on feeding sprayed mulberry from day 3 to day 6 could be due to the stepwise induction of these enzymes by a greater association of their oligomers (Kulkarni and kulkarni, 1987(Table2). Increase in these enzyme activities could indicate the structural reorganization of amino acids or for the incorporation of keto acids into TCA cycle to favor gluconeogenisis and / or energy production.

A higher level of ALAT and AAT in fat bodies than in haemolymph indicates the activity involvement of former tissue in the transmination reactions. The AAT activity decreased at day 5 and day 6 and activity break down and AAT marks towards the mobilization of amino acids into TCA cycle (Davison and Longslow, 1975(Table3). The decrease in AAT in the fat bodes of tukra sprayed mulberry leaves fed to silkworms partly could provide an evidence for the transamination and their incorporation into TCA cycle. The natural insecticides-sprayed mulberry leaves did not show any adverse effect on rearing of silkworm, feeding silkworms with mulberry leaves harvested from natural extracts after safe waiting period showed significant improvement in respect of larval weight, cocoon weight and shell weight as compare to the infested control (Karippa and Narasimhanna,1978).

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