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Effect of Botanical Extracts against the Tukra (*Maconellicoccus Hirsutus)* Infested Mulberry on Trehalose and Phosphorylase Activity in Silkworm, *Bombyx Mori* L.

Sambanaik[1]Jagadish Naik
Department of Zoology, Acharya Nagarjuna University, Guntur, AndhraPradesh-522510 India

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 botanical extract sprayed to tukra infested mulberry leaves fed to the silkworms and its impact on Trehalose, Phosphorylase activity in tissues like fat bodies and haemolymph was studied. For the study, good healthy leaves(Control)and plant extracts viz., Azadirachta indica,Ocimum Sanctum,&parthenium hysterophorus were sprayed to tukra infested V1 mulberry variety and fed to Silkworm (CSR2 Bivoltine hybrid). The trehalose activity gradually increased, this increase however was significant (P>0.05). The elevation in total phosphorylase activity gradually progressed from day 3 to day 6, in the order: day 3<4<5<6; and the magnitude of increase between one day to the other were statistically significant when fed with natural extracts sprayed mulberry to silkworm 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 aqueous Extract sprayed infested mulberry leaves can be effectively utilized for the silkworm rearing instead of pesticides insecticides for mulberry sericulturistes.*
Key Words*: Tukra, plant extracts, Trehalose, phosphorylase.*

I. 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.Manjunath (2003) reported that 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 Different concentrations of botanicals were reported effective in suppression of Tukra i.e. mealy bugs in mulberry (Mukhopadhyay 2008; 2009). Several natural enemies were recorded from mulberry agro-ecosystem (Bandyopadhyay & Santha Kumar 2007). The changing scenario in mulberry poses newer threats with pests like mealy bugs becoming serious and regular. In the recent years serious damage to mulberry by tukra has been reported in rain fed sericulture tract of Karnataka and Andhra Pradesh. The commonly employed chemicals used for control of tukra are dimethoate, dichlorvos hardly control the disease. Moreover, chemical control of disease leads to environmental pollution as well as bio degradation in soil leads to toxicity (purohit et al., 1978).There is overwhelming support at the global level to use either biological control to eradicate the disease or to employ plant extracts having potency of controlling or eradicating the disease. plant extracts from variety of plants have been reported to posses the inhibiting of mulberry diseases (govindachari, 1992). The present study explores to assess the plant extract sprayed to tukra infested mulberry leaves fed to silkworm and to analyze the role of enzyme activity in tissues of silkworm of cross breed CSR2 (Bivoltine hybrid) silkworm.

II. Materials And Methods

Maintenance of Silkworms

For the present investigation, the popular south Indian cross breeds (CB) silkworms CSR2 of Bivoltine breeds of Mulberry silkworms variety, *Bombyx mori* (L) was used as test materials. The disease free laying (DFLS,) of this cross breed CSR2 (Bivoltine hybrid) were produced under field conditions and brought to the laboratory.

III. Maintenance of botanical Extract Sprayed on mealybugs 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 Tukra 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 Azadirachta indica, Ocimum Sanctum, &parthenium hysterophorus.

Preparation of aqueous plant extract

Aqueous plant extracts were prepared by homogenizing 5 g of the plant material (leaf) in 100ml of distilled water using pestle &mortar. The homogenate was filtered using 3-layered muslin cloth. The resulting clear solution was used as foliar spray. The aqueous extract was sprayed using hand sprayer twice a week for 45days on mulberry until the solution ran down the leaf plants.

Enzymatic Studies in tissues of CSR2Silkworm 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, CSR2. 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 (4^0C) 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. Trehalose concentration was determined by Schmidt& Platzer,(1980).Phosphorylase activity in the Fat bodies were estimated using the method of Sutherland(1955).The activity is expressed as mg/100ml&mg/g wet wt of tissue. The Phosphorylase activity is expressed as uMpi liberated/mg protein/h, using phosphate standards.)

IV. Stastical 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.

V. Results

The Trehalose level in the haemolymph and fat bodies fed with botanical sprayed tukra infected mulberry leaves batch increased activity levels at all the days relative to respective haemolymph controls (152.400, 154.450, 159.700 and 162.200) and also activity levels increased gradually relative to respective fat bodies controls (6.310, 6.330, 6.390 and 6.400). The increases in the Trehalose level were almost Non Significant (Table1). The Phosphorylase activity level in fat bodies levels fed with tukra affected leaves gradually increased relative to controls (0.136,0.152, 0.156and 0.160). The increases in the phosphorylase activity in fat bodies at day 3 and almost significant ($P<0.05$) (Table2).

VI. Discussion

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 ((Sriharan *et al*., 1979; Muralikumaran and Baskaran, 1992:Mukhopadhy et.al,2006). leaf curling with mealy bug are the symptoms of tukra infested mulberry and to find out whether spray of the aqueous plant extracts of Azadirachta indica,Ocimum Sanctum,&parthenium hysterophorus extract were sprayed to tukra infested V1 mulberry variety and fed to Silkworm (CSR2 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 (yamamoto et al.,2007) and sathayaseelan &Baskaran(2010). 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). Analysis of Trehalose (Table1) showed that gradual increase was recorded in sprayed plant extracts fed to the silkworm and also some what better performance was observed over control. 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; Muthuswami *et al.*, 2010). A key control step in glycogenolysis is catalyzed by glycogen phosphorylase. The phosphorylase 'a' form and the inactive dimeric phosphorylase 'b' form requires C-5-AMP for its activity (Cori and Cori, 1945; Cori *et al.*, 1955). Progressive increase of phosphorylase activity (Table2) from day 4 to day 6 indicates the the percentage of active glycogen phosphorylase in the fat body increased within hours of starvation and its glycogen content decreased gradually. Misra et al, (2003) reported that some insecticides-sprayed laves were effective against mealybugs. 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 (Kariappa and Narasimhanna, 1978). Banken&Stark (1998) and Dickson (2006) reported that some insecticides-sprayed leaves were effective against mealy bug (Tukra).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).

Table-1 Percent change over control in Trehalose in haemolymph and fat bodies of silkworm at days of Vth in star larvae fed with extracts sprayed Mulberry leaves.

Name of the tissue		Days of Vth in star			
		3	4	5	6
Haemolymph	Control	152.400	154.300	159.700	162.200
	S.D.±	5.250	5.150	3.990	5.356
	Sprayedmulberry	153.800	155.600	160.900	164.610
	fedbatchS.D.±	3.990	4.550	4.890	5.312
	%change	0.967	0.940	0.890	1.489
	't' test	N.S	N.S.	N.S.	N.S.
Fatbodies	Control	6.310	6.337	6.390	6.451
	S.D.±	0.261	0.215	0.198	0.279
	Sprayedmulberry	6.390	6.440	6.471	6.563
	fed batchS.D.±	0.231	0.241	0.254	0.279
	%change	1.290	1.294	2.110	2.148
	't' test	N.S.	N.S.	N.S.	N.S.

Table.2 Percentage change over control in Phosphorylase activity in fat bodies of silkworm at days of Vth instar larvae fed with extract sprayed Mulberry leaves

Name of the tissue		Days of Vth instar			
		3	4	5	6
Fat bodies	Control S.D.±	0.136	0.152	0.156	0.160
		0.0054	0.0051	0.0049	0.0055
	Sprayedmulberry	0.140	0.159	0.162	0.167
	fed batchS.D.±	0.0056	0.0038	0.0040	0.0049
	%change	2.940	4.640	4.560	4.453
	't' test	N.S	N.S.	N.S.	N.S.

VII. Conclusion

Based on the results of the study mulberry growers may use botanical extracts instead of chemicals which is used for the suppression of mealy bugs in mulberry fields. Feeding of silkworm with the mulberry leaves treated with botanical extracts showed marked improvement in the silkworms instead of feeding with tukra infected mulberry

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