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ORIGINAL ARTICLE

Impact of Botanical Extracts on the Incidence of Major Pest (tukra) in Mulberry leaves on Carbohydrate metabolism in Silkworm, *Bombyx Mori* L.

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ABSTRACT
The continuous use of pesticides over a period of time cannot sustain the crop yield and also harmful affects on soil and environment. Mulberry leaves are the predominant food source for silkworm, Bombyx mori rearing. The incidence of Pink mealy bug occurring in mulberry plantation can cause tukra disease that leads to qualitative loss of mulberry plantation. The present study was undertaken to study the effect of local botanical plant extracts having potential against the pests and insects as natural botanicals origin by foliar spray. The botanical plant extracts viz., Azadirachta indicia, pongamia pinnata and occimum sanctum were sprayed to occurring mealy bugs at the early cause of infection to V1 mulberry variety and reared to Silkworm. The total carbohydrates and glycogen and glucose parameters were studied in tissues like silk gland and haemolymph. The carbohydrate and glucose activity gradually increased this increase however was significant at (P>0.05).There were a gradual decrease of Glycogen level from day 3 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 the carbohydrate metabolism in silkworms.
***Key words**: Tukra, botanical plant extracts, carbohydrates*

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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 [1]. 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. Recently discussed on early diagnosis method for the tukra incidences in mulberry [2] 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 [3-5].
Different concentrations of botanicals were reported effective in suppression of Tukra i.e. mealy bugs in mulberry [6,7]. Several natural enemies were recorded from mulberry agro-ecosystem [8]. 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 Andhrapradesh. 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 [9].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 [10]. 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 PMxNB4D2 (Bivoltine hybrid) silkworm.

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 PMxNB4D2 (Bivoltine hybrid) 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 *Azadirachta indica, Pongamia pinnata and occimum sanctum*.

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.

Carbohydrate metabolism 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 (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.

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 carbohydrate level in the haemolymph and silk gland fed on leaves treated with botanical sprayed mulberry leaves gradually increased at all the days relative to respective haemolymph controls 190, 194.1,1 99.3, 205.7 Silk gland 15.6,16.6,17.4,18.5(Table1) and glucose levels increased gradually relative to respective controls haemolymph 27,27.3, 28.3, 29. And Silk gland 0.66,0.69,0.73,0.74 (Table 2).The glycogen activity level gradually decreased at day 5 to day6 relative to controls haemolymph 11.2, 12.3, 12.8, 13.2 Silk gland 9.22, 9.4, 10.1, 10.6, (Table 3).

DISCUSSION

The tukra infected mulberry leaves with symptoms of minute mealy bugs, infected mulberry garden, curling of apical leaves 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 [12, 6]. Leaf curling with mealy bug is the symptoms of tukra infested mulberry and to find out whether spray of the aqueous plant extracts of *Azadirachta indica*, *Pongamia pinnata, occimum sanctum plant* extract were sprayed to tukra incindence at earlier of 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. [6, 7] 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. Carbohydrates are 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 [12].

There is a gradual increase in total carbohydrate from day 3 to day 6 when reared with botanical foliar spayed extract against the occurring mealy bugs and found that the sprayed proved the best maximum food consumption, larval body weight and cocoon recovery Mohammad Aslam and Mohammad Ashtag [13]. The foliar application of mulberry has impact and did not affect the feeding or nutritional status of mulberry with incidence of suppression of bugs influencing on metabolic activity of silkworms or digestive ability enhanced during rearing condition. Elevation in total carbohydrates and glucose level through the decrease in glycogen in haemolymph and silk gland of the PMxNB4D2 hybrid at day 3 to day 5 indicated increased gluconeogenisis in order to possess the energy reserves. The decrease through insignificant, in glycogen levels in haemolymph than in fat bodies in the silkworm *B.mori* $PMxNB_4D_2$ at sprayed mulberry leaves fed to silkworm could be due to the rapid absorption of them from the haemolymph into the tissues of silkworms and lack of nutrional products in the mulberry or mobilization of glucose to the required tissues from the silkworm for energy releasing purposes.

Several reports revealed that application of seri boost extracts has good efficacy on suppression of mealy bugs and also having good potential application in sustainable development in agriculture biotechnology by Bucker field *et al.,* [14].Raman Suresh babu *et al.,*[15] and Venkataramana *et al.*, [16] reported that aqueous leaf extract *of Azadiracta indica, Rhizophora apiculata and parthenium hystophorus* used as foliar spray in control of tukra disease. Jadhave *et al*., [17] who reported that the nutritional sources effects not only the growth and development of silkworm larvae but also its final silk produce. The foliar spray proved best with ready availability of the material at the real site of action, i.e. the leaves which are to be fed to larvae compared with other application.

Table 1: Effect of local traditional plant extracts on the incidence of tukra mealy bug M*aconelli coccus hirsutus* on total carbohydrates in haemolymph and silk gland of silkworm.

RACE BREED	**Name of the tissue**		**Days of Vth instar**			
			3	4	5	6
	Haemolymph	Control	190.00 7.52	194.10	199.30 6.96	205.70
		S.D.±		8.73		7.79
		Sprayedbatch	196.00	200.10	200.20	209.20
		S.D.±	7.02	6.28	7.05	6.86
PMxNB4D2		%	2.63	3.09	2.96	1.7
		't' test	P<0.01	N.S	N.S	N.S
	Silkgland	Control	15.60	16.60	17.40	18.50
		S.D.±	0.59	0.66	0.6	0.74
		Sprayedbatch	17.00	18.00	19.20	20.00
		S.D.±	0.57	0.63	0.66	0.68
		%	8.97	8.43	10.34	8.10
		t' test	P<0.01	P<0.01	P<0.01	P<0.01

S.D.±: Standard deviation P: level of significance. N.S: Non Significant

Table 2: Effect of local traditional plant extracts on the incidence of tukra mealy bug M*aconellicoccus hirsutus* on Glucose level in haemolymph and silk gland of silkworm

RACE BREED	**Name of the tissue**		**Days of Vth instar**			
			3	4	5	6
	Haemolymph	Control	27.000	27.300	28.300	29.000
		S.D.±	1.080	1.200	0.950	0.980
		Sprayedbatch	28.200	29.600	30.200	32.100
PMxNB4D2		S.D.±	0.850	1.150	1.350	1.280
		%	4.440	8.420	6.710	10.680
		't' test	N.S	P<0.05	P<0.05	P<0.01
	Silk gland	Control	0.661	0.692	0.733	0.745
		S.D.±	0.026	0.024	0.032	0.036
		Sprayedbatch	0.672	0.699	0.738	0.750
		S.D.±	0.018	0.016	0.021	0.020
		%	1.660	1.010	0.680	0.670
		't' test	N.S	N.S	N.S	N.S

S.D.±: Standard deviation P: level of significance. N.S: Non Significant

Table: **3:** Effect of local traditional plant extracts on the incidence of tukra mealy bug M*aconellicoccus hirsutus* on Glycogen level in haemolymph and silk gland of silkworm

RACE BREED	**Name of the tissue**		**Days of Vth instar**			
			3	4	5	6
	Haemolymph	Control	11.200	12.300	12.800	13.200
		S.D.±	0.450	0.520	0.380	0.420
		Sprayed batch	11.300	12.200	12.700	13.000
PMxNB4D2		S.D.±	0.370	0.290	0.310	0.450
		%‘	0.890	-0.810	-0.780	-1.510
		t’ test	N.S	N.S	N.S	N.S
	Silk gland	Control	9.220	9.460	10.100	10.600
		S.D.±	0.360	0.280	0.220	0.260
		Sprayed batch	9.230	9.440	10.000	9.9600
		S.D.±	0.290	0.350	0.360	0.410
		%	0.100	-0.210	-9.990	-9.430
		‘t’ test	N.S	N.S	N.S	P<0.001

S.D.±: Standard deviation P: level of significance. N.S: Non Significant

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 or contaminated mulberry.

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