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STUDIES ON ENZYME ACTIVITY IN SILKWORM, *BOMBYX MORI* L. FED WITH THRIPS (*PSEUDODENDROTHRIPS MORI)* NIWA INFESTED MULBERRY LEAVES

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In mulberry, thrips is a dominant pest and causing a lot of damage to the mulberry. The effect of pest infected mulberry leaf feeding with Silkworm and role of enzyme activity in Silk worm have been studied. For the study, good healthy leaf without thrips infestation batch and infested with thrips leaves were fed to cross breed Silkworm (CSR2 Bivoltine hybrid). SDH, LDH and GDH activities were estimated in haemolymph and fat bodies of the silkworm. The SDH and LDH activity gradually increased, this increase however was significant ($P>0.05$). The GDH activity decreased in both the tissues from day 4 to day 6 when fed with infested mulberry to silkworm. The results indicate that thrips infected mulberry feed has adverse effect on silkworm rearing and Moreover, overall improvement was not noticed in thrips infected leaves fed batch. This can sturdily suggest that thrips infested mulberry leaves cannot be effectively utilized for the silkworm rearing in acute shortage of healthy mulberry leaves.

Key words : *Bombyx mori,* Mulberry thrips, *Pseudodendrothrips mori,*

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 on 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 tó 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. The growth and development of silkworms were also found to be affected when they were fed with infested mulberry leaves .This focuses on major pest *i.e.* thrips (*Pseudodendrothrips mori*), which effects the mulberry leaves.Thrips, is a highly oligophagous pest and a native of northern hemisphere (Mound, 2003, 1999). Mulberry thrips (*Pseudodendrothrips)* is a major pest of mulberry trees recorded from different sericulture regions of the world and in Southern Parts of India.

Infestation of mulberry garden by different species of thrips was reported by different scientists (Palmer, 1990; Wan & Zhang, 1997; Mound, 1999 Etebari *et al.,* 2000), *Pseudodendrothrips mori* (Thysanoptera : Thripidae) was found to be most dominant species in different parts of world. It is also reported from Africa, Australia, China, India, Japan, Korea Sri Lanka, and Vietnam. *P. mori* is introduced as dominant species in the insect fauna of mulberry field in the India, whereas this species was dispersed in all regions of world with mulberry orchards (Etebari *et al.*, 1998). Mulberry, *Morus alba* L. is the sole food land for silkworm, *Bombyx mori* L. and is grown under varied climatic conditions ranging from temperate to tropical. Mulberry leaf is a major economic component in sericulture since the quality and quantity of leaf produced per unit area have a direct bearing on cocoon harvest (Etebari, *et al.*, 2000). *P. mori* feed on fully expanded leaves and young tissue in the bud. Infested leaves dry out and have a stippled or silver lecked appearance. Small brownish specks of excrement will usually be noticed on the underside of the leaves (Lewis, 1997).

Estimation of crop losses are required to determine the relative importance of particular pests, and thus decide upon the level of resources that should be devoted to research and pest management inputs and provide a basis for understanding the often complex interaction between pest and its host plant. Bhattacharya *et al.* (1993) reported thrips on mulberry in West Bengal. Devaiah & Kotikal (1983) reported the incidence of thrips on mulberry in Karnataka and Andhra Pradesh. The damage by thrips infestation causes numerous tiny dots of wounds leading to quick evaporation from leaf surfaces especially during summer seasons (Venugopalapillai & Krishnaswami, 1983). The damage by thrips cause loss of moisture from leaves besides causing appreciable reduction in nutritive value of leaves by inducing biochemical changes in leaves. The present study explores to assess the damage caused by thrips infestation in mulberry leaves and effort has been made to analyze the role of enzyme activity in tissues of silkworm on feeding with thrips infected leaves on popular cross breed CSR_2 (Bivoltine hybrid) silkworm during the 5th instar over the healthy leaves fed batch.

MATERIALS AND METHODS

Maintenance of Silkworms : For the present investigation, the popular south Indian cross breeds (CB) silkworms CSR_2 of Bivoltine breeds of Mulberry silkworms variety, *Bombyx mori* (L) was used as test materials. The disease free laying (DFLS) of this cross breed CSR_2 (Bivoltine hybrid) were produced under field conditions and brought to the laboratory.

Collection of thrips infested mulberry leaves **:** Effect of infestation on two dominant mulberry varieties of the area *viz* V1/local variety were selected at mulberry gardens of Sericulture Department (Anantapur), for the present study. For each mulberry variety, four plots of $200m^2$ areas in randomized block design were utilized. Two of them were sprayed with 0.5% Metasystox at the peak period of occurrence of second nymph population to obtain healthy leaf without any infestation while another two plots of both the varieties were left without any spray to obtain the infested leaves according to Lim *et al.*, 1990).The both infected and healthy mulberry leaves were utilized for the experiment was brought from well maintained and irrigated mulberry garden.

Silkworm Rearing **:** The popular disease free laying of the cross breeds CSR2 (Bivoltine hybrid) was utilized for the present study. Silkworm laying of CSR2 was incubated, brushed on the freshly chopped mulberry leaves and reared under standard rearing conditions (Krishna swami, 1975). Relative humidity and the late age larvae (4th and 5th instars) were reared at 24-26°C with a relative humidity of 70-80%. From 1st to

4th stages, healthy V1/local mulberry leaves variety were fed under standard silkworm rearing conditions. After resumption from 4th moult, 300 larvae were counted and kept in a plastic tray and fed with fresh thrips infected V1/local varieties of mulberry leaves harvested separately and fed to the experimental batch till spinning. Proper care was taken during feeding, bed cleaning and spinning under hygienic conditions. Fifth in star larvae was taken for experimentations.

Mode of isolation of tissues for activities : 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. Succinate dehydrogenase activity in the tissues was estimated using the colorimetric method of Nachlas *et al.* (1960). Lactate dehydrogenase activity in the tissue was estimated using the method by Govindappa & Swami (1965).GDH activity was estimated by Lee & Lardey (1965).The activity is expressed as u formazon/ mg protein/ hour.

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 AND DISCUSSION

The SDH activity in the haemolymph and fat bodies fed with thrips infested mulberry leaves batch increased activity levels at all the days relative to respective haemolymph controls (0.035, 0.038, 0.042and 0.051) and also activity levels increased gradually relative to respective fat bodies controls (0.106, 0.115, 0.130 and 0.140)and almost insignificant (P>0.05) (Table I). The LDH activity level in haemolymph and fat bodies levels fed with thrips affected leaves gradually equal to control batch the days relative to respective haemolymph controls (0.013, 0.015, 0.027and 0.036) respective fat bodies controls (0.136,0.144, 0.152and 0.155)and insignificant (P>0.05) (Table2). The GDH activity decreased in the haemolymph and fat bodies of the silkworm fed batch at day 6 relative to respective haemolymph controls (0.029, 0.036, 0.040 and 0.045) decreased at day 5 and day 6 relative to respective fat bodies controls (0.102, 0.115, 0.120 and 0.126).were non-significant (P> 0.05).

The thrips infested mulberry leaves, commonly known as Thunder Flies or Thunder Bugs, are tiny insects that range in length from one twenty-fifth of an inch to one-eighth of an inch. Thrips feeds on plant sap and decreases the leaf protein and moisture contents by 17.8 and 3.57%, respectively. *P. mori* negatively affected the quality of leaves consumed by silkworms. The mulberry thrips, (*P. mori)* is a major pest of mulberry orchards in southerner parts of India and has become regular pest of mulberry in Andhra Pradesh and Tamil Nadu and other southern states especially during warmer months (Misra2003). The damage by thrips infestation causes numerous tiny dots of wounds

Table I : Percent change over control in SDH activity in haemolymph and fat bodies of silkworm at days of Vth instar larvae fed with thrips infested Mulberry leaves.

Name of the tissues		Days of Vth instar 3	4	5	6
	Control	0.035	0.38	0.042	0.051
	S.D. ±	0.0014	0.0016	0.0022	0.0017
Haemolymph	Thrips fed batch	0.400	0.046	0.049	0.059
	S.D. ±	0.0016	0.0022	0.0025	0.0029
	%	14.280	21.050	16.660	15.680
	't' test	p<0.001	p<0.001	p<0.001	p<0.001
	Control	0.105	0.115	0.130	0.140
	S.D. ±	0.0042	0.0038	0.0036	0.0029
Fat bodies	Thrips fed batch	0.116	0.176	0.139	0.150
	S.D. ±	0.0054	0.0061	0.0052	0.0049
	%	10.420	0.560	6.920	7.140
	't' test	p<0.01	p<0.01	p<0.01	p<0.01

S.D.± : Standard deviation; p : Level of significance.

Table II : Percent change over control in LDH activity in haemolymph and fat bodies of silkworm at days of Vth instar larvae fed with thrips infested Mulberry leaves.

Name of the tissues		Days of Vth instar 3	4	5	6
	Control	0.013	0.015	0.027	0.030
	SD±	0.00052	0.0049	0.00059	0.00061
Haemolymph	Thrips fed batch	0.014	0.016	0.029	0.032
	S.D. ±	0.00055	0.00045	0.0057	0.00058
	%	34.460	24.660	12.810	14.660
	't' test	p<0.001	p<0.001	p<0.001	p<0.001
	Control	0.136	0.144	0.152	0.155
	S.D. ±	0.00610	0.00590	0.00680	0.00720
Fat bodies	Thrips fed batch	0.143	0.145	0.158	0.160
	S.D. ±	0.00590	0.00620	0.00690	0.00696
	%	1.940	2.470	4.570	4.800
	't' test	N.S.	N.S.	p<0.05	N.S.

S.D.± : Standard deviation; p : Level of significance;. N.S : Non Significant.

leading to quick evaporation from leaf surfaces especially during summer seasons (Venugopalapillai & Krishna Swami, 1983).The thrips infestation affects the qualitative and quantitative characters of mulberry leaf Das *et al.* (1994). Decrease in chlorophyll and carotenoid contents in infested leaves may have been due to loss in chlorophyll synthetase activity in response to thrips infestation (Das *et al.*, 1994). Paul *et al.* (1992) reported that leaves moisture is one of the most important factors in silkworm nutrition. Mulberry thrips reduces the moisture by 3.57%, therefore has a negative impact on the quality of the leaves consumed by silkworm (Etebari *et al.*, 1998). Kariappa & Narashimanna (1978) reported that Feeding silkworms with mulberry leaves harvested from dimethoate plots after safe waiting period showed a significant improvement in respect of larval weight, cocoon weight and shell weight as compared to the infested control and spraying show marked improvement in larval weight and cocoon parameters than feeding the larvae with thrips infested leaves. It has been reported that most of the mulberry varieties were susceptible for the thrips attack (Naik, 1997). There are no sufficient information available on the effects of thrips damage on silkworm rearing (Paik & Lee 1984; Etebari & Matindoost, 2004).

Etebari & Bizhannia (2006) opined that during dry season due to excess evaporation through the wounds caused by thrips, the infestation can seriously reduce mulberry yield. Lactate dehydrogenase (LDH) is the key enzyme responsible for the conversion of pyruvate to lactate it is located at the vital point between glycolysis and TCA cycle. Succinate dehydrogenase (SDH), a flavin linked enzyme of TCA cycle catalyses the reversible oxidation of succinate to fumarate and serves as a link between electron transport and oxidative phosphorylation (Singer *et al.*, 1973). Since the activity of SDH in mitochondria is for greater than the other enzyme of TCA cycle, an insight into the alteration of this enzyme activity may be taken to reflect the function of the TCA cycle. Progressive decrease of GDH activity from day 4 to day 6 indicates the gradual elevation of oxidative deamination of amino acid through ketoglutarate which facilitates division of amino acid in to TCA cycle to meet the energy from the nutritive demand. Hiarayama & Nakamuram (2002) reported the regulation of glutamine metabolism during starvation with effect to development of *Bombyx mori.* The symptoms of an animal can be

Table III : Percent change over control in GDH activity in haemolymph and fat bodies of silkworm at days of Vth instar larvae fed with thrips infested Mulberry leaves.

Name of the tissues		Days of Vth instar 3	4	5	6
Haemolymph	Control	0.029	0.036	0.040	0.045
	S.D. ±	0.0013	0.0021	0.0023	0.0022
	Thrips fed batch	0.028	0.032	0.039	0.036
	S.D. ±	0.0019	0.0023	0.0031	0.0032
	%	-3.440	-10.000	-3.500	-19.000
	't' test	N.S.	p<0.05	N.S.	p<0.001
Fat bodies	Control	0.102	0.115	0.120	0.126
	S.D. ±	0.0041	0.0044	0.0045	0.0052
	Thrips fed batch	0.100	0.114	0.116	0.111
	S.D. ±	0.0059	0.0062	0.0065	0.0053
	%	-1.960	-0.960	-3.330	-10.900
	't' test	N.S.	N.S.	N.S.	p<0.01

S.D. ± : *Standard* deviation; p : Level of significance;. N.S. : Non Significant.

manifested, when pests contaminate largely in mulberry leaves when the silkworm feeds on mulberry leaves. Such symptoms are manifested in terms of morphological, physiological and biochemical variations in the silkworm.

Conclusion : If mulberry garden is infected with thrips, the sericulture farmers or the errer can discard the thrips infected mulberry leaves may not be effectively utilized for hybrid silkworm rearing since it is affecting the dietary to silkworm feeding. It may be due to the presence or reduction of nutrients by the stimulation of the pathogen in thrips infected leaf than that of healthy mulberry leaf and emphasis for further research may be given on actual biochemical/biotechnological process.

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