RESEARCH PAPER:

Impact of enzyme activity in silkworm, *Bombyx mori* (L.) feeding with tukra affected mulberry leaves

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SUMMARY

In the present study fifth instar larvae PMxNB4D2 (F1 Multix Bivoltine hybrid) silkworm were selected for feeding with tukra leaves due to the rapid growth at this period, AAT and ALAT enzyme activities in haemolymph and fat bodies of the silkworm when fed with 25%, 50%, 75% and 100% tukra affected leaves were normal from day 3 to day 6 and in the both the tissues. The contents gradually increased, this increase however was significant (P>0.05). This increase gradually progressed from day 3 to day 6 in the order of day 3<4<5<6. The GDH activity decreased in both the tissues from day 4 to day 6 at 75% and 100%. tukra fed silkworm larvae. Hence, there was a situation of nutritive imbalance and physiological status created in the tissues of silkworm. Estimation of enzyme activities indicated the stepwise break down by the bio molecules in the tissues of silkworm due to vast infestation of mealy bugs in mulberry leaves which lead to very low nutritive value in mulberry at 75% and 100% tukra affected leaves which is the dietary source to the silkworm, *Bombyx mori* (L.).

Key words :
Bombyx mori, tukra, AAT, ALAT, GDH

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Cultivation of mulberry (*Morus* sp.) and rearing of silkworms are the basis of sericulture industry that is dependent primarily on mulberry leaves. The only source of nutrition for the silkworm, the growth and development of the larvae and subsequent cocoon production are very much influenced by its nutritive value (Anonymous, 1975; Krishnaswamy, 1994).

Sericulture has really come to age and is now poised for a great leap forward. The success of sericulture depends industry upon several environmental factors which play major role. There are biotic and abiotic factors which influences upon silkworm and leaf quality, seed quality, fecundity etc. Among these, environment plays a greater role at various stages during the course of insect development. There are no silkworm races at present which be deemed totally resistant to contaminated food, but different races of silkworm shows variation in their susceptibility to different pests and pesticides (Liu,1984). Moore (1967) has discussed in detail the problem of pests and its management in ecological research. The mealy bug, *Maconellicocus hirstus* (Homiptera; Pseudococcidae) is reputed as a vector of the viral disease, popularly known as tukra disease of mulberry (Rangaswami *et al*.,1996). Mealy bug infestation of mulberry by *Maconellicoccus hirsutus* (Green) causes malformation of terminal buds and the appearance of small curly leaves on the shoots. The food and dietary water intake and utilization were studied by feeding diseased mulberry leaves to the silkworms, *Bombyx mori* (L.) during the fifth instars, caused shortening of the level duration and significant in conversion rate, conversion efficiencies, water absorption efficiency, water retained in the body and water retention efficiency. In continuation of this, a significant increase in biomass, cocoon, pupal and shell weights followed by their efficiencies were noticed inspite of lesser wet food consumed and water intake and utilization were decreased (Aftab Ahamed *et al.*, 1999). The efficiency is the amount of food required to reach its potentiality will be manifested in various ways and degrees (Waldbauer, 1968). Therefore an effort has been made to find out the effect of feeding tukra affected leaves by silkworm rearing performance.

MATERIALS AND METHODS

For the experimental materials, the popular south Indian F_1 cross breed (CB) silkworm PM x NB4D2 race hybrid F1 variety of silkworm *Bombyx mori* (L.) was used as test insects, which are abundantly available in and around Anantapur district, A.P.

Maintenance of silkworm:

The disease free layings of the cross breed PMxNB4D2 race Multi x Bivoltine hybrid were taken from the Government grainage, Anantapur, Andhra Pradeshare brought to laboratory. The method of incubation of disease free laying silkworm and silkworm rearing was followed that as advocated by Krishnaswamy *et al*. (1978). The insects were collected in pre-disinfected rearing trays, where they were fed four times a day (6.00, 1.00, 16.00 and 22.00hrs) on fresh mulberry and tukra affected mulberry leaves (mori species – V_1 variety and local variety) leaves except during moulting period. Mealy bug, *Maconellicoccus hirsutus* (Family: Pseudococcidae) presence has been reported in and around Anantapur district, Andhra Pradesh. Each adult female deposits 350-500 eggs in a loose cotton terminal. Eggs are elongated in shape and orange in colour, hatching takes place in about 5-10 days depending upon the climatic conditions. Nymphs are covered with mealy substances. The females have three while males have four nymphal instars. Adults reproduce parthenogenetically, they mate but do not feed and die in 2-3 days. Occurrence of tukra is mostly in summer. Severe infestation of mulberry occurs in nurseries by *M.hirsutus* in April-May which corresponds to the summer season.Fifth instar silkworm larvae were selected for feeding, due to rapid growth during this period and the preference of tukra affected leaves to silkworm. Mulberry being a perennial crop and forms sole food of silkworm which is affected by several insects and pests, among them is *M. hirsutus* is of late severe one (Manjunath *et al.,* 1992)

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. The amino transferases were estimated by the method of Reitman and Frankel (1957). The values were expressed as µ moles of pyruvate per mg protein per hour.

Results and Discussion

The ALAT activity in the haemolymph and fat bodies of the silkworm fed with 25%, 50%, 75% and 100% tukra affected leaves increased at all the days relative to respective haemolymph controls (0.113, 0.121, 0.136 and 0.151) and also increased at all days relative to respective fat bodies controls (0.421, 0.653, 0.721 and 0.723). The increase in the enzyme activities was less at day 3 and almost insignificant (P>0.05) (Fig. 1 and 2).

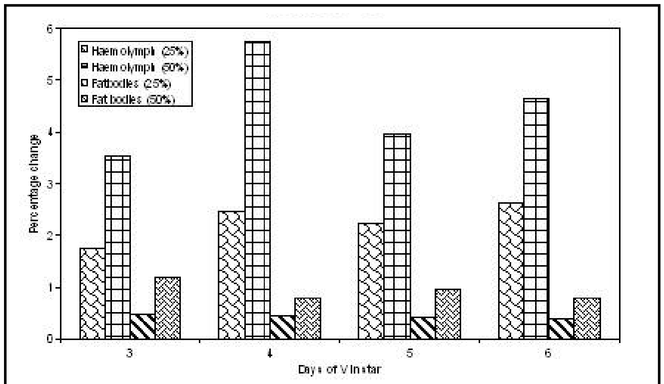

Fig. 1 : Per cent change over control in the ALAT of haemolymph and fat bodies of PMXNB$_4$ D2 hybrid silk worm at different days of v instar larvae fed with 25% and 50% tukra affected leaves

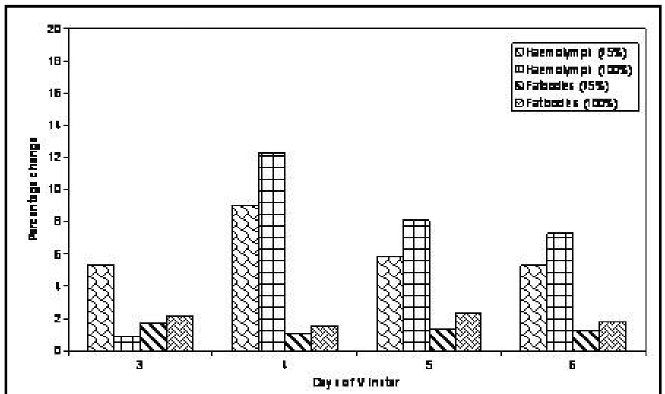

Fig. 2 : Per cent change over control in the ALAT of haemolymph and fat bodies of PMXNB$_4$ D2 hybrid silk worm at different days of v instar larvae fed with 75% and 100% tukra affected leaves

The AAT activity in the haemolymph and fat bodies of the silkworm fed with 25%, 50%, 75% and 100% tukra affected leaves increased at all the days relative to respective haemolymph controls (0.124, 0.131, 0.138 and 0.156) and also increased at all days relative to respective fat bodies controls (0.625, 0.641, 0.714 and 0.882). The increase in the enzyme activities was less at day 3 and almost non significant (P>0.05). Contrary to it, the degree of increase of AAT activity was significantly greater in the fat bodies than that of haemolymph. (Fig. 3 and 4).

The GDH activity in the haemolymph and fat bodies of the silkworm fed with 25%, 50%, 75% and 100%

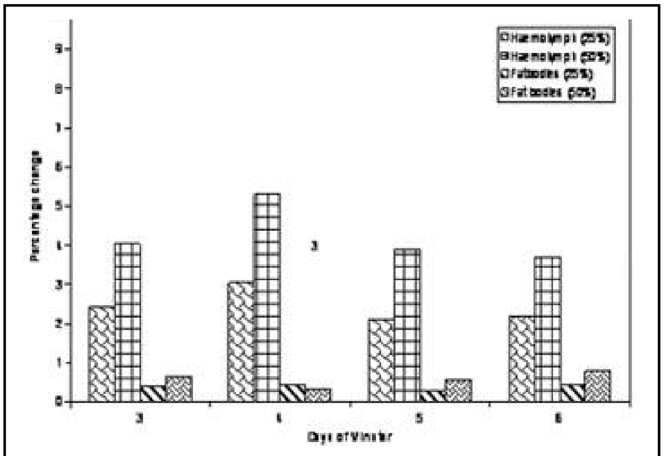

Fig. 3 : Per cent change over control in the AAT of haemolymph and fat bodies of PMXNB$_4$ D2 hybrid silk worm at different days of v instar larvae fed with 25% and 50% tukra affected leaves

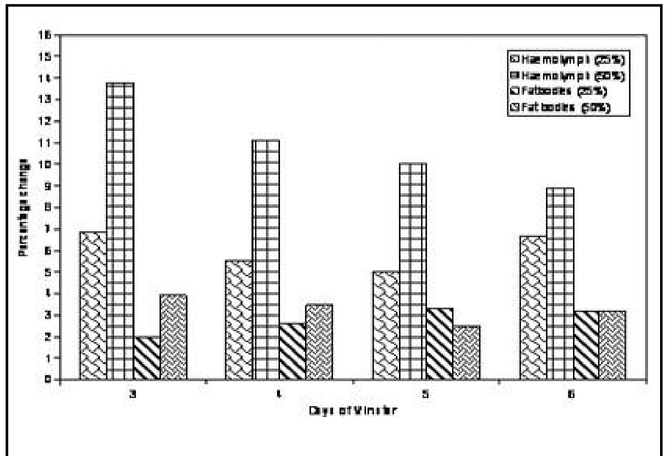

Fig. 5 : Per cent change over control in the GDH of haemolymph and fat bodies of PMXNB$_4$ D2 hybrid silk worm at different days of v instar larvae fed with 25% and 50% tukra affected leaves

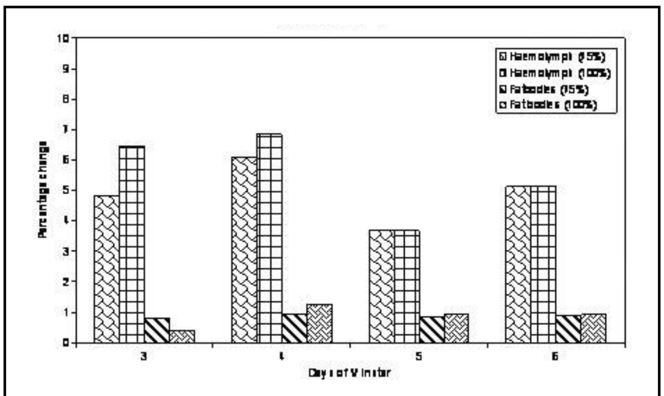

Fig. 4 : Per cent change over control in the AAT of haemolymph and fat bodies of PMXNB4 D2 hybrid silk worm at different days of v instar larvae fed with 75% and 100% tukra affected leaves

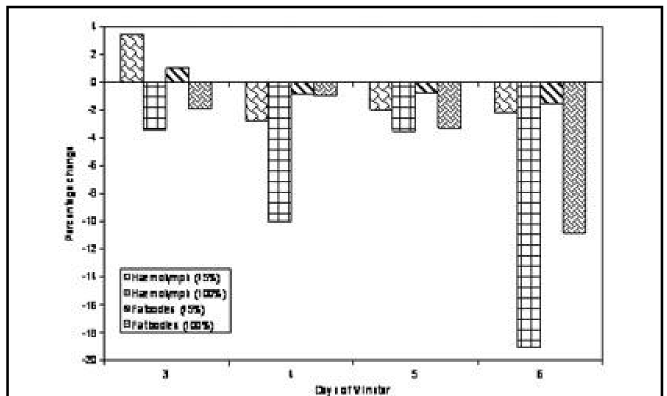

Fig. 6 : Per cent change over control in the GDH of haemolymph and fat bodies of PMXNB$_4$ D2 hybrid silk worm at different days of v instar larvae fed with 75% and 100% tukra affected leaves

tukra affected leaves decreased at day 4 to day 6 relative to respective haemolymph controls (0.029, 0.036, 0.040 and 0.045) and also decreased at day 5 and day 6 relative to respective fat bodies controls (0.102, 0.115, 0.120 and 0.126). The GDH activity in both the tissues of silkworm fed with 25%, 50% tukra leaves increased slowly and also non significant ($P > 0.05$). the silkworms. The GDH activity level in haemolymph was mostly statistically significant ($P > 0.05$) (Fig. 5 and 6).

The amino transferases function as strategic link between carbohydrate and protein metabolism. AAT and ALAT are located in both mitochondrial and cytosol fractions of the cell. A close relation appears to exist between the mitochondrial integrity and transaminase levels (Bonitenko, 1974). Amino transferases are pyridoxal phosphate dependent enzymes, catalyzes the transfer of amino group from alpha amino acids to alpha keto acid without the liberation of ammonia.

The alanine and aspartate amino transferases which function as a strategic link between the carbohydrate and protein metabolism are known to alter under several pathological and physiological conditions (Ramana Rao *et al*., 1990). AAT catalysis the interconversion of asparatate and ketoglutaric acid to pyruvic acid and glutamate (Goldstein and Newhome, 1980; Martin *et al*., 1983). In the present study, significant variations were observed in the ALAT, AAT and GDH activities of the fifth instar larva of silkworm PMxNB4D2 on feeding of tukra affected chawki leaves. On feeding with 75% and 100% tukra affected mulberry leaves could be harmful infected by the *M. hirsutus* (mealy bug) in mulberry resulting low leakage enzymes into the cytosol disturbs the biochemical functioning of cellular activities and impose protein synthetic potential (Sreedevi *et al.*, 1992).

A steady increase in the activities of ALAT and AAT in haemolymph and fat bodies of hybrid silkworm on feeding with 25%, 50%, 75% and 100% tukra leaves from day 3 to day 6 could be the stepwise induction of these enzymes by greater association of their oligomers (Kulkarni and Kulkarni, 1987). The increase in enzyme activities could indicate the active transamination reaction in both tissues either for structural organization of aminoacids in corportion of ketoacids into TCA cycle to favour gluconeogenesis or energy products. The ALAT activates ketoacids a general index of amino acids break down and AAT marks mobilization of amino acid into TCA cycle (Adibi 1968; Davison and Longslow, 1975).

Progressive decrease of GDH activity from day 4 to day 6 indicates the gradual elevation of oxidative deamination of aminoacid through ketoglutarate which facilitates division of amino acid in to TCA cycle to meet the energy from the nutritive demand. Hirayama and Nakamura (2002) reported the regulation of glutamine metabolism during starvation with effect to development of *Bombyx mori*. Venkatarami Reddy *et al*. (1992) reported a decrease in protease, GDH activity and an increase of ALAT, AAT activities in both the tissues when *M. hirstus* insect infected in mulberry when fed by silkworms.

All our findings suggest that instead of discarding tukra affected leaves, the farmers can make use of 25%, 50% and can discard the leaves at the level of 75% and 100% affected tukra and they found to be some what pathological to the hybrid silkworm.

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