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Original Scientific Article

THE EFFECT OF COLD-STRESS ON BIOCHEMICAL COMPONENTS OF THE LARVAL FAT BODY, HAEMOLYMPH AND EXCRETA IN TROPICAL TASAR SILKWORM, *Antheraea mylitta* Drury (LEPIDOPTERA: SATURNIIDAE)

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Abstract

Studies on cold acclimation of insects including silkworms have shown significant variations in the levels of various biomolecules to cope with thermal shock. The present study has been carried out on cold-stressed larvae of Daba Trivoltine (TV) of tasar silkworm, *Antheraea mylitta* Drury to analyze the mortality rate, variations in the biomolecules and excretory products at varied durations. The results revealed that exposure to low temperatures (10± 1°C) for seven days leads to 100% mortality, five days exposure caused 50% mortality and two days exposure resulted in 38% mortality on reverting the larvae to normal temperature. The treatment of Daba T.V larvae with low temperatures resulted in increased aminoacid content (2% – 5%) in the haemolymph and decreased in fat body (37% – 94%) of all the three treatments in comparison with control group reared at 28±2°C. Significant decrease in urea (68% – 77%) and uric acid (46% - 50%) was observed in the larvae exposed to cold stress in comparison with control group.

Keywords: Haemolymph, Fat body, Amino acids, Urea, Uric acid, Daba T.V.

INTRODUCTION

It is well known that temperature plays a major role in their physiological behavior of the insects. The insects will get acclimatized to the low temperatures by the production of various cryoprotectants like glycerol, trehalose, sorbitol etc (Sinclair *et al*., 2003; Storey, 1990).

In insects, the growth and development is associated with protein metabolism (Singh and Baquaya, 1971). In silkworms, the protein synthesis activity of the body wall and the midgut decreased when the larvae began to moult and increased from the midstage of the moulting period (Nagota, 1976). Marked increase in protein, pyruvate, total free aminoacids, total lipids, phospholipids and triglycerols was observed in response to cold exposure (Pant, 1984). A recent study has also revealed the involvement of aminoacids in interaction with tyranine in *Bombyx mori* (Ohta *et al*., 2004).

In the silkworm larva, the nitrogenous waste products of metabolism are mainly excreted as urine, together with faecal pellets. The excretory pattern depends upon a number of environmental factors such as temperature and humidity (Alexandria and Stanchion, 1981; Dhinaker, 1990). The excretory pattern of silkworm larvae on exposure to F2 alpha increased the nitrogenous end products (Bharathi, 1993). Similarly larvae feeding with trace elements like cobalt increased the pattern of excretion (Sailaja *et al*., 1997). Excretion forms an important factor for the balance of nitrogen in the body. The excretion of

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nitrogenous waste products has been studied in a number of insects (Wigglesworth, 1950; Prosser and Brown, 1965; Craig, 1960). Uric acid contains comparatively less hydrogen than any other nitrogenous compound excreted by animals and it is therefore well adapted for conservation (Wigglesworth, 1965). Urea is present in small quantities in insects. The excretion in insects, its energetic and functional principles has also been worked out (Florey, 1982).

Although the adaptive strategies exhibited by various insects in response to temperature variations are well known, only a few studies show the change in rate of acclimation in insects in response to cold stress. The present study focused on molecules which were available in the metabolic pool of fifth instars larvae of Daba T.V during cold stress conditions.

MATERIALS AND METHODS

Newly hatched larvae of Daba T.V (250) were reared on tender fresh leaves of *Terminalia arjuna* in the laboratory Sericulture Unit, Kakatiya University, Warangal, Andhra Pradesh, at 28±2ºC and humidity 70% to 75%.To study the effect of cold stress on mortality, biochemical components and excretory products of Daba T.V, fifth instar larvae were divided into four treatments based on temperature exposure for different durations of which each containing 50 larvae. The details of the treatment are : Treatment at 28±2ºC – T1 (Control), Treatment at 10±1ºC for 2 days – T2, Treatment at 10± 1ºC for 5 days – T3, Treatment at 10 ±1ºC for 7 days – T4. After 7 days of treatment the haemolymph was collected in the test tubes and stored in the deep freezer. The fat body was isolated in cold condition by using Bodenstein's Ringer solution and weighed in chilled state using "Dhona" electrical balance and was used for subsequent biochemical studies. Free amino acids were measured at micro grams $100mg^{-1}$ of wet weight of tissue as Moore and Stein (1954). The excretory pellets of fifth instar larvae of all the four treatments were collected separately and homogenized in precooled mortar and pestle thoroughly. Urea was estimated according to the standard procedure of Natelson (1971) and uric acid (Brown, 1945; Oser, 1965) estimated as micro grams$100mg^{-1}$ of wet weight of the pellets. Centrifugation was done by using Remi centrifuge T.8 model (20,000 rpm). The estimations were based on calorimetric principle of Beer- Lambert's law in which the absorbances of coloured complexes are proportional to the concentration of reaction products. The mortality rate was recorded for all the four treatments after reverting back to normal temperature.

Each assay was replicated 3 times. Values were expressed as mean ± SE of replication and Student's t-test was applied to locate significant ($P \leq 0.05$) differences between treated and control groups.

RESULTS AND DISCUSSION

The exposure of the insect larvae to low temperature is expected to lead some changes in its mortality, biomolecules and excretory products. Hence, the present experiment was conducted to estimate the mortality rate, levels of amino acids in haemolymph and fat body and also excretory products at low temperature for different durations by using standard procedures as described in the materials and methods section. Table I explains the mortality of Daba T.V larvae which were incubated at low temperature (10±1ºC) for varying treatment durations. Low temperature exposure of Daba T.V larvae for 7 days lead to 46% mortality on the 8th day and a total loss at the end of the 10th day. Anitha Singh *et al.*, (2010) have reported the similar results in *Philosamia ricini*. Larvae subjected to 5 days cold stress have shown 64% mortality on sixth, seventh, eighth and ninth days and 2 days cold stress have

Table I. Mortality (in number) of Daba T.V larvae exposed to low temperature for different durations after reverting back to normal temperature (28± 2ºC)

Day of rearing at Normal Temp after treatment duration	No. of larvae died on 2 days exposure (T2) (50 larvae)	No. of larvae died on 5 days exposure (T3) (50 larvae)	No. of larvae died on 7 days exposure (T4) (50 larvae)
First	2	2	23
Second	5	16	16
Third	6	9	11
Fourth	8	5	
Fifth	-	-	-
Sixth	-	-	-
Seventh	-	-	-
Eigth	-	-	-

Table II. Effect of exposure to low temperature on amino acids in the haemolymph and fat body of the 5th instar larvae of Daba T.V for 2, 5, and 7 days (in µg 100 mg^{-1} of fat body, mg 100 ml^{-1} of haemolymph).

Location	Control(T1)	T2	T3	T4
Haemolymph	955.0±3.0	971.0±4.0	989.0 ±3.0	999.0 ±3.8*
		(+1.7)	(+3.6)	(+4.6)
Fat body	2.51± 0.14	1.58± 0.05*	1.18± 0.04	0.14± 0.02
		(- 37.0)	(-53.0)	(-94.4)

Each value represents the mean ± SEM of 3 different observations. The values presented in parentheses indicate the percentage increase (+) or decrease (-) over control. *Significantly different at $P \leq 0.05$ (Students' t-test)

Table III. Effect of exposure to low temperature on Urea and Uric acid of the 5th instar larvae of Daba T.V for 2, 5, and 7 days (in µg 100 mg^{-1} excretory pellet).

Excretory Product	Control(T1)	T2	T3	T4
Urea	10.2 ±0.076	3.25± 0.04	3.11 ± 0.02*	2.35 ± 0.03
		(-68.0)	(-69.5)	(-77.0)
Uric acid	6.28 ± 0.061	3.38± 0.059	3.28 ± 0.01	3.11 ± 0.06*
		(-46.2)	(-47.8)	(-50.5)

Each value represents the mean ± SEM of 3 different observations. The values presented in parentheses indicate the percentage increase (+) or decrease (-) over control. *Significantly different at $P \leq 0.05$ (Students't-test).

shown 42% mortality in third, fourth, fifth and sixth days on reverting the larvae to normal temperature.

The results shown in Table II indicate that cold stress profoundly affects the levels of amino acids in different parts of the insect ($P \leq 0.05$). When compared with the control, the levels of free amino acids were found to be high (2% – 5%) in the haemolymph of cold-stressed larvae. Shamitha and Purushotham Rao (2008) have reported that, the increase in amino acid content in the haemolymph of Antheraea mylitta from first crop to the third crop was mainly because of the environmental factors. Singh *et al.*, (2010) have reported a drastic increase in the amino acid content in the haemolymph of *Philosamia ricini* larvae exposed to cold stress.A significant variation was observed in aminoacid content of the haemolymph of cold stress larvae and it was found to be high in the larvae kept under seven days treatment. The high amino acid content in the haemolymph can be attributed to high proteolytic activity. Sinha *et al.*, (1987) have reported that variation in the concentrations of aminoacids in the haemolymph of *A.mylitta* can be attributed to the climatic changes. Watanabe and Kobayashi (1976) have reported that the low transaminase activity or high proteolytic activity results in high amino acid content. Present results suggest that the impact of the cold stress on the amino acid content of haemolymph was dependent on the duration of treatment.

In comparison with the control, the fat body of cold-stressed 5th instar larvae has shown a remarkable decrease ($P \leq 0.05$) in the level of amino acids and reduced drastically in seven days exposure (94%). Thus the aminoacid content of haemolymph and fat body were found to be in the reverse order. Singh *et al.*, (2010) working on *Philosamia ricini* have reported the decrease in amino acid content in the fat body of the larvae under cold stress conditions. Decrease in the free amino acid content of fat body may indicate the possibility of active feeding of amino acid in Kreb's cycle and glycolytic pathway to meet the emergent energy needs as well as their utilization in the production of some new proteins synthesized to cope with the low temperature stress (Zhao, 1997; Forcella, 2007)

The results shown in Table III depicts that cold stress significantly affects the levels of urea and uric acid in the fifth instar larvae ($P \leq 0.05$). When compared with the control, the levels of urea (68% – 77%) and uric acid (46% – 50%) were found to be decreased in the cold-stressed larvae. Shamitha and Purushotham Rao (2008) have reported high level of excretory products in the outdoor worms during winter season than indoor worms reared at 25°C - 30°C. The present investigation shows high content of urea compared to uric acid in the cold stressed larvae. In comparison with the uric acid, high level of urea was noted in the outdoor worms of *A.mylitta* reared in the winter season than the worms reared at 25°C - 30°C (Shamitha and Rao, 2008). Present results show that, the levels of urea and uric acid decreases as the duration of exposure increases. Yamada and Kato (1991) have reported that the variation in the excretory products of insects is due to their habitat. Barsagade and Tembhare (2004) have studied the excretory metabolism of lepidopteron larvae at different stages of larval life and concluded that the end products fluctuate enormously from day to day.

Thus in conclusion the larvae exposed to low temperature (10±1°C) for 7 days have shown high aminoacid content in its haemolymph, low amino acid content in its fat body and low levels of urea and uric acid than the larvae reared at 10±1°C for 5 and 2 days.

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