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EFFECT OF TREHALOSE AND AMYLASE ON ECONOMIC CHARACTERS OF TASAR SILKWORM, *ANTHERAEA MYLITTA* D (SUKINDA ECORACE) IN *EX-SITU* CONDITIONS

SREENIVAS.M AND SHAMITHA.G*

Department of Zoology, Kakatiya University, Warangal-506009, Telangana, India

ABSTRACT

The tasar silkworm *Antheraea mylitta* D., a wild sericigenous and polyphagous insect of India, basically feeding on *Terminalia sp*, is a trivoltine, tropical variety. In the present study, biochemical and economic parameters of Tasar silkworm, (Sukinda ecorace) are analysed. It was observed that a lowest capacity of silk yield in sukinda suggests for a more viable method of crop stabilisation as a cause of concern though Daba and Sukinda ecoraces are mostly commercially applied for cocoon production.

KEYWORDS: ***Antheraea mylitta*, Sukinda, trehalose, amylase, cocoon parameters**

***Corresponding author**

SHAMITHA.G
Department of Zoology, Kakatiya University,
Warangal-506009, Telangana, India

INTRODUCTION

Tasar silkworm*, Antheraea mylitta Drury*, is a sericigenous, polyphagous insect distributed in the form of about 43 ecoraces in varied geographical tropical zones in India[1] like Bihar (Singhbhum, Santhal), Madhya Pradesh (Jagdalpur), Orissa (Mayurbhanj), West Bengal (Bankura), Maharashtra (Bhandara), Andhra Pradesh (Adilabad, Warangal, Karimnagar and Khammam) and Karnataka (Belgaum). Among fifty races of sericigenous insects 25 races of *Antheraea mylitta* Drury alone are seen[2]. The tropical tasar silkworm feeds on eight primary food plants and more than two dozen secondary food plants. The primary food plants are *Terminalia Arjuna*, *T. tomentosa*, *Shorea robusta*, *Lagerstroemia parviflora*, *L. speciosa, L. indica, Zizyphus* and *Hardwickia binata*[3] . Sukinda is an ecorace, collected from Kunda, Ankurapal, Sukaran and Kans area of Sukindagarh (85.62°N and 20.88°E) of Jajpur district of Orissa, known as 'Sukinda ecorace' was introduced to other areas by the state Department of Sericulture and was cultivated commercially during 1970s. The race being trivoltine was suitable for warmer climates, cocoons were predominately yellow with Silk Ratio ranging between 10.5 to13.1% in different seasons and fared well in semi-domesticated conditions. It had also advantages over the wild tasar races in low pupal mortality during preservation, higher coupling and cocoon: dfl ratio and less deterioration of economic characters in progenies. Sukinda ecorace also originates from Sukindergarh plain, Orissa at altitude of 300'AMSL is the most commercially exploited trivoltine for tasar silkworm rearing. Daba of bordering Singhbhum area, Jharkhand at altitude of 500'AMSL is another commercially exploited ecorace in the bivoltine areas of Orissa state[4,5]..Among other ecoraces of Tasar silkworm, *A mylitta,* Andhra local ecorace is a tropical tasar variety available only in Warangal and Karimnagar districts of Telangana state. It is an exclusive race of this region and is well known for its superior commercial qualities like hard and compact cocoons, high reelability (69%), high shell ratio (16.85) and low denier (7%). It is on the verge of extinction due to certain weaknesses like poor egg - laying behavior, voltinism, erratic emergence, non-uniform silk deposition in cocoons and pupal mortality. It shows heavy mortality of larvae due to predators, parasites and climatic hazards[6,7&8]. Due to these reasons robust ecoraces like Daba and sukinda are commercially exploited in these regions by the farmers. In an attempt to overcome the problems faced by Andhra local ecorace, a comprehensive breeding program was suggested by earlier works[9,10]. Hence the present work on rearing of Tasar silkworm, *A mylitta* (Sukinda) is undertaken with a view to study its tendency of acclaimatisation and stabilisation of this population by analysing rearing performance and the possibility of future breeding with Andhra local ecorace in this geographical region. The present investigation is taken up to study the influence of environmental conditions in *ex-situ* conditions. The instar wise mortality, larval and cocoon parameters were calculated and the study also involves a comparative analysis of trehalose content in haemolymph, fatbody and silkgland and amylase activity in the midgut region of fifth instar tasar silkworm *Antheraea mylitta* D (Sukinda).

MATERIALS AND METHODS

In present studies, the tasar silkworm, *A.mylitta* (Sukinda), was collected from Baripada district of Orissa state. Out of 300 eggs, about 200 newly hatched larvae were raised in the *Terminalia* plantation at Kakatiya University Campus, Warangal.To monitor the environmental factors i.e., temperature and relative humidity during the course of rearing, lab thermometer and hygrometer were used and the readings were noted and presented in tabular form. The average length and weight of five larvae from I to V instars, selected at random from the rearing lot were measured using graph paper and an electronic balance of Shimadzu-make respectively. The mortality was calculated by counting the number of worms lost due to various reasons during each instar. The cocoon parameters like cocoon weight, pupal weight, shell weight was also weighed and recorded (in grams) and shell width, shell thickness and shell length were measured using standard procedures.

ESTIMATION OF TREHALOSE CONTENT

Estimation of trehalose content was done by done by[11] method and its content was expressed as micrograms/100 mg wet weight of the tissue. Centrifugation was done by using Remi centrifuge T-8 model. The estimations were based on colorimetric principle of Beer-Lamber's Law in which the Absorbance of coloured complexes are proportional to the concentration of reaction products. The data was statistically analysed and the results have been discussed by comparing the various tissues.

Collection of digestive fluid to determine the enzyme activity of amylase in vitro:

Digestive fluid was collected from the 4th day of fifth larval stadium. Larvae were starved for about 4 hr and digestive juice was collected from midguts after dissection of larvae. Digestive fluid was collected into precooled tubes. Five individual larvae were used for sample collection. The digestive fluid was centrifuged at 10,000 rpm for 10 min to remove undigested leaf particles and stored at -20°C until use .Amylase activity was measured with the standard procedure using soluble starch as substrate[12,13]. Maltose was used as a standard and the enzyme activity was expressed as mg of maltose released/ml/min at 37°C. Samples were diluted prior to assay to maintain linearity. Enzyme assays were carried out for five individual larval samples calibrated against controls for two trials and means of all the five values and trials were taken as the final values.

RESULTS

For the present study, the natural habitats of the sukinda ecorace of Tasar Silkworm, *Antheraea mylitta* Drury, were explored in their natural habitats and the geographical parameters were recorded. The eggs which were collected from those forest areas were grown under an optimum temperature of 26-30°C and a relative humidity of 70-80% in the Tasar Plantation at Kakatiya University, Warangal (Fig 1).

Figure 1
V-Rearing of *Antheraea mylitta* D (Sukinda) on *Terminalia arjuna* plantation at Kakatiya University, Warangal

Table-1
Estimation of trehalose content in tasar silkworm, *Antheraea mylitta.* D.,Sukinda ecorace (mg/ml or mg of 50mg tissue)

Sl.No	Haemolymph	Silkgland	Fatbody
1.	13.72 ± 1.80	4.87 ± 1.27	6.37 ± 1.35

The values are expressed in terms of standard Error of the Mean

The total trehalose content and its standard deviation in the fifth instar *haemolymph; fat body and silk gland* of Tasar silkworm(Sukinda),*Antheraea mylitta*, were 13.72 ± 1.80 (S. D), 4.87 ± 1.27 (S. D) and 6.37 ± 1.35 (S. D) mg/ml for fifth instar respectively (Table1).

Table-2
Estimation of digestive Amylase activity in tasar silkworm, *Antheraea mylitta* D, Sukinda ecoraces (expressed in mg/ml/min)

Sl.No	Enzyme activity expressed in mg/ml/min	Digestive juice
1.	Amylase activity	0.003 ± 0.0007

The values are expressed in terms of Standard Error of the Mean.

The digestive amylase activity in the fifth instar digestive juice of Tasar silkworm (Sukinda), *Antheraea mylitta*, was 0.003 ± 0.0007 (S. D) mg of maltose released mg/ml/min at 37°C for fifth instar (Table 2).

Table 3
Instar-wise Rearing Average Temperature (°C), Average Relative Humidity (%) and Mortality of Tasar silkworm, *Antheraea mylitta* D (Sukinda ecorace)

Instar	Temperature (ºC)	Humidity (%)	Mortality	Larval life span/days	Avg. larval length (cm)	Avg. larval weight (g)
I	31.4±3.15	29±4.06	30	6	1.7±0.2	0.09±0.02
II	33.3±1.58	42.6±2.88	26	5	2.24±0.11	2.3±0.27
III	30.54±0.97	48.2±3.83	21	7	4±0.21	5.66±0.46
IV	28.16±0.86	52.4±2.07	48	8	7.26±0.42	8.6±0.5
V	29.02±1.86	48.8±4.08	22	20	11.3±073	11.3±0.73

The values are expressed in terms of Standard Error of the Mean.

The instar wise average temperature and its standard deviation of Tasar silkworm *Antheraea mylitta* (Sukinda) were 31.4±3.15 (S. D), 33.3±1.58 (S. D), 30.54±0.97 (S. D), 28.16±0.86 (S. D) and 29.02±1.86 (S. D) while that of rearing were I, II, III, IV and V instar respectively . The instar- wise average Relative Humidity and its standard deviation of were 29±4.06 (S. D), 42.6±2.88 (S. D), 48.2±3.83 (S. D), 52.4±2.07 (S. D) and 48.8±4.08 (S. D) in the I, II, III, IV and V instars respectively. The instar wise mortality of was 30, 26,21, 48,22 respectively and the instar wise larval life span/days was 6, 5, 7, 8, 20 respectively (Table 3).The instar wise average larval length (cm) and its standard deviation of Tasar silkworm *Antheraea mylitta* (Sukinda) were 1.7±0.2 (S. D), 2.24±0.11 (S. D), 4±0.21 (S. D), 7.26±0.42 (S. D) and 11.3±073 (S. D) while that of rearing were I, II, III, IV and V instar respectively. The instar wise average larval weight (g) and its standard deviation of was 0.09±0.02 (S. D), 2.3±0.27 (S. D), 5.66±0.46 (S. D), 8.6±0.5 (S. D) and 11.3±0.73 (S. D) while that of rearing were I, II, III, IV and V instar respectively (Table 3).

Table-4
Post cocoon characters of Tasar silkworm (Sukinda)*, Antheraea mylitta*

S.No	Cocoon Weight (Gm)	Pupal Weight (Gm)	Shell Weight (Gm)	Shells Width (Cm)	Shell Thickness (mm)
1	6.402±1.43	6.53±1.24	1.38±1.62	0.089±0.020	0.13±0.051

The average cocoon weight, Pupal weight, Shell weight (gm), Shell width, shell length(cm), Shell thickness(mm) of Tasar silkworm *Antheraea mylitta* (Sukinda) were 6.402±1.43 (S. D) 6.53±1.24 (S. D), 1.38±1.62 (S. D), 0.089±0.020 (S. D)) and 0.13±0.051 (S. D) respectively (Table 4).

DISCUSSION

Earlier, several studies were conducted on Sukinda based on utilisation of economic wild life, conservation of the associated environment

for sustainable rural and tribal development[14]. It was also revealed that semi domestication for commercial exploitation can enhance potential of sukinda under *in situ* because of better performance levels than the stocks maintained *ex situ*[4]. The trehalose content in the haemolymph, silkgland and fatbody in the tasar silkworm were 13.72, 4.87 and 6.37 mg/ 50 mg of tissue respectively. A high content of trehalose content in the heamolymph suggests the energy demand of the insect. It is well known that trehalose in the major carbohydrate and important reserve metabolite of insects. It is also notable that the trehalose content of fat body is next to that of heameolymph. Fatbodies of insects were the main site for active biosynthesis of trehalose[15].The present investigation revealed a higher trehalose content in haemolymph of sukinda reared in *ex-situ* , which is on a higher side when compared to that of other ecoraces like Sarihan, Modal, Raily , Daba and Sukinda as seen in a study conducted on variability of trehalose in various ecoraces on natural variation, which suggested breeding as the need of the hour to produce new varieties, other than commercially exploited Daba and Sukinda, with desirable characters[16] .Amylase is one of the key enzymes involved in digestion and carbohydrate metabolism in insects[17,13] . The amylase activity increases steeply during III & IV instars and reaches highest value at the last period of IV and V instars and decreases during spinning[18] .The increase in amylase activity in indoor reared worms is consistent with the report on amylases in silkworm, which has revealed an increased amylase activity and efficient starch digestion in non-diapausing strain which may have adaptive significance and better survivability than diapausing strains[19].From the present studies, it is observed that the hatching percent was 66%, which was less when compared to its normal range of 80-82% in its natural habitats. The larval parameters like- larval life period and larval weight; cocoon parameters like cocoon weight, and shell width were observed lesser than the worms grown under natural habitats. The low cocoon yield of 48 for the 200 worms hatched (*i. e.,* 24%), also suggests poor adaptability in *ex-situ* conditions. However, a recent study conducted on multilocational trials, rejuvenation and preservation of Sukinda TV revealed that a pure line of Sukinda ecorace could be developed which started behaving equally at all the supplied places, which could result in the rejuvenation of dying natural treasure of Odisha[20]. Based on the significant observation that the shell weight of the cocoons obtained as considerably higher than those studied under natural conditions and the fecundity was also higher in these moths, the studies needs further probing in cocoon parameters and also improvement of the crop yield.

CONCLUSION

From the present studies it can be concluded that trehalose content is high in haemolymph followed by fat body, which suggests a greater energy demand for the silkworm in fifth instar and a low amylase activity in the midgut is attributed to low yield traits when compared to other ecoraces.

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