Studies on the High Temperature Induced Stress on the Biochemical Profile and Fecundity of Daba and Laria Ecoraces of Tropical Tasar Silkworm *Antheraea mylitta* Drury (Lepidoptera: Saturniidae)

G. Lokesh¹*, P. K. Kar¹, A. K. Srivastava¹, Saloni Swaroopa², and M. K. Sinha¹

¹Silkworm Breeding & Genetics, Central Tasar Research & Training Institute, Central Silk Board, Ranchi, India, Jharkhand-835303
²Department of Biotechnology, Ranchi Women College, Ranchi University, Ranchi

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Tropical tasar silkworm *Antheraea mylitta* Drury experiences extreme temperature stress conditions during its lifecycle particularly during diapauses and first crop. The present study witnessed the impact of high temperature on some biochemical profiles and egg production (fecundity) of semi-domesticated Daba and *Shorea robusta* (Sal) based wild ecorace Laria during seed cocoon (pupa) preservation. Cocoons of Daba and Laria were treated with high temperature at 40°C for 10 days in a BOD incubator. The protein profile and carbohydrate content in the hemolymph and fat body and total haemocyte count (THC) in the hemolymph of pupa were investigated. Further, the fecundity and fertility of egg was assessed. Significant increase in the protein concentration was observed in the hemolymph with reduction in the fat body (p<0.05). The difference in protein concentration was highly significant between the semi-domesticated Daba and wild ecorace Laria (p<0.05). High pupal mortality (20%) and reduced fecundity (10-15%) in Daba was noticed compared to wild Laria. Also an increased THC (>28000) was recorded in Laria. The study infers the potentials of wild ecoraces in sustaining the extreme temperature conditions and need of adopting suitable package of practices for the preservation of diapause seed cocoons during extreme summer conditions. There is possibility to introgression thermal stress resistant traits in the semi-domesticated races of tasar silkworm by resorting to conventional breeding plans with wild races and keeping the thermal stress induced response as markers.

**Key words:** Daba, Fecundity, Haemocyte, Laria, Protein concentration, Temperature stress

### 1. Introduction

Many sericigenous insect species have been extensively exploited for the extraction of silk protein fibers due to high commercial value. Tropical tasar silkworm *Antheraea mylitta* Drury is reared in central and eastern parts of India, experiences extreme thermal conditions during its lifecycle (Hansda *et al*., 2008; Sinha and Srivastava, 2004; Suryanarayana *et al*., 2005). Forty four ecoraces of *A. mylitta* have been identified with significant phenotypic and behavioral variations (Srivastava *et al*., 2002; Srivastava *et al*., 2004; Lokesh *et al*., 2012). Tropical tasar silkworms primarily feeds on leaves of Sal (*Shorea robusta*), Asan (*Terminalia tomentosa*) and Arjun (*T. arjuna*), besides many other secondary food plants. Among the ecoraces, Daba is a ruling commercially exploited semi-domesticated ecorace reared in almost all tasar growing states of India. On the other hand, Laria being a Sal based wild ecorace predominantly exists in the tropical moist deciduous forests of Jharkhand, less amenable to human interference but has good commercial cocoon characters (Suryanarayana and Srivastava, 2005).

The environmental conditions prevailing during pupal diapauses play a vital role as the pupae have to survive the extremes of climatic conditions during preservation of seed cocoons this affects about 30% of the stock (Narain...
et al., 2001). Large quantities of cocoons are lost due to high temperature especially during peak summer i.e., May-June (Kapila, et al., 1992; Ojha and Saxena, 1997). The high temperature stress induces the changes in the physiology and biochemical composition and in turn the fecundity and other commercial traits in the silkworm (Malik and Malik, 2009; Omana and Gopinathan, 1995; Harjeet and Kumar, 2010).

High temperature affects nearly all biological processes including structure of proteins, biological membranes and rates of biochemical and physiological reactions (Hazel, 1995; Willmer et al., 2004; Pezhaman and Kumar, 2010.). The composition of hemolymph is variable in response to different range of thermal stress in insects. The fat body synthesizes a number of proteins and releases them into the hemolymph during active feeding stage and the same stored fat body nutrition is utilized for the metabolic activity during inactive stage (hibernation) of the insect (Kumar et al., 1998). Stress condition induces the changes in hemocytes of hemolymph in many insect species and particularly in silkworms. Hemocyte profile increases with an increased temperature and reduced hemocytes in the hemolymph when the silkworm exposed to lower temperatures (Blacklock and Ryan, 1994; Tiwary and Shukla, 2000; Chaubey, 2002; Pandey et al., 2010). The present study was intended to investigate the level of physiological damage in pupa and subsequent egg production (fecundity and fertility) due to high temperature stress and comparative analysis of wild and semi-domesticated tasar silkworm seed cocoons in sustaining the high temperature during pupal hibernation and summer preservation.

Materials and Methods

Tasar silkworm and temperature treatment

Experiments were undertaken at Silkworm Breeding and Genetics laboratories of Central Tasar Research & Training Institute, Ranchi, during April-July 2011. The cocoons of semi-domesticated Daba ecorace were obtained from Tasar silkworm germplasm bank and wild ecorace Laria cocoons collected from forest area of Peterbar, Jharkhand. The good cocoons were sorted based on the sex. About 50 male and 50 female cocoons each of Daba and Laria were incubated in the BOD incubator at 40°C with 55-65% of relative humidity for ten days. A similar set of cocoons were maintained at normal room temperature condition in the grainage house as control. After ten days of incubation, about 20 pupae from each set and sex were used for the biochemical studies and remaining cocoons (pupa) allowed for the moth eclosion and to study the fecundity and egg fertility performances. Mortality of the pupae were recorded separately in the treated sets and assessed in percentage. The treated and control pupae were used for the collection of hemolymph and fat body for the biochemical studies.

Preparation of Samples for biochemical studies:

Hemolymph and fat body of both Daba and Laria of both the sexes were collected separately for the biochemical studies.

i. Hemolymph was collected in a pre-chilled test tube containing a few crystals of phenyl thiourea by cutting the anterior part of the pupa. The hemolymph was centrifuged at 3000 g for 10 min at 4°C and the supernatant was collected and stored at −20°C until further use.

ii. Fat body was isolated by dissecting the pupae. Homogenate the fat body in a homogenizer using phosphate buffer pH 7. The homogenate was transferred to a clean centrifuge tube and centrifuged at 10000 rpm for 30 min in cooling condition. The supernatant was collected in a clean test tube and were stored at −20°C until further use.

The total protein concentration and total carbohydrates were estimated in both hemolymph and fat body (Lowry et al., 1951; Sinha et al., 1998). The total hemocytes count (THC) was carried out in the hemolymph (Pandey et al., 2010).

Fecundity and Egg Fertility rate: The fecundity was calculated as total number of eggs laid by a single mother moth in three days of egg laying (Sinha, 1998). Egg fertility rate was recorded (Saheb et al., 2009) and expressed in percentage.

Statistical Analysis

One-way analysis of variance was used to test the significance of differences between the mean values of independent observations of proteins and carbohydrates in the hemolymph and fat body of silkworm pupae. Comparisons were performed with Duncan’s Multiple Range Test (DMRT) to find significance differences between the ecoraces and treatments. Differences were considered significant at p < 0.05 (Duncan, 1955).

Results

The impact of high temperature induced stress on the cocoons (pupa) of Daba and Laria and its biochemical profiles, Total hemocytes count, fecundity and egg fertility rate were recorded as follow.

Impact of high temperature stress was initially assessed and found that some of the pupae were dead (generally considered as melt cocoons) because of high temperature,