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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)

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Tropical tasar silkworm *Antheraea mylitta* Drury experiences extreme temperature stress conditions during its life cycle 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

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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; Pezhman 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 40ºC 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 4ºC and the supernatant was collected and stored at –20ºC 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 –20ºC 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,

Table 1. Pupal mortality (%) in response to high temperature stress induced on cocoons of Daba and Laria ecoraces of *A. mylitta* D

	Daba		Laria	
	Male	Female	Male	Female
Control	1.00	1.00	2.00	1.00
Treated	24.00	14.00	16.00	10.00

Percentage of total pupal mortality with the sample size of 50 cocoons in each set

also found difference in the mortality between the ecoraces and between the sexes (Table 1). Where, more mortality was found with Daba male cocoons (24%) compared to Laria (16%) and most of the male cocoons (20%) were affected than female (12%).

High temperature stress, total protein and carbohydrate concentration: the quantitative assay of proteins in both Daba and Laria showed significant increase in the hemolymph protein concentration due to high temperature stress in the treated sets. Higher protein concentrations were recorded in the treated males (484.63 mg/ml and 513.97 mg/ml) compared to females (Table 2). High temperature has negative impact on the fat body proteins and observed it was decreased in the treated sets (165.17 and 157.92 mg/ml). Among the races studied, the proteins profile showed significant variations and was at higher end in the wild Laria ecorace.

High temperature has similar effect in the total carbohydrates as of protein concentration (Table 2). Where, higher carbohydrates recorded with treated set and maximum was recorded in treated Laria female (161.99 mg/ml). Higher rate of carbohydrates increase was observed with the female compared to males. Carbohydrates in fat body affected with the high temperature stress and the decreased values were observed.

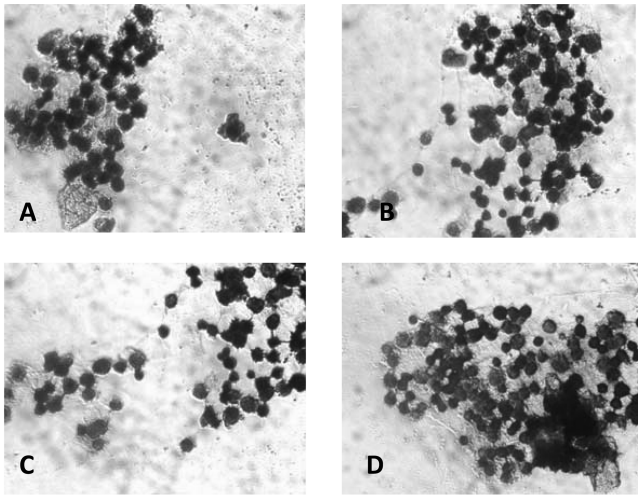

Fig. 1. Microscopic observation of hemocytes in the pupal hemolymph, **A**. Daba Control, **B**. Daba Treated, **C**. Laria Control and **D**. Laria Treated.

Total haemocyte count (THC): Observations on the Total number of hemocytes (Fig. 1) in the pupal hemolymph showed highly significant variability in control and treated sets (16753±325 and 28177±279), found significantly higher counts influenced by high temperature stress. Also comparatively different total haemocyte counts (16753±325 and 17363±183) were recorded between Daba and Laria ecoraces (Table 3).

Fecundity and fertility: High temperature has negative impact on the productive behavior of tasar silkworm. The fecundity of mother moth and the rate of egg fertility were

Table 2. Effect of high temperature stress on total protein and carbohydrate concentration (mean±SEM) in the pupal hemolymph and fat body of Daba and Laria

Race and sex		Total proteins		Total carbohydrates	
		hemolymph (mg/ml)	Fat Body (mg/g)	hemolymph (mg/ml)	Fat Body (mg/g)
Daba male	Control	145.3±0.88^{g}	197.79±7.82^{e}	27.58±0.97^{h}	21.97±0.99^{c}
	Treated	484.63±15.79^{b}	165.17±1.69^{f}	110.11±3.85^{d}	11.41±0.83^{g}
Daba female	Control	235.83±10.24^{e}	281.4±6.26^{a}	32.83±1.29^{g}	27.81±0.44^{a}
	Treated	369.83±8.70^{d}	228.92±3.52^{d}	127.62±3.65^{c}	10.65±1.10^{h}
Laria male	Control	138.35±2.64^{h}	161.53±2.9^{g}	35.83±0.96^{f}	16.89±0.97^{e}
	Treated	513.97±20.28^{a}	157.92±1.14^{h}	142.76±3.88^{b}	12.56±1.80^{f}
Laria female	Control	219.72±15.33^{f}	252.63±3.79^{b}	42.05±0.61^{e}	26.3±0.73^{b}
	Treated	386.01±8.05^{c}	236.09±0.93^{c}	161.99±1.44^{a}	19.12±0.86^{d}

Small alphabets are significantly different according to Duncan's multiple range test ($P<0.05$).

Table 3. Effect of high temperature stress on total haemocyte count (cells/mm^2) in the pupal hemolymph of Daba and Laria

	Daba		Laria	
	Control	Treated	Control	Treated
Mean	16753^{d}	26335^{b**}	17363^{c}	28177^{a**}
SD	±325	±490	±183	±279

The values represent the mean of three replications with standard deviation, values with different small alphabets are significantly different according to Duncan's Multiple Range Test (**- P<0.01)

Table 4. Effect of high temperature stress on fecundity and fertility of Daba and Laria (Mean±SD)

	Daba		Laria	
	Control	Treated	Control	Treated
Fecundity	225.8±8.0^{a}	205.6±8.0^{b}	212.60±7.0^{c}	197±11.0^{d}
Fertility (%)	81.00±3.00^{a}	67.00±6.30^{c}	70.00±3.00^{b}	66.00±2.70^{d}

The values represent the mean of three replications with standard deviation, values with different small alphabets are significant different according to Duncan's Multiple Range Test (P<0.05).

adversely affected in the treated sets of Daba (205±8.0 & 67%) and Laria compared to controls (225.8±8.0 & 81.00). Fecundity and fertility when compared among the ecoraces, semi-domesticated Daba was greatly affected than wild ecorace Laria (Table 4).

Discussion

The silkworms like other living organisms, face different environmental conditions during its lifecycle in which the intensity of dominant factors and their combination vary to different degrees causing reversible or irreversible changes in the metabolism. These changes cause survival or death of an organism. The observation of pupal mortality in the present study was due to the high temperature stress was imminent. The differential mortality among the ecoraces may be due to the genetic capacity to sustain high temperature stress. Laria considered as wild ecorace of tropical tasar silkworm, has sustained less damage compared to the semi-domesticated Daba and this may be due to higher level of tolerance to different biotic and abiotic stress in wild ecoraces. Also, there was sexual dimorphism in the expression of tolerance to the stress. Higher mortality in male population is attributed to higher the metabolic stress corresponding to lower fat composition as stored energy in male pupae compared to females. The protein levels in the hemolymph increased significantly in response to high temperature due to high metabolic rate and synthesis of new proteins by the tissues and release into hemolymph. In contrast, the protein level in the fat body was decreased in the treated sets this is because of increased stress and organism struggle for the survival where, supplementation of heat shock proteins and carbohydrates synthesized by fat metabolism which transported to the hemolymph (Joy and Gopinathan, 1995). This also confirms that proteins are not a source of energy in the stress condition but are involved in the modulation of silkworm physiological activity to protect from temperature stress (Anitha *et al*, 2010.). Similarly, the total carbohydrate levels in the hemolymph was increased in the high temperature exposed sets and converse to the decreased levels in the fat body this is in concomitant with the earlier works (Joy and Gopinathan, 1995; Harjeet and Kumar, 2010; Zhao, 1997). Carbohydrates are essential components for the energy demand during stress condition. The biomolecules such as glycerol, sorbitol and other polyols acts as thermoprotectants (Gregory *et al.*, 1998.; Chen and Haddad 2004; Shamitha and Rao, 2008) are synthesize in the tissues and release into the hemolymph. Other commonly reported compounds are trehalose, glucose, fructose and mannitol, these compounds are believed to protect organisms during adverse conditions like temperature stresses (Forcella *et al*., 2007). Decrease in the carbohydrate level in the fat body may indicate the possibility of utilization of fat body during non-feeding stage of the silkworm as a source to meet the emergent energy needs as well as their utilization in the production of some new proteins/ biomolecules to cope with the temperature stress.

It has been reported that the increase in the total hemocyte count in response to high temperature and also cause a physiological damages in the hemocytes Omana and Gopinathan, 1995; Pandey *et al*., 2010)). Similar results were recorded in the present study, about four time increase in the hemocyte count observed in treated sets of both ecoraces studied. However, higher counts were with wild ecorace Laria compared to semi-domesticated Daba.

Fecundity is considered as one of the most desired quantitative traits of commercial importance in silkworms (Reddy *et al*., 2010). The genotype-environment interaction has highly significant influence on the fecundity of the silkworms, extreme temperatures adversely affects the total egg productivity of the mother moth (Singh and Kumar, 2008; Kumar *et al*., 2008; Srivastava *et al*., 2001). The significantly reduced fecundity due to high temperature stress in the present study would be due the diversification of protein metabolism and energy to synthesize new proteins in the hemolymph to support the physio-

logical mechanism to tolerate high temperature stress condition. The differential performance of the Daba and Laria with reference to fecundity attributed to the genetic endowment of the two ecoraces. Similar results were reported (Omana and Gopinathan, 1995) in the *Bombyx mori* bivoltine NB_4D_2 and multivoltine C-nichi silkworm breeds. A synonymous effect was also observed in case of fertility rate of eggs with differential impact in Laria and Daba ecoraces.

Quality seed (eggs) is the basic requirement for the success of sericulture industry. There are many efforts in the improvement of productivity and production of tasar silkworm seed and silk. Summer preservation of seed cocoons is critical for subsequent seed production in tasar industry. Proper management practice during this period with control on heat stress, about 15-20% of seed cocoons can be saved. Thus, from the present study, following conclusions can be made. Firstly, there is need to adopt a suitable management practices during silkworm seed cocoon preservation especially in summer seasons. Secondly, high temperature stress induces many proteins related to family of heat shock response proteins. This could be of valuable study with respect to development of temperature tolerant tasar silkworm races/breeds. Thirdly, semi-domesticated and wild ecoraces of tasar silkworms have shown heterogeneity in response to the high temperature along with expression in commercial traits. Thus, there is a possibility in obtaining hybrid combinations in semi-domestic Daba ecoraces with introgression of thermal resistant traits of wild Laria ecorace and further, this can also be verified through biochemical profiling and hemocyte using them as markers.

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