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COMPARATIVE STUDIES OF JUVENILE HORMONE III IN OUTDOOR AND INDOOR REARED TASAR SILKWORM, *Antheraea mylitta* D.

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ABSTRACT

The Tasar silkworm, *Antheraea mylitta* Drury (Daba TV), is an important wild, sericigenous insect and is commercially exploited in the nine states of tropical India. It encounters the vagaries of nature, pests and predators resulting in low crop yield. Rearing being outdoors, there is no control over the natural vagaries leading to irregular hatching of eggs and prolonged larval period. Not only the climatic hazards, its indefinite period of diapause leading to erratic moth emergence followed by inadequate seed support and other grainage problems also lead to severe crop loss. In order to overcome these difficulties, the concept of indoor rearing has been adopted. In the present investigation, a comparative analysis of Juvenile hormone (control larval period) extracted from the haemolymph of outdoor and indoor reared fifth instar 10th day larvae of tasar silkworm has been taken up. The studies has revealed that JH III levels decreased in indoor reared worms in the second and third crops when compared to that of the outdoor reared ones.

KEYWORDS: Juvenile Hormone III, Indoor rearing, Tasar silkworm, HPLC, Larval P eriod

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INTRODUCTION

Tasar silk is of two types *viz.,* tropical and temperate. China is the largest producer of tasar silk in the world followed by India. China produces only temperate tasar silk, while India has the distinction of producing both tropical and temperate varieties. India is the only country where tropical tasar silk is produced[1].The Indian tropical tasar silkworm is *Antheraea mylitta* D. and temperate silkworm or Indian Oak tasar silkworm is *Antheraea proylei* J. Other tasar silkworms include *Antheraea hubner* (Oriental region), *Antheraea Pernyi* (China, Japan and U.S.S.R) and *Antheraea yamami* (Japan) based on habitat. The *Antheraea mylitta* Drury (Daba TV) a forest-grown, commercial variety of Indian Tasar silkworm undergoes crop loss due to vagaries of nature, pests, parasites[2] and predators affecting cocoon yield to a great deal. Rearing being outdoors, there is no control over the natural vagaries leading to irregular hatching of eggs and prolonged larval period. The climatic hazards[3] and indefinite period of pupal diapause leads to erratic moth emergence followed by inadequate seed support and other grainage problems[4]. There is a dearth of appropriate technologies especially in the post cocoon sector and marketing facilities. In the post cocoon stage this race suffers certain drawbacks like lack of uniformity in cocoon structure, silk deposition and cocoon boiling due to their hardness which account for 50% silk loss in spinning.In the present investigation, an attempt has been made for total indoor rearing of *Antheraea mylitta* Drury (Daba TV), in which rearing of silkworm has been undertaken from brushing stage to the cocooning stage in the controlled conditions for the three crops, *i.e*., from June to December in the laboratory of sericulture unit, Kakatiya University campus. Simultaneously, outdoor rearing was also done in the field of *Terminalia arjuna* plantation raised in Sericulture Unit, Kakatiya University. The physical parameters of larvae like length, weight, colour and mortality and larval duration and studied for both indoor and outdoor reared larvae. The cocoon, post-cocoon and peduncle parameters of both are studied and compared. A comparative analysis of biochemical estimations was done in the late fourth and fifth instar worms and presented in the results.

Juvenile hormone is secreted by *corpora allata* which is an endocrine gland associated with the brain in the head region. Juvenile hormone is three types viz., JH I and JH II which is directly involved in the larval development and JH III presumed as gonadotropin hormone[5]. Juvenile hormones are a homologous series of sesquiterpenoids that are involved in embryogenesis, moulting, metamorphosis and reproduction[6]. The rate of insect metamorphosis is controlled by terpene JH, from CA, is effectively a hormone that keeps the animal's cuticle in the juvenile form, i.e., it maintains the expression of juvenile genes and represses adult genes. Its levels susally fall off progressively to allow increasing expression of adult characters. Removing the corpora allata in fifth instar larva gives rise to a miniature adult and adding extra JH gives an extra (sixth) and abnormally large larval stage and then a giant adult[7]. Juvenile hormones are a class of sesquiterpenoids which regulate embryogenesis, the progress of larval and adult development, metamorphosis, and reproduction in insects[8]. They are also involved in the control of diapause, migration, polyphenism, behavior, and metabolism[9, 10].

MATERIALS AND METHODS

Indoor rearing set-up consists of earthen pots / conical flasks or any wide mouthed water containers which can ensure constant water supply to the branches. The mouth of the conical flask or bottle was plugged with cotton to protect the larvae from drowning and also check any increase in humidity due to evaporation of water (Fig.1). The average number of worms on twigs of conical flask for I, II, III, IV and V instars are 100, 75, 50, 25 and 15 respectively. A paraffin paper was used to collect the faecal pellets and to maintain

cleanliness and healthy atmosphere in the rearing set[11]. This rearing set-up was surrounded by *vetifera* curtains to maintain more humidity in the indoor rearing environment (Fig.2)

(i) Collection of samples

The samples of haemolymph was collected from ten randomly selected late fifth instar larvae of outdoor and indoor reared Tasar silkworm, *Antheraea mylitta* D (Daba TV), for the extraction of juvenile hormone III, secreted by corpora allata. The haemolymph samples of silkworms were collected in cold conditions by adding phenylthiourea to prevent melanosis of haemolymph and stored at -20^{0}C until use.

Figure 1

The third instar Tasar silkworm, Antheraea mylitta D. (Daba TV) growing on the Indoor door rearing set-up containing a) mud-pots, b) Vetifera curtains, c) tables, d) bamboo stick stands and e) inserted twigs of Terminalia arjuna. 6 bamboo stick stands and e) inserted twigs of)

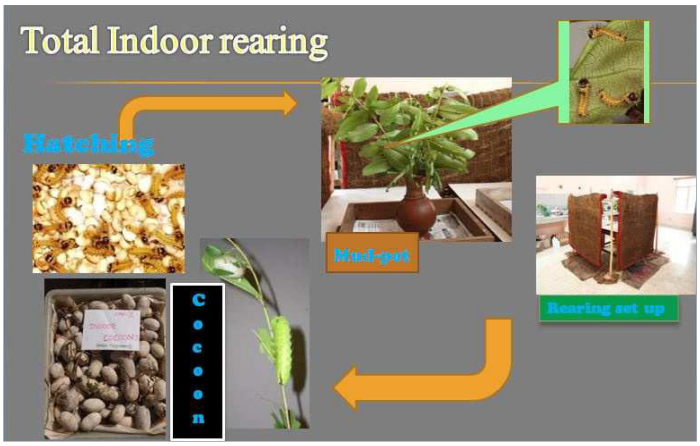
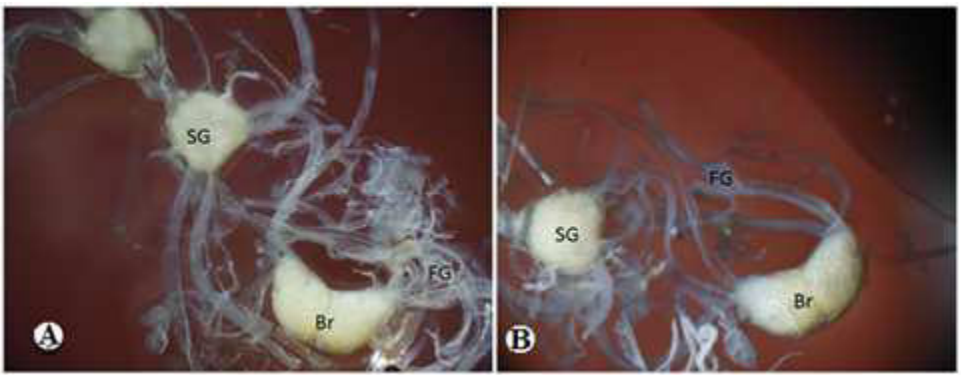


Figure 2

Total indoor rearing of tasar silkworm Antheraea mylitta D (Daba TV) from brushing of young silkworms to cocoon formation.

Figure 3
Brain of Fifth instar Tasar Silkworm Antheraea mylitta D (Daba TV) A) Outdoor reared larva B) Indoor reared larva



Br: Brain; SG: Sub esophageal ganglion; FG: Frontal ganglion.

(ii) Separation of Juvenile Hormone III from Haemolymph

The sample of JH III was prepared from the Insect haemolymph by the method of Stephanie et al.[12], with modifications. 5-10 µl was collected with a glass capillary and blow down into methanol/isooctane (1:1, v/v). The haemolymph and solvent ratio was 1:10 (v/v). The mixture was vortexed for 20 sec. and allowed to stand at room temperature for 30 min. Sample were centrifuged at 8,500xg for 15 min. The isooctane phase was transferred to a new glass vial, the methanol phase was vortexed and centrifuged at 10,000Xg for 30 min and combined with the isooctane phase and stored at-20^{0}C until use.

(iii) Hormonal Quantification

For the quantification of insect hormone which manipulate the larval period and growth is juvenile hormone III, standard were brought from the sigma Aldrich chemical pvt. Ltd. USA. Test samples were collected from the indoor and outdoor reared tasar silkworm, *Antheraea mylitta* Drury Daba TV haemolymph. The quantitative estimations of the JH III hormone were done by Beydon method[13].

(iv) Quantitative and qualitative analysis of JH III by HPLC

Extracted sample diluted in methanol was analyzed by HPLC type Jasco HPLC system UV-2075 with UV detector. The type of column used was Hypersil BDS C18, 250 mm X 4.6 mm, 5 um. The mobile phase used was a mixture of methanol-water (9:1) and the flow rate was 1ml/min and the wavelength used to detect JH III was 230 nm[13]. JH III standard used as a comparison was JH III (obtained from Sigma Ltd. USA). Ecdysone standard was diluted with methanol pro analysis. Quantitative analysis was conducted by comparing the retention time between standard JH III and the sample. If the sample contained compounds that had the same retention time with the standard JH III, then the compound was assumed to be Ecdysteroid. Co injection of standard and sample were conducted to confirm the existence of JH III in the sample.Quantitative analysis to determine JH III concentration was conducted by comparing area width of the sample to that of the standard on a standard calibration curve. The curve was made by plotting the area width of the Ecdysteroids standard to its concentration.

RESULTS AND DISCUSSION

Table 1

Instar-wise larval period (in days) of tasar silkworm, Antheraea mylitta Drury (Daba TV)

Crop	Rearing / Instar	I	II	III	IV	V	Total
I	Outdoor	4	4	7	9	14	38
(June-July)	Indoor	4	3	6	11	15	39
II	Outdoor	4	4	5	5	15	33
(Aug – Sep)	Indoor	3	4	6	7	13	33
III	Outdoor	5	5	7	9	15	41
(Dec- Jan)	Indoor	5	4	7	8	13	37

Hormone quantification of juvenile hormone III of tasar silkworm, *Antheraea mylitta* Drury (Daba TV) ecorace during the three crops of 2010 presented in the table. The juvenile hormone III hormone level in outdoor reared silkworms was 0.210, 0.271 and 0.289 µg while than that of indoor reared silkworms was 0.578, 0.137 and 0.279 µg (Fig.5).The silkworm growth, development and reproduction are regulated by the hormones *viz*., the Moulting hormone or Ecdysone (20-hydroxyecdysone) which has ecdysteroidal structure (secreted by Prothoracic glands), that activate the epidermal cells to produce both a new exoskeleton and moulting fluid and Juvenile hormone which has terpenoid structure and secreted by corpora allata. When both ecdysones and JHs are present, growth and moulting occur, but the larval characteristics are perpetuated into the next immature instar. When JH is absent or in low concentration, ecdysones many induce metamorphosis of the immature into adult. Ecdysteroids and juvenile hormones are the principal hormones regulating moulting, reproduction and diapause in insects[14].These are the chief hormones which control the larval moulting and larval duration apart from activation hormone or Brain hormone (secreted from brain or neurosecretory cells), diapause hormone (secreted from sub-oesophageal ganglion), PTTH(prothoracicotropic hormone (secreted by neurosecretory cells of protocerebrum), eclosion hormone and other peptidic hormones involved in regulating the ecdysis and emergence[15, 16, 17 and 18]. As JHs play a crucial role in insect development, reproduction and behaviour and regulate larval and pupal moulting and ecdysone promotes the growth by initiating the moulting process, pupa or larval span and, these two hormones are quantized in the outdoor and indoor reared fifth instar larvae. In insects, growth occurs during nymphal or larval life. The timing of moulting and metamorphosis is coordinated by a rise in the titre of ecdysteroids. With the last moult, the adult size is reached. Body size is intimately linked to nutritional, environmental and genetic cues[19]. In the present investigation, a comparative account of larval weight, length, larval duration and moulting duration have been studied and presented in the results. The larval size (weight and length) were observed to be more in outdoor rearing condition, larval and moulting duration was also more in the outdoor reared ones. This can be ascribed to ecdysone which is the one which initiates moult and controls or directs fate of metamorphosis[15]. However, in the third year, the pattern of larval duration was that it was more in the indoor conditions in the first crop, equal in both in the second crop and greater in the outdoor in the third crop , where, the ecdysone levels were greater in the first and third crops in indoor while greater in second crop of outdoor rearing conditions also relates to the role played by ecdysteroids, which are essential for driving the molecular and cellular events that lead to moulting and metamorphosis[20, 21, 22]. Neurohormones are the master regulators of all life processes in insects and employ a strategy of triggering stress-protective events and are synthesized mainly in insect brain

neurosecretory neurons. Despite the wide differences observed in insects, all life processes are coordinated by only a few neurohormonal compounds which regulate molting hormones (ecdysteroids) and juvenile hormones (JHs, sesquiterpenoids).The role of neurohormones and involvement of ecdysteroid or JH in response to stress was explained by Vesna Peric[23], according to whom the stress induces changes in release of ecdysoregulatory and allatoregulatory neurohormones and modifies ecdysone and juvenile hormone synthesis in prothoracic gland and *corpora allata.* The involvement of hormones of an ecdysteroid or JH type in response to stress creates the danger of an untimely induction of morphogenetic process in target cells. In the present investigation, as the two factors *i.e.,* larval duration and size, can affect the cocoon yield *i.e.,* if the larval period is more, the intake of food and development of larvae also will be more which ultimately helps in spinning of good quality of cocoons, this aspect needs to be further explored as there may be a possibility of stress involved in change from natural environment resulting in earlier completion of morphogenetic processes.As the majority of the crops in the three years have shown that outdoor reared worms have better larval size and greater larval period than those of indoor reared ones, these phases of weight and size control have been elucidated by feeding experiments and variation of environmental factors[24], which revealed a combination of nutritional and environmental determinants guiding insect development. The present studies includes provision of selected leaf and optimum conditions of temperature and RH, minimized pest and predator menace, improved crop production and decreased mortality. However, further efforts to produce robust indoor reared silkworms are the need of the hour.

The duration of the larval period varies between outdoor and indoor rearing. The indoor reared larval period was found less when compared to that of outdoor rearing. It might be due to the reason that sufficient good quality food was supplied twice per day to indoor reared worms and no climatic fluctuations made continuous feeding leading to early spinning. The outdoor reared silkworms during the fifth instar feed voraciously and empty the twigs early and busy searching for leaves until it finds a good leaf. The Juvenile hormone is a very important hormone which controls the larval period of the silkworm. Low levels of Juvenile hormone were observed in indoor reared worms of during the third year in the fifth instar of second and third crops.

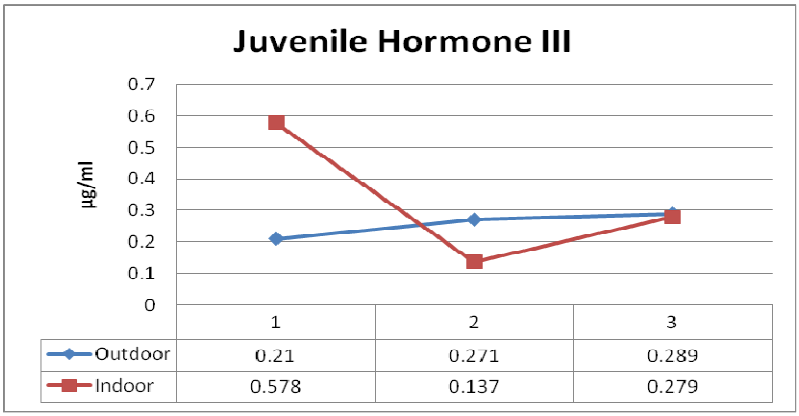


Figure 5
Quantification of Juvenile hormone III in the haemolymph of fifth instar outdoor and indoor reared Tasar silkworm, Antheraea mylitta Drury (Daba TV) by HPLC.

The protein content in haemolymph of fourth and fifth instars was found to be higher in all the three crops of the indoor reared tasar silkworms. On the contrary, in the fat body it

was higher in outdoor reared ones. The proteins, which form the major constituents of insect haemolymph, undergo both qualitative and quantitative changes during development of insects and such fluctuations suggest their regulation by morphogenetic hormones. The trehalose content in the haemolymph of fourth and fifth instars was mostly found to be higher in indoor reared tasar silkworms rather than outdoor reared ones. Carbohydrates present in haemolymph are used principally as a source of energy for synthesis of fat and glycogen. Both haemolymph proteins and carbohydrates levels were found to be influenced by the Juvenile hormones during larval-pupal metamorphosis of lepidopteron[25]. The increase in protein and carbohydrate levels in the haemolymph of indoor reared worms may be attributable to lower levels of JH. The attributed increase in protein concentration was due to prevention of sequestration of storage proteins by fatbody[26]. On the other hand, the increase in carbohydrate content may be due to mobilisation of trehalose from fat body to haemolymph[27] which is also supported by other reports of increased JH levels interfering with carbohydrate metabolism[28, 29]. These studies indicate that carbohydrate and protein levels in the haemolymph have significantly decreased in JH treated fifth instar larvae of *Bombyx mori*. Silkworm's fat body produces storage proteins will be released in to haemolymph which found in the earlier instars but their major accumulation occurs during the latter part of the feeding phase for which a decline in the juvenile hormone is necessary[30].

CONCLUSION

The quantification of juvenile hormone has revealed that in the first crop the indoor worms have shown greater quantity than that of outdoor reared ones, while in second and third crops it was lower than that of outdoor reared tasar silkworms. The corresponding decrease in the larval period of indoor rearing might me due to the reason that sufficient good quality leaves were supplied to silkworms, twice a day and no climatic fluctuations made continuous feeding leading to early spinning. However, the study opens an avenue to study further as protein and carbohydrate content were found to be higher in the haemolymph of indoor reared tasar silkworms, which may be due to fluctuations in JH.

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