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Comparative studies of ecdysone in outdoor and indoor reared tasar silkworm, *Antheraea mylitta* Drury

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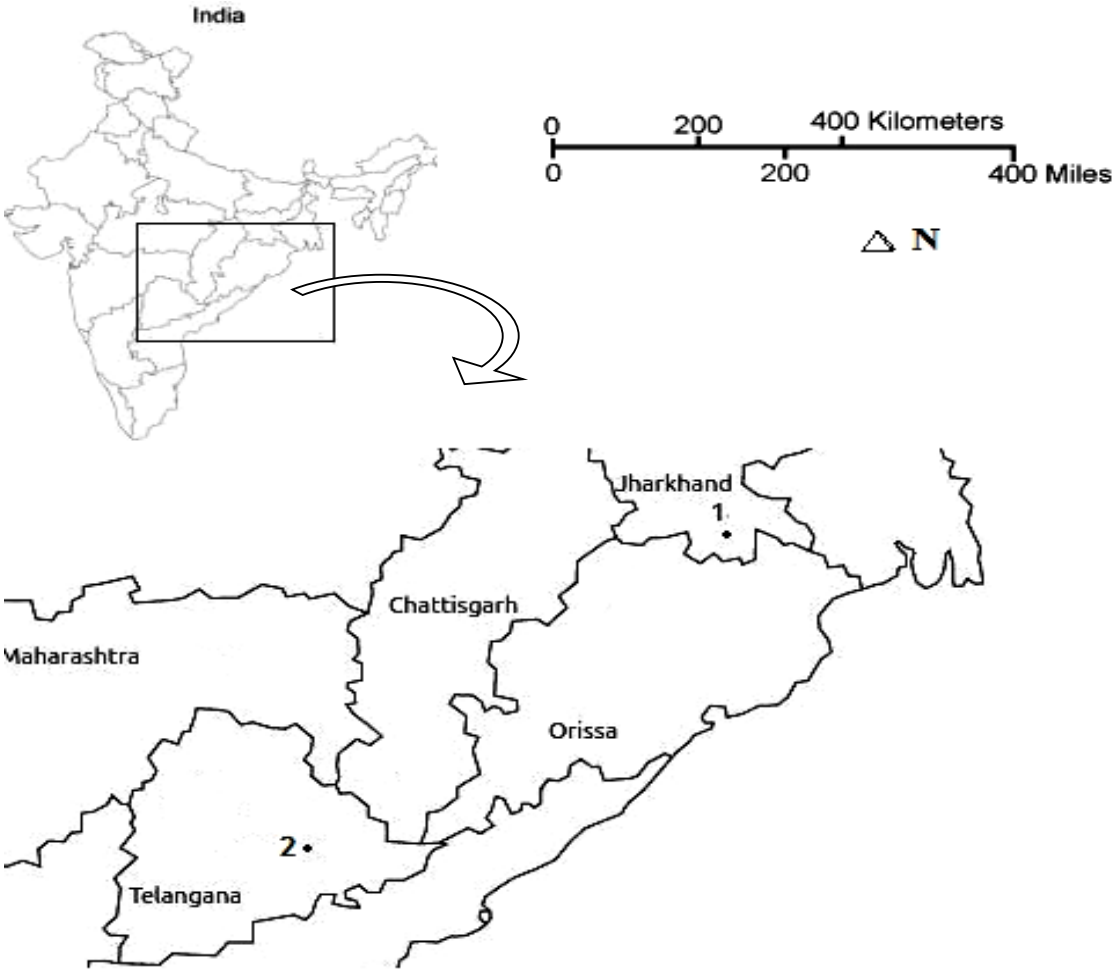
Abstract
The Indian tropical forest-grown, Tasar silkworm, *Antheraea mylitta* Drury Daba TV (Tri-voltine) ecorace has been facing problems during outdoor rearing, heavy mortality of larvae due to predators, parasites; irregular hatching of eggs leading to prolonged larval period, climatic hazards; indefinite period of diapause leading to erratic moth emergence followed by inadequate seed support and other grainage (egg production) problems. In order to overcome these problems indoor rearing concept has been evolved. In the present investigation, a comparative analysis of ecdysone in outdoor and indoor reared fifth instar, 10th day larvae of tasar silkworm has been taken up as they control moulting period. In spite of feeding same quality of leaves for both rearing methods of outdoor and indoor reduced larval period observed in Vth instar stage of indoor rearing. As early pupation in the fifth instar larvae of indoor rearing is mainly owing to a rise in the hormone concentration, the present study based on quantification of ecdysone hormone level in the haemolymph of tasar silkworm of outdoor and indoor on fifth instar 10th day was taken up. Ecdysone levels of fifth instar larvae in the first and third crops of indoor rearing found to be increased by 15% when compared to those of outdoor reared ones.

Keywords: Ecdysone**,** Tasar silkworm. *Antheraea mylitta,* Daba TV, indoor rearing, Vth instar

1. Introduction
The tasar silkworm *Antheraea mylitta* Drury is a species widely distributed in India between the range of 16^0-24^0 N latitude and 80^0-88^0 E from West Bengal in the East to Karnataka in the South with its natural inhabitation in the forest areas of Jharkhand, Bihar, Orissa, Madhya Pradesh, Maharashtra and Andhra Pradesh [1]. The *A. mylitta* D, Daba ecorace, native of Singhbhum of Jharkhand (Fig. 1) is available in two forms based on voltinism *viz*., bi and tri-voltine. According to the latest survey on tasar silkworm done by Srivastava and Suryanarayana [2], the features of *A. mylitta* D., Daba ecorace include an emergence capacity of 91.19%, mating capacity of 76.10%, average fecundity of about 250, cocoon weight of 16.39 gm and shell weight of 2.65 gm. It has reel ability, shell ratio, filament length and denier of 16.76%, 69%, 1010 m and 9% respectively. Some studies have revealed that the Daba TV has been facing problems during outdoor rearing. Heavy mortality of larvae is caused due to predators, parasites [3], irregular hatching of eggs leading to prolonged larval period, climatic hazards [4], indefinite period of diapause leading to erratic moth emergence followed by inadequate seed support and other grainage problems [5]. There is a dearth of appropriate technologies especially in post-cocoon sector and marketing facilities. In the post-cocoon stage this ecorace suffers certain drawbacks like lack of uniformity in cocoon structure, silk deposition and cocoon boiling due to their hardness which account for 50% of 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 was 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 Department of Zoology, Kakatiya University campus, Warangal, Telangana state (Fig. 1). Simultaneously, outdoor rearing was also done in the field of *Terminalia arjuna* plantation raised in Kakatiya University. The physical parameters of larvae like length, weight, moulting period and ecdysone hormone levels in the Vth instar were studied for both indoor and outdoor reared larvae and presented in the results.

Ecdysone (20-hydroxyecdysone) is a steroid hormone which is responsible for insect moulting to promote growth, also called as moulting hormone. For the first time, Moulting hormone (MH) was extracted, purified and crystallized from *Bombyx mori* [6]. Later, Karlson [7] showed by X-ray analysis, that it is steroid in nature, with a molecular weight of 464, having empirical formula $C_{22}H_{44}O_6$



Location indicators of site of sample collected and research area map

Sl. no	Location Name	perpose of site
1	Singhbhum of Jharkhand state	Native place of Daba TV ecorace
2	Warangal of Telangana state	Research experimented spot

Fig 1: Research area map

In the recent times, the concept of indoor rearing of Tasar silkworm *A. mylitta* by use of artificial diet was successfully done for young age worms [8, 9]. Semi-domesticated rearing of modal ecorace in its natural habitat was made possible by Nayak [10]. A recent study has shown that the indoor rearing method is an alternative to outdoor rearing method, to avoid the main problems of pest and predators which exposes the tasar silkworms to natural climatic vagaries during outdoor rearing. It has also resulted in increasing the crop yield compared to outdoor traditional rearing method. Though indoor reared cocoons are not as compactly formed as the outdoor reared ones, the larval characteristics like length and weight were on par with those of outdoor reared ones, which is an encouraging aspect of these studies [11]. There was also a considerable improvement in biochemical parameters like high proteolytic activity and amylase activity in the digestive juice, increase in protein and carbohydrate content in the tissues of silkworm leading to improvement in cocoon characteristics when compared to that of outdoor rearing [12, 13, 3, 14, 15].

Insects have a rigid exoskeleton and must moult to grow, and moulting is the basis for metamorphosis given that transformations take place through the moults. The moulting hormone has an ecdysteroid structure[16] and during juvenile stages, it is synthesized by the prothoracic glands. Towards the end of each stage, ecdysteroid production increases rapidly, reaches maximal values and then decreases and remains low until the next moulting cycle [17].

The hormones, Juvenile hormone and Ecdysone (moulting hormone) play a key role in the larval duration, moulting, pupation and they influence other physiological functions including cocooning. Juvenile hormone is mainly responsible for larval duration in the silkworm. The fifth instar lasts about 10-15 days, during which stage they feed voraciously leading to greater improvement of its body size. The quantification of juvenile hormone from the haemolymph revealed lower levels in the indoor reared silkworms that of outdoor rearing [18]. Ecdysone hormone plays a key role in the ecdysis (Fig. 2) and final pupation. Early pupation and

Less larval period are responsible for the less quality cocoons in the silkworm rearing. The present investigation on the quantification of ecdysone hormone extracted from haemolymph during the late fifth instar of outdoor and indoor reared Tasar silkworm helps in better understanding of the formation of feeble quality of cocoons in the indoor rearing compared to that of outdoor rearing.

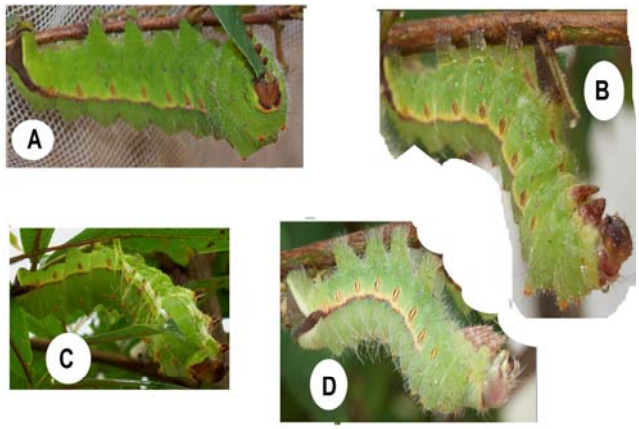


Fig 2: Moulting stages of Tasar silkworm, *Antheraea mylitta*

A: Feeding larva, B: Initiation of moulting (Non-feeding stage) C: Ecdysis (casting-off of old skin) D: Larva out-of-moult (with fresh integument in pale colour)

2. Materials and Methods

Fig 3: The fifth instar Tasar silkworm, *Antheraea mylitta;* a) Indoor Rearing set-up b) Outdoor reared conditions on *T. arjuna* plantation (Kakatiya University, Warangal)

Indoor rearing set consists of mud pot or wide mouth bottles rather, any water container which can ensure constant water supply to the branches. The mouth of the mud pot or bottle was plugged with cotton to protect larvae from drowning and also to check any increase in humidity due to evaporation of water. The average number of worms on twigs of conical flask for I, II, III, IV and V instars were 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. This rearing set-up was surrounded by *vetifera* curtains to maintain more humidity in the indoor rearing environment [12]. Simultaneously outdoor rearing was also done by net method (Fig. 3).

During the rearing period 10 healthy worms were selected at random from the rearing lot of both indoor and outdoor worms, which were feeding on the *Terminalia arjuna* plants for measurement of length and weight. These parameters were taken daily for first to fifth instars except on their moulting period. Larval weights were measured in an electronic balance of Shimadzu-make and length of the larvae was measured in centimeter by using a graph paper. The instar-wise average of the larval weight and length were noted down.

2.1 Collection of Haemolymph – The haemolymph was collected from ten each of outdoor and indoor reared, late fifth instar larvae of Tasar silkworm, *A. mylitta* D. (Daba TV), selected at random for the extraction of hormones during three crops of a year. The haemolymph of silkworms was collected in cold conditions; phenyl thiourea was used to prevent melanosis and stored at -20 ^{0}C until use.

2.2 Separation of 20-Hydroxyecdysone from Haemolymph – 20 ml of Haemolymph was collected from ten late Vth instar larvae simultaneously reared in outdoor and indoor conditions for three crops. Added phenyl thiourea to prevent the melanisation and kept at -20^{0}C until use. The haemolymph was allowed to melt at room temperature and 10 times of this haemolymph, acetone-ethanol (1:1) added and therefore extraction was done three times. The acetone-ethanol extracts were combined, dried under reduced pressure in a constant temperature chamber held at 25-30 ^{0}C, and the residue sent to the next step. Equal amounts of 70% methanol and petroleum ether were then added to the residue to transfer the lipids to the petroleum ether fraction. The methanol fraction is used for the next step. The 70% methanol fraction was allowed to dry in a constant chamber maintained at 30-35 ^{o}C to obtain a residue. This residue was partitioned in water and Butanol, the procedure was done three times and the Butanol extract was used as the ecdysone extract and stored at -20 ^{0}C until use. This extract was dried under reduced pressure, re-dissolved in 10 ml of methanol, kept in dried and cold condition until the HPLC quantification.

2.3 Detection and conformation of 20-Hydroxyecdysone: Before proceeding further analysis for HPLC analysis, the compound was detected by TLC (Thin Layer Chromatography) technique. Chloroform: methanol: water (60:40:10) solvent was used for separation, P- anisaldehyde was used for color reaction against standard. The conformation of the protons in the 20-Hydroxyecdysone, H1 NMR spectra was recorded on Jeol 400-MHz NMR (Model JNM-400) spectrometer and CDCl3 as internal standard.

2.4 Quantitative analysis of 20-Hydroxyecdysone by HPLC – Ecdysteroid 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 µm. 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 Ecdysteroid was 254 nm [19]. Ecdysteroid standard used as a comparison was ecdysone (obtained from Sigma Ltd. USA). Ecdysone standard was diluted with methanol pro analysis. Quantitative analysis was conducted by comparing retention time between standard Ecdysteroid and the sample. If the sample contained compounds that had the same retention time with the standard Ecdysteroid, then the compound was assumed to be Ecdysteroid. Co-injection of standard and sample were conducted to confirm the existence of Ecdysteroid in the sample. Quantitative analysis to determine Ecdysteroids 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.

3. Results

Table1: Instar-wise average larval length, of outdoor and indoor reared tasar silkworm, *A. mylitta* D., during three crops of a year.

Crop	Rearing Method	Instar				
		I	II	III	IV	V
I (June – July)	Outdoor	1.07 ± 0.35	1.97 ± 0.12	2.61 ± 0.44	4.15 ± 0.79	8.89 ± 1.86
	Indoor	1.07 ± 0.35	1.85 ± 0.19	2.76 ± 0.49	4.45 ± 0.84	9.01 ± 1.74
II (Aug – Sep)	Outdoor	0.6 ± 0.68	1.23 ± 0.38	2.06 ± 0.36	4.98 ± 0.44	8.81 ± 2.4
	Indoor	0.6 ± 0.68	1.23 ± 0.38	1.99 ± 0.36	4.8 ± 0.4	8.77 ± 2.56
III (Dec – Jan)	Outdoor	0.3 ± 0.5	2.24 ± 0.57	3.45 ± 0.21	5.01 ± 0.71	9.21 ± 2.1
	Indoor	0.3 ± 0.5	2.06 ± 0.38	3.42 ± 0.51	5.14 ± 0.55	9.16 ± 1.74

Note: Length in cms; Mean of randomly picked 5 silkworms; after ± Standard Error.

The first crop average larval length and its standard deviation of tasar silkworm, *A. mylitta* D. outdoor rearing were 1.07 ± 0.35, 1.97 ± 0.12, 2.61 ± 0.44, 4.15 ± 0.79 and 8.89 ± 1.86 while than that of indoor rearing were 1.07 ± 0.35, 1.85 ± 0.19, 2.76 ± 0.49, 4.45 ± 0.84 and 9.01 ± 1.74 of I, II, III, IV and V instar respectively.

The second crop average larval length and its standard deviation of tasar silkworm, *A. mylitta* D. outdoor rearing were 0.6 ± 0.68, 1.23 ± 0.38, 2.06 ± 0.36, 4.98 ± 0.44 and 8.81 ± 2.4 while than that of indoor rearing were 0.6 ± 0.68, 1.23 ± 0.38, 1.99 ± 0.36, 4.8 ± 0.4 and 8.77 ± 2.56 of I, II, III, IV and V instar respectively.

The third crop average larval length and its standard deviation of tasar silkworm, *A. mylitta* D. outdoor rearing were 0.3 ± 0.5, 2.24 ± 0.57, 3.45 ± 0.21, 5.01 ± 0.71 and 9.21 ± 2.1 while than that of indoor rearing were 0.3 ± 0.5, 2.06 ± 0.38, 3.42 ± 0.5, 1 5.14 ± 0.55 and 9.16 ± 1.74 of I, II, III, IV and V instar respectively (Table 1).

Table 2: Instar-wise average larval weights in grams, of outdoor and indoor reared Tasar silkworm, *A. mylitta* D. (Daba TV), during three crops of a year.

Crop	Rearing Method	Instar-wise average weights				
		I	II	III	IV	V
I (June – July)	Outdoor	0.235 ± 0.01	2.132 ± 0.31	4.813 ± 0.35	8.418 ± 1.23	24.11 ± 7.27
	Indoor	0.224 ± 0.02	1.985 ± 0.32	4.522 ± 0.35	7.713 ± 1.09	20.13 ± 6.45
II (Aug – Sep)	Outdoor	0.192 ± 0.01	2.215 ± 0.07	4.522 ± 0.31	8.635 ± 1.75	25.01 ± 6.92
	Indoor	0.190 ± 0.01	2.189 ± 0.08	4.110 ± 0.36	7.921 ± 1.65	20.62 ± 5.19
III (Dec – Jan)	Outdoor	0.215 ± 0.03	2.312 ± 0.14	4.013 ± 0.47	9.215 ± 1.82	26.51 ± 7.52
	Indoor	0.201 ± 0.03	2.103 ± 0.14	3.910 ± 0.58	8.129 ± 1.43	21.43 ± 6.69

Note: Weight in Grams; Mean of randomly picked 5 silkworms; after ± Standard Error.

The first crop average larval weights and its standard deviation of tasar silkworm, *A. mylitta* D. outdoor rearing were 0.235 ± 0.01, 2.132 ± 0.31, 4.813 ± 0.35, 8.418 ± 1.23 and 24.11 ± 7.27 while than that of indoor rearing were 0.224 ± 0.02, 31.985 ± 0.32, 4.522 ± 0.35, 7.713 ± 1.09 and 20.13 ± 6.45 of I, II, III, IV and V instar respectively.

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Table 3: Moulting period of tasar silkworm, *Antheraea mylitta* Drury (Daba TV), during three crops of a year (in Hours).

Crop	Rearing	1st moult	2nd moult	3rd moult	4th moult	Total moulting duration in hrs	Total larval duration in days
I (June – July)	Outdoor	34	24	27	29	114	38
	Indoor	24	23	26	31	104	39
II (Aug – Sep)	Outdoor	34	34	35	42	145	33
	Indoor	23	34	28	27	112	33
III (Dec – Jan)	Outdoor	35	35	47	48	165	41
	Indoor	25	34	37	38	134	37

Note: Moulting duration in hours; Mean of randomly picked 5 silkworms.

The total moulting period in the first crop of outdoor rearing were 114, 145 and 165 hrs, while those in indoor rearing were 104, 112 and 134 hrs in the first, second and third crops respectively The total larval period in the first crop of outdoor rearing were 38, 33 and 41 days, while those in indoor rearing were 39, 33 and 37 days in the first, second and third crops respectively (Table 3).

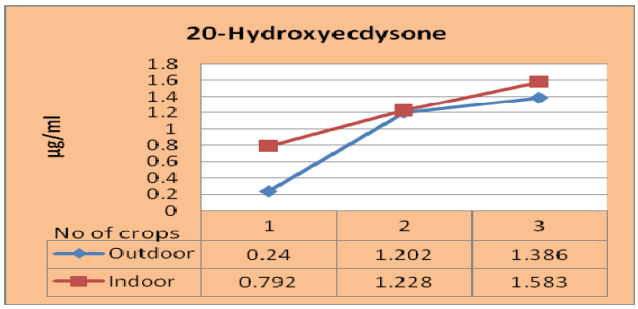

Fig 4: Quantification of Ecdysone hormone in the haemolymph of outdoor and indoor reared tasar silkworm, *A. mylitta* D. (Daba TV) during the three crops.

Hormone quantification of 20-Hydroxyecdysone of tasar silkworm, *A. mylitta* D. (Daba TV) ecorace during the three crops was presented in the Figure 4. The 20-Hydroxyecdysone hormone level in outdoor reared silkworms was 0.240, 1.202 and 1.386 µg while than that of indoor reared silkworms was 0.792, 1.028 and 1.583 µg. The juvenile hormone III 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.

The 20-hydroxyecdysone, with a molecular formula $C_{27}H_{44}O_7$ extracted from haemolymph was detected by the colour reaction on TLC (Thin layer chromatography) with p-anisaldehyde. NMR study results showed the structural conformation of 20-Hydroxyecdysone. Hormone quantification results of 20-Hydroxyecdysone of tasar silkworm, *Antheraea mylitta* Drury (Daba TV) ecorace during the three crops of a year presented in Figure 5.

The 20-Hydroxyecdysone H^1 NMR Spectrum data as follows: 0.85 (S, 3H, C18-CH), 0.87 (s, 3H, C19-CH_3), 0.94 (s, 3h, C21-CH_3), 0.96 (q, CH2, C16), 0.98 (t, CH_2, C12), 1.25(s, 6H, C27, C26-H), 1.54 (q, CH_2, C11), 2.05 (t, CH2, CH15), 2.06 (d, 2CH_2, C11, C12), 2.27 (q, CH, C17), 2.79(t, CH2,CH4), 3.16 (t, CH, C5), 3.47(d, CH_2, C1), 3.60(m, CH, C20), 3.73 (t, CH, C9), 3.85 (t, CH, C22), 4.23 (BRS, 6OH)5.12 (t, CH, C23), 5.34(t, CH, C24-H), 5.78 (t, 3CH, C2, C3, C25) and (s, CH, C7-H) (Fig 5)

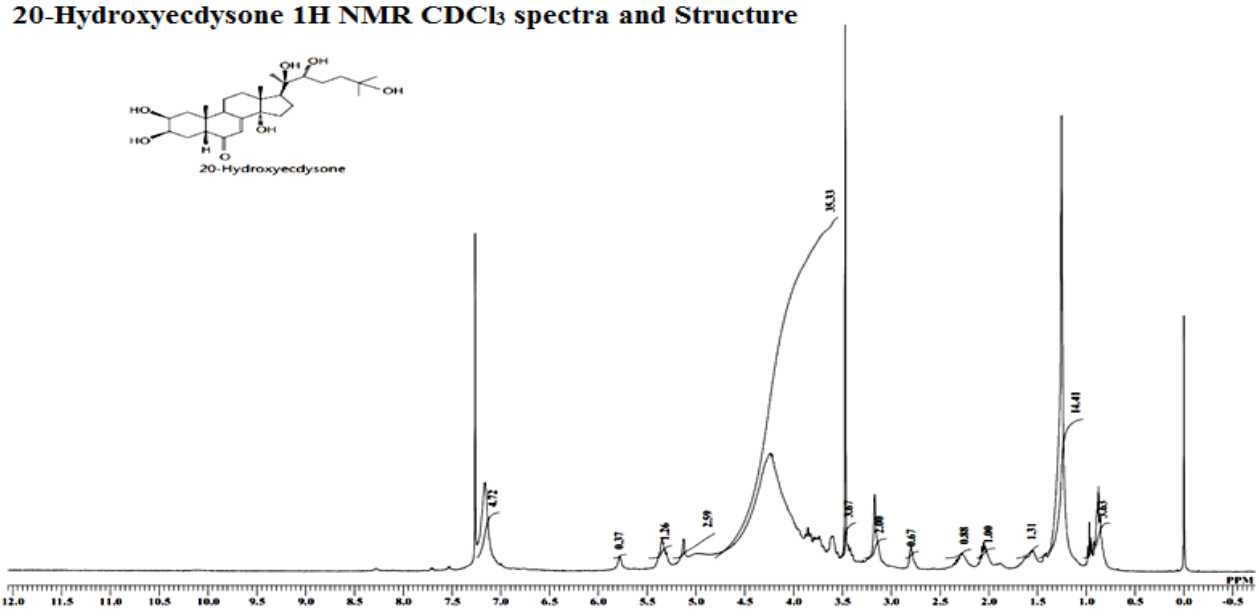

Fig 5: The 20-Hydroxy ecdysone ^{1}H NMR CDCl3 spectra and Structure: The 20-hydroxy ecdysone with molecular formula $C_{27}H_{44}O_7$ extracted from haemolymph was detected by the colour reaction on TLC (Thin layer chromatography) with p-anisaldehyde and ^{1}H NMR (Proton Nuclear Magnetic Resonance) analysis. The methyl resonances in the ^{1}H NMR spectrum at δ 1.25, 1.54 and 2.05 were assigned to 18th, 19th and 21st methyl groups respectively. The 2.06 location was due to 26th and 27th methyl groups; 3.16 doublet was due to C22 carbon; C11 as quartet at δ 1.54; C2 and C3 carbons as triplets at 5.78; OH group on C25 carbon appeared as broad singlet at 4.23 which indicated that the compound was a 20-Hydroxyecdysone.

4. Discussion

The silkworm growth, development and reproduction are regulated by the hormones *viz*., the Moulting hormone or Ecdysone (20-hydroxyecdysone) which has ecdysteroid structure (secreted by Prothoracic glands), that activate the epidermal cells to produce both a new exoskeleton, 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 may induce metamorphosis of the immature into adult. Ecdysteroids and juvenile hormones are the principal hormones regulating moulting, reproduction and diapause in insects [20].

The quantification of ecdysone in outdoor and indoor reared tasar silkworms has revealed that in the first and third crops it was more in the indoor reared worms; on the other hand it was found to be more in the outdoor reared worms in the second crop. The fluctuations in the ecdysone quantities may be explained in relation to the environmental variations, availability of food and quality of leaf during the rearing period. Ecdysone has essential roles in co-ordinating major developmental transitions, such as larval moulting and metamorphosis and its levels are significantly affected by environmental conditions like high temperature and nutritional shortage result in an increase in ecdysone levels [21].

Ecdysone hormone is secreted by prothoracic gland (PTG), promotes the growth by initiating the moulting process, pupa or larval span and also an important factor for the promotion

and maintenance of fibrin synthesis in the posterior silk gland [22]. It stimulates the enzymatic reactions in the metabolic processes like cytochrome oxidase system which regulate the normal metabolism and growth [23]. DOPA decarboxylase is a key enzyme for glycogen synthesis in the fat body [24]. It also influences the ovarian development during the larval stage of many insects and also increased rate of mutation in silkworm by Ecdysone [25].

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 [26]. In the present investigation, we have studied a comparative account on larval weight, length, larval duration and moulting duration 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 (Table 1, 2 and 3). This can be ascribed to ecdysone which is the one which initiates moult and controls or directs the fate of metamorphosis [16].

The moulting process is very crucial in the development of the exo-skeletonised insects both ecdysone (20E) and hormones governed this process. The Ecdysone hormone levels promote the moulting process within high concentrations. The juvenile hormone and juvenoids regulate the quality of the moult [27]. The moulting period of outdoor reared tasar silkworm, *Antheraea mylitta* Drury (Daba TV) showed long duration when compared to that of indoor reared ones. Enough care was taken during the rearing period towards silkworms which were under moult, by separating them from the rearing environment and keeping undisturbed until the moulting period was over. In outdoor rearing, the larvae encountered certain disturbances due to wind blow, pests and rainfall, which may enhance the moulting period. The moulting process is controlled by the moulting hormone or Ecdysone (precursor of 20-Hydroxyecdysone) [16]. In the present studies, the level of the 20-hydroxyecdysone was found to be more in the haemolymph of indoor reared worms when compared to that of outdoor worms, which may be the cause for early moulting in the former.

However, in the third year, significant observations were recorded, the pattern of larval duration was that - it was more in the indoor conditions in the first crop, equal in both outdoor and indoor during the second crop and greater in the outdoor during the third crop. The moulting period of outdoor reared worms showed long duration when compared to that of indoor reared ones (Table 3). Further, the ecdysone levels were greater in the first and third crops and lesser in second crop of the haemolymph of indoor reared larvae when compared to that of outdoor reared ones (Fig. 4). It is observed from the present studies that, as the ecdysone level increased, the moulting period decreased, subsequently the larval period also decreased in the indoor rearing, except in the first crop. However in the second crop, the ecdysone level and larval duration were same in both outdoor and indoor rearing. This aspect relates to the role played by ecdysteroids, which are essential for driving the molecular and cellular events that lead to moulting and metamorphosis [28, 29, 30].

Earlier reports indicated that exposure of *Bombyx mori* to 20E and loss of the sensitivity of the epidermal cells to JH are required for the completion of pupal commitment, and suggested the unusually long process of 3 days could be due to the presence of the detectable JH during the commitment [31]. As the two factors *i.e.,* larval duration and size, can affect the cocoon yield, and, 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 explored further as there may be a possibility of stress involved change from natural environment resulting in earlier completion of morphogenetic processes. The role of neurohormones and involvement of ecdysteroid or JH in response to stress was explained by Vesna [32] in 2006.

Application of increasing proportions of 20-Hydroxyecdysone hormone for larval treatment in *Bombyx mori,* multivoltine silkworm resulted in the maximum level of larval weight and improvement in larval survival was noticed by Prasad and Upadhyay [33]. The nutritional shortage induces rising ecdysone hormone levels which lead to apoptosis in the oogenesis and low concentrations are essential for normal oogenesis in *Drosophila melanogaster* [34, 35]. In the present studies 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 [36], which revealed a combination of nutritional and environmental determinants guiding insect development. This explains that though indoor rearing can be successfully done till cocooning stage, a clear requirement in terms of nutritional and environmental proportions can be carved out for definite hormonal regulation which influences moulting and growth.

The 20-Hydroxyecdysone hormone quantification was done from outdoor and indoor reared tasar silkworm, *Antheraea mylitta* Drury Daba TV on the 8th day of fifth instar larval haemolymph for the three crops of a year by the HPLC method. The 20-Hydroxyecdysone concentration was found to be increasing from first crop onwards in both outdoor and indoor rearing. A recent study in the tropical tasar silkworm *Antheraea mylitta* (D), Bhandara ecorace [37] has revealed that the concentration of protein and carbohydrate increased after the treatment of JH-III because of its growth stimulating effects while, reduction in the protein as well as carbohydrates after the 20-HE treatment indicated its utilization for the cocoon formation due to which early larva – pupa metamorphosis has occurred, which is in corroboration with the present studies.

Certain studies have revealed a stimulating capacity of the JH analogue hormones on various characteristic of the silkworm contributing to the quality silk yield [38]. The present studies emphasizes the provision of selected leaf and optimum conditions of temperature and RH, minimized pest and predator menace which has improved crop production and decreased mortality. However, further efforts in this direction can be sought after to produce robust indoor reared silkworms.

5. Conclusion

The present study reveals that the ecdysone hormone was one of factors responsible for reducing the larval duration in indoor rearing of tasar silkworm, *A. mylitta* D. The study can be further explored by the application of juvenile analogues during the indoor rearing of tasar silkworms in order to improve the larval duration, which may ultimately give rise to good, compact and quality cocoons for commercial purposes.

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7. References

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