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**COMPARATIVE STUDY OF REARING PERFORMANCE IN F1 AND F2  
HYBRID POPULATIONS OF TASAR SILKWORM, *ANTHRAEA*  
*MYLITTA***

**RENUKA G AND SHAMITHA G\***

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

\*Corresponding Author: Dr. Shamitha Gangupanthula: E Mail: [vodithalashamitha@gmail.com](mailto:vodithalashamitha@gmail.com)

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**ABSTRACT**

*Antheraea mylitta*, a wild sericigenous insect is a species widely distributed from West Bengal in the in the East to Karnataka in the South with its natural inhabitation in the forest area of Bihar, Orissa, Madhya Pradesh, Maharashtra and Andhra Pradesh and Telangana. It is a polyphagous insect feeding on a number of food plants primarily on *Terminalia arjuna* and *T. tomentosa* and a host of secondary food plants. The wide range of distribution of the species has encountered diverse geographic and climatic variations of the distinct areas, leading to marked differences in not only phenotypical and physiological traits but also in the commercial and technological aspects. *Antheraea mylitta* Drury (Andhra local ecorace), is an exclusive race of State of Telangana and Andhra Pradesh. The present study is on The improved varieties of these silkworms can be evolved by hybridization between Andhra local and Daba TV ecoraces by backcross method, breeding of tasar ecoraces is aimed at the improvement of to be bestowed with superior characters *i.e.*, survivability and fecundity of Daba TV and also as one of the probable strategies for enhancing the productivity and quality of tasar raw silk by breeding method to improve the traits in the upcoming generation by selective parental selection.

**Keywords: *Antheraea mylitta* Drury, Tasar silkworm, Ecoraces, Breeding, Backcross, phenotypical and physiological traits**

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## INTRODUCTION

Non-mulberry sericulture known as forest or wild sericulture holds great promise for agro industries and forestry as supplementary activity. Tasar, Eri and Muga are the principal non-mulberry silk commercially exploited in India. Tasar silk worms are reared in the tropical and temperate zones and the global production is dependent on *Antheraea* species. The tropical tasar silkworm *A. mylitta* Drury belongs to the family Saturniidae and order Lepidoptera.

**Tasar silk** is produced from the tasar silkworms, which has many eco-races principally controlled by prevailing environmental conditions. The tasar silkworm has two varieties viz., temperate, mainly in Central, Northern and Eastern regions like in many States like Jharkhand, Bihar, Assam, Jammu and Kashmir, Madhya Pradesh, Karnataka, Kerala, Maharashtra, Andhra Pradesh and Telangana also have good population as well as variety of tasar food plants available, mostly on *Terminalia arjuna* (Arjun) and *T. tomentosa* (Asan) forest plants as well as economic plantation. Besides, large number of tasar cocoons is also collected from *Shorea robusta* (Sal) and other secondary food plants. Tasar silkworm are in dire need to be explored, catalogued, conserved and characterized for its

commercial utilization by breeders and geneticists [1]. The Tasar silkworm, *A. mylitta* has rich genetic resource as 44 ecoraces, however, the Tasar culture, an important co-discipline of applied forest biology, needs special understanding and addressing towards breeding perspective to promote the sustainable utilization of this precious natural resource. The basic information on ecology, environmental factors, climatology, flora, fauna and their inter relationship, the life cycle, diapause, reproductive biology and voltinism and population dynamics of tasar silkworm [2].

Among other ecoraces of Tasar silkworm, *A. mylitta* D, **Andhra local ecorace** is a tropical tasar variety available only in Warangal and Karimnagar districts of Telangana state. Andhra local, the exclusive ecorace of Telangana state is well known for its remarkable commercial characters like compact and hard cocoons, high reelability, high shell ratio, low denier and high filament length. 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 [3, 4, 5]. Due to these

reasons robust ecoraces like Daba and Andhra local are commercially exploited in these regions by the farmers. A recent study, which primarily focused on post-cocoon parameter of Tasar silkworm Andhra local ecorace, it is found that it has low denier, high reelability and shell ratio, which are important commercial qualities of silk production, which opens an avenue to take up measures to overcome its weaknesses like low hatchability, irregular emergence, disease incidence, etc. [6].

*A. mylitta* Drury, **Daba TV** is a commercial and wild sericigenous insect, Daba ecorace is being used for commercial rearings for their amenability to human handling during rearings and seed production operations. Native of Maharasta and Telangana states. Exists in nearly 17 states of our country. Daba-TV is trivoltine having three crops, it undergoes diapause from February to June cocoons are grey coloured. It is found in tropical, moist deciduous forests [7]. It is polyphagous insect feeding on a host of food plants but primarily feeding on *Terminalia arjuna*, *Terminalia tomentosa* and *Shorea robusta*. The secondary food plants are *Terminalia paniculata* etc. The performance of Daba ecorace was mixed and comparatively inferior under commercial rearings than its in-situ habitat, which is

common exposition of ecoraces under ex-situ habitats [8]. The high genetic variability present within wild Daba population of *Antheraea mylitta* is of much importance for conservation as well as utilization in systematic breeding program [9].

**Backcrossing** is a well known and long established breeding plan where a character is introgressed from a domesticated or wild relative donor parent into the genomic background of a recurrent parent, which progress better with selection of genetically diverged parental breeds. The backcross breeding of silkworm using parents with preferred traits and selection in subsequent generations offer superior varieties [8].

Earlier reports [10] revealed that the genetically weak characters of this ecorace could be overcome if methodical breeding activities are undertaken probing into the binding capacities and combining abilities of *Antheraea mylitta*. Identification of several potential markers that contribute to develop genetic characteristics of silkworm population and reveal genetic divergence within low and high yielding strains, could have potential practical utility in prospective silkworm breeding program [11].

The present breeding programme, by exploiting the genetic proximity of the ecoraces of *A.mylitta*, viz., Andhra local

ecorace and Daba TV, envisages evolution of a viable cocoon crop of tasar silkworm, *Antheraea mylitta* (Andhra local ecorace) with better egg -laying behavior, uniform emergence, reduced larval and pupal mortality without losing their beneficial commercial characters also suggests methods to overcome its weaknesses and conserve the dwindling population. The selection of parental strains for a breeding program is based on economically desirable quantitative traits of the parental ecotypes. The economic parameters of hybrid populations reared on *T. arjuna* were also recorded in the present study.

## MATERIAL AND METHODS

### Collection of Tasar Silkworm *A. mylitta* cocoons for breeding

The cocoons of selected ecoraces of Tasar silkworm, *A. mylitta* D, (Andhra Local and Daba TV) were collected from various ecopockets and were maintained in well ventilated grainage chambers in the laboratory till the moth emergence. The date of emergence of each of the ecorace (male/female) was noted. From the data collected, it is observed that there is a chance of coupling between Andhra local and Daba TV ecoraces due to corresponding/synchronising dates of emergence, seasonal coincidence and physiological compatibility

towards breeding.

The total number of eggs, fertilized eggs of one Dfl in respect of parents as well as hybrids was considered as one replication and single shell weight, silk ratio were calculated with equal number of random cocoon samples. The observations recorded on seed and cocoon commercial characters like fecundity, egg fertility, shell weight and silk ratio were subjected for statistical analysis.

### Coupling Behaviour

On reaching the female moth, the male moth starts courtship behavior with raised antennae, fluttering wings around the female followed by mating. Mating lasts for 10-12 hr but it continues up to 24 hr of the next day if not disturbed. In copulation one male moth is enough for complete fertility of the female moth. Male moths were utilized for second time mating when there is shortage of female moths. The optimum temperature and relative humidity for moth emergence and coupling were recorded (**Table 2**).

### Egg Laying and Oviposition of Hybridized Female moth

Female moths were decoupled and kept in mud cups or nylon net bags (15 × 20 cm) and kept in dark condition at 22±2°C and 75±5 % R.H, (Ovi-position/egg laying device) for ovi-position upto 72 hrs. The number of eggs

laid by individual mother moth in three days (72 hrs.) was counted and recorded as fecundity. The average fecundity ranges from

185-230 eggs in both the crop seasons. The coupled moths detached at a slight mechanical disturbance (**Figure 3.a, b**).

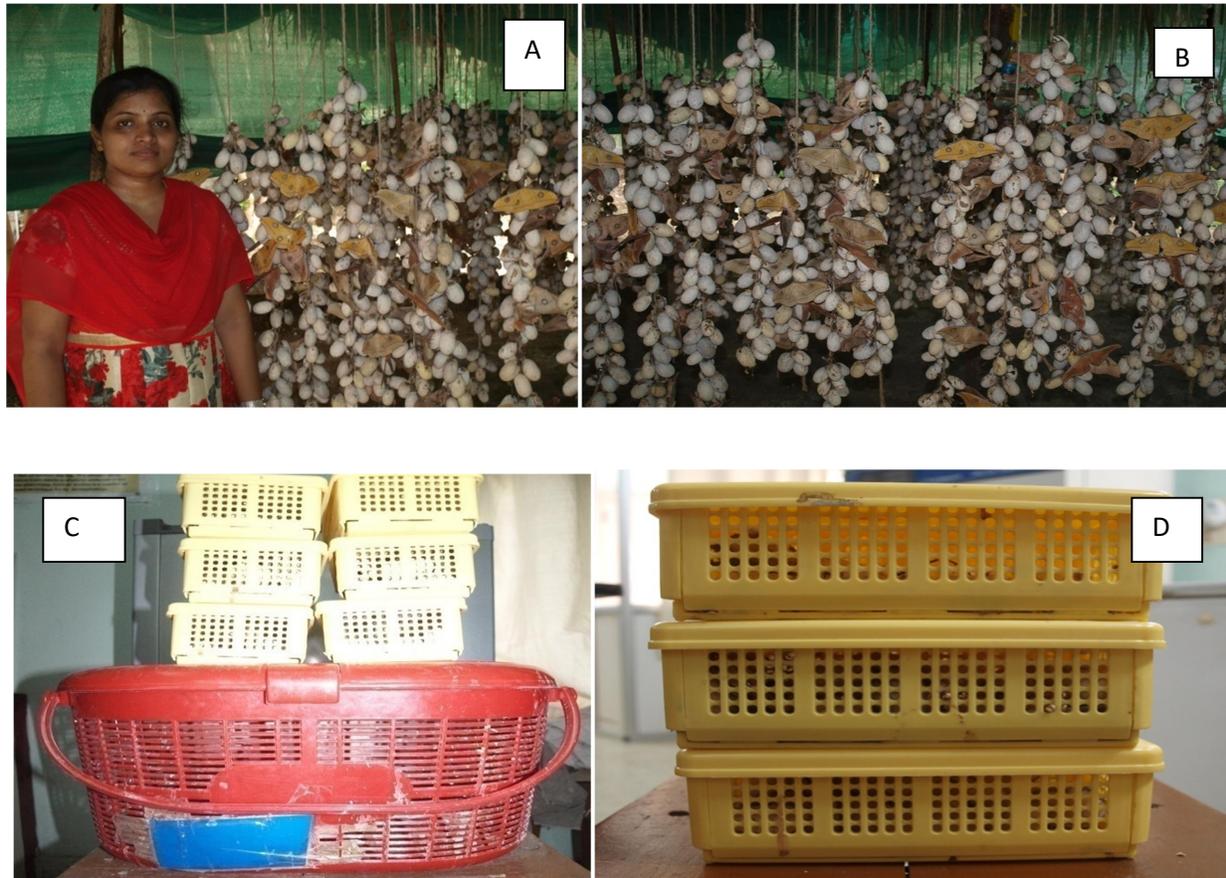


Figure 1: A. Collection of seed cocoons Telangana State Silk Board; B. Daba- TV Seed cocoons; C,D: Grainage activity of Tasar Silkworm, *A. mylitta* D, Andhra local and Daba- TV ecoraces (Seribiotech lab, KU)

### MOTH EMERGENCE PATTERN

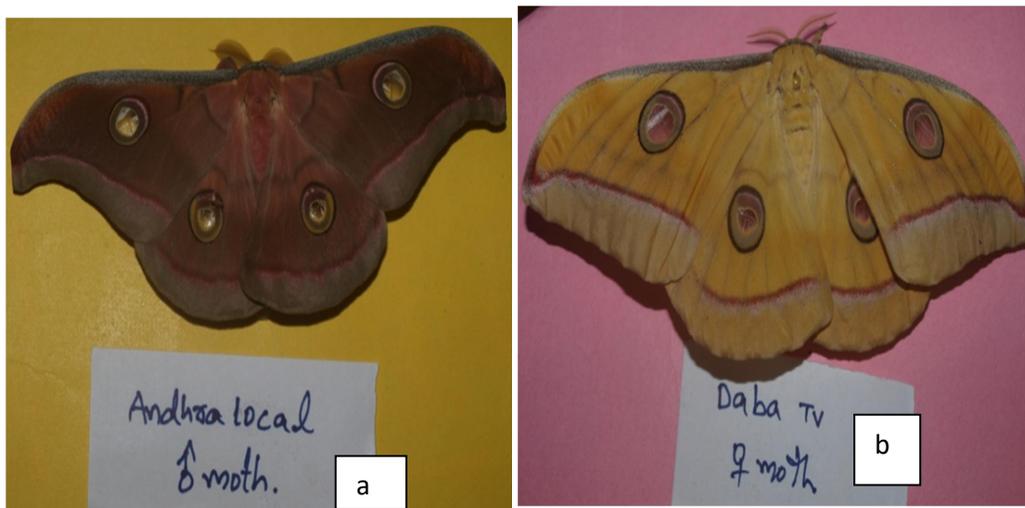


Figure 2: (a) Andhra local-Male; (b) Daba TV Female Moths of Tasar Silkworm, *A. mylitta*



Figure 3: (a) Moths Coupling (b) Egg laying (C) Oviposition of hybridized female moths of Tasar Silkworm, *A. mylitta*

### Egg washing

The eggs were washed to ensure removal of outer meconium (gummy coated) layer the

egg surface was sterilized to prevent disease contamination as eggs are one of the vital pre-requisites for successful rearing. The

occurrence of disease is prevented by using formalin (5%) and surface sterilized to prepare disease free layings- DFLs.

### Hatching performance

Larval hatching was recorded on the 7th or 8th day of incubation after date of coupling. Comparative hatching performance was calculated in percentage. Hatching percentage (brushing calculation) was calculated by using the formula Total number of larvae hatched / total no. of eggs  $\times$  100.

### Rearing breeding larvae of Tasar silkworm *A. mylitta* (Daba TV, Andhra local Ecoraces) Influence of Temperature

Larvae survive over wide range of temperature *i.e.*, from a of maximum 34° - 35°C and a minimum of 9- 10°C in rearing site. Larvae react to temperature, *i.e.*, above 30°C they become restless while when the temperature is below 20°C they become inactive. The temperature also determines larval life span. In the present study, the temperature was recorded with the help of laboratory thermometer. The average of lowest and highest values were taken and recorded instar-wise. The present investigation is taken up to study the influence of environmental conditions like optimum temperature during the rearing period were F1and F2 populations Instars wise are 29-31°C and 28-30°C respectively

(Table 2). During rearing, environmental fluctuations were seen, which sometimes involved unfavorable conditions like rain, storm or hail.

### Humidity

Larvae survive over wide range of humidity *i.e.*, a maximum of 90- 100% and a minimum of 30-40% in rearing site. Relative humidity also determines larval life span, *e.g.*, early instars need higher humidity of 75-85% while late instars require a lesser humidity of 60-70%. In the present study, the humidity was recorded with the help of lab hygrometer. During the rearing period were F1and F2 populations instars wise are 85-87% and 89-93% respectively (Table 2).

### Larval parameters of Length, weight, colour, size and shape of larvae

At the beginning of each instar, 5-10 healthy worms were selected at random from the rearing lot of Tasar Silkworm, *A. mylitta* (F1 and F2 Hybrid populations) rearing on *Terminalia arjuna* plantation at Kakatiya University, Warangal, Telangana, India for measurement of length and weight. These parameters are taken just after hatching for the first instar and just before moulting for second instar and daily for fourth and fifth instar. Larval weights were measured in an electronic balance of Citizen (Model: CG 203). The weights were measured for first

instar to fifth instar, the length of the larvae were measured in cm by using a graph paper. The larval lifespan was also recorded. The larval mortality was calculated by counting the number of worms lost due to various reasons during each instar (Table 4).

Plantation uniformly after one day of completion of 2nd moult, by cutting off branches with young worms with a help of secateurs, putting gently on sheet of polythene spread in a basket. Transfer the worms in basket to another plantation in rearing garden, one by one with the branch of the plants during early morning or evening cooler hours. Larvae should not be disturbed during moult/ spinning.

#### **Early age rearing (I to III instars) of F1 and F2 hybrid populations of Tasar Silkworm, *A. Mylitta*.**

Two DFLs (F1) and One DFL (F2) of the young age larvae (I to III instars) of F1 and F2 population were reared on *T. arjuna* plants maintained separately to avoid mixing of parental stocks. The young age larvae prefer tender leaves. It was observed that the young age worms crawl up to the tip of the branches and start eating tender leaves. The young age rearing was conducted in rearing

garden under cover of nylon net to provide protection to the young larvae from heavy rain, hail storm, pest and predators *etc.*, to minimize larval loss during early instars. (Figure 4. a, b, c, d), (Table 1).

#### **Late age rearing (IV and V instars) of F1 and F2 hybrid populations of Tasar Silkworm,**

##### ***A. mylitta* D.**

For the healthy growth of the larvae, semi-mature and mature leaves were provided to the 4th and 5th instars respectively. The mature larvae 1-2 days stop feeding and either rest on the food plant or come down to the tree trunk near the strips of plastic sheet tied around the tree trunk. The larvae remained there by raising their head upwards till evening and released their last excreta. Afterwards the larvae started searching suitable location for cocooning and pupation. At this stage, the mature larvae were picked up and kept in the bundle of dry twigs or in the bamboo made moutage for cocooning. The pupation was completed inside the (F1) cocoon within 9-10 days, (F2) cocoon within 10- 15 days and cocoon formation was completed (Figure 5 a, b, c, d, e).

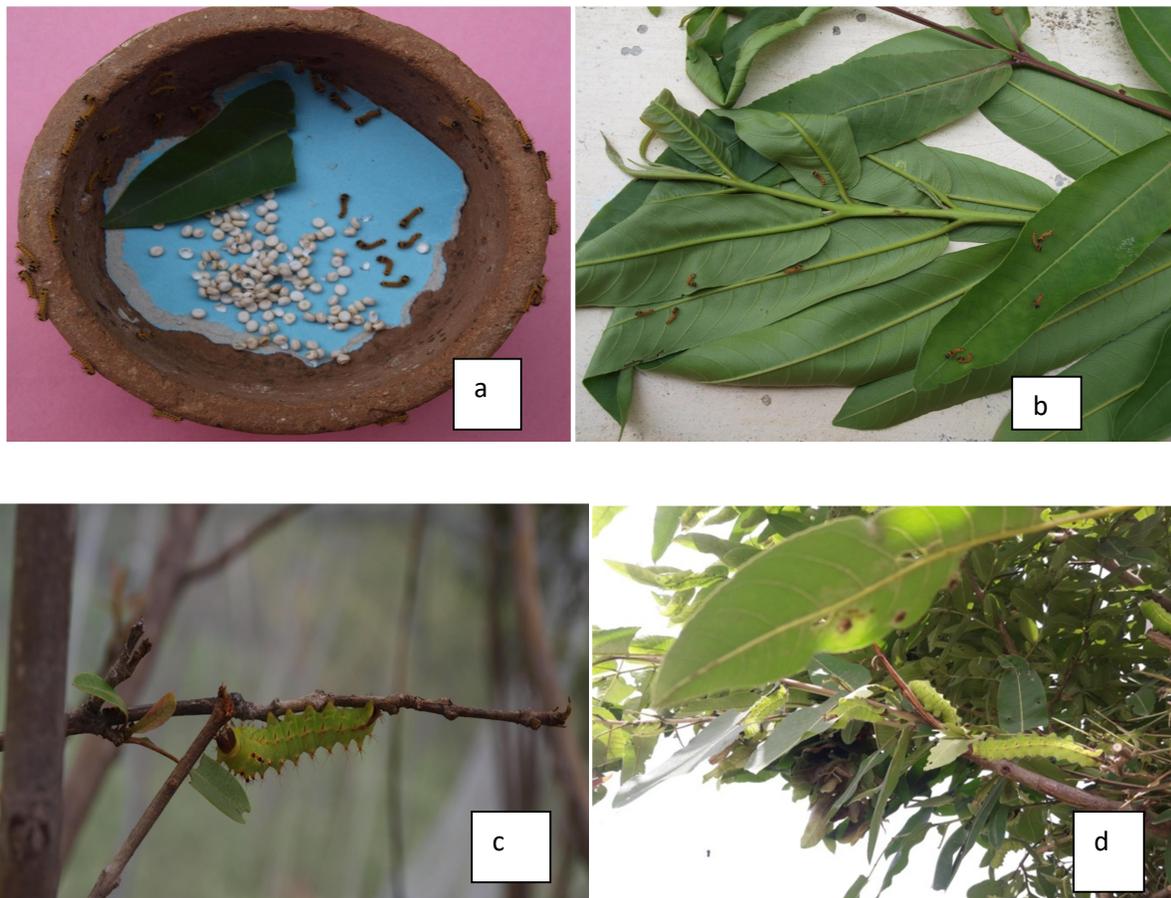


Figure 4: (a) Early age rearing newly hatched larvae (b) I instar larvae (c) II instar larvae (d) III instar larvae of F1 and F2 hybrid populations of Tasar Silkmoth, *A. mylitta* D

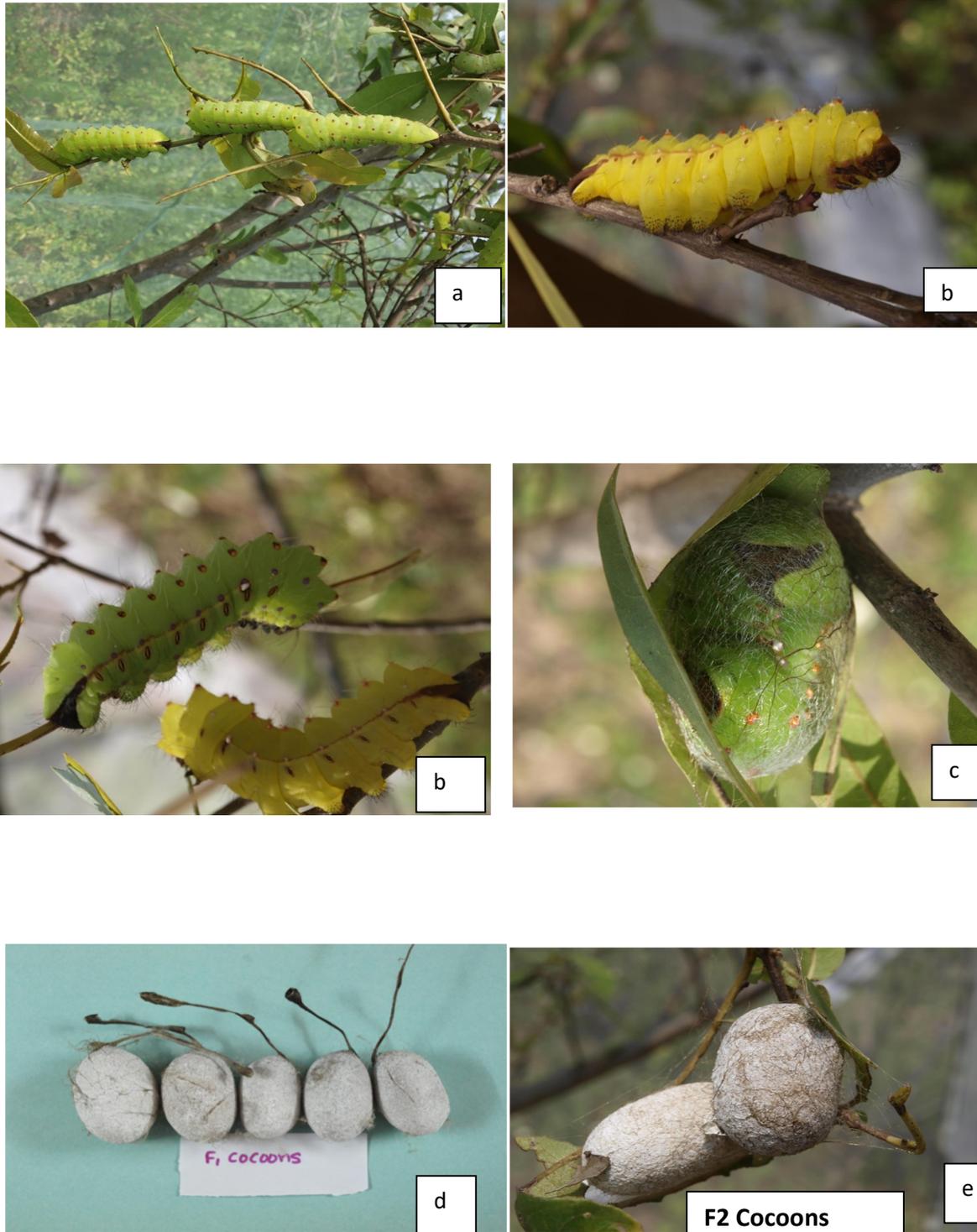


Figure 5: Late age rearing (a)IV instar (b) V instars (c) spinning larvae (d) F1 cocoons (e) F2 cocoons of hybrid populations of Tasar Silkworm, *A. mylitta* D

## RESULTS & DISCUSSION

The Instar wise average Temperature ( $^{\circ}\text{C}$ ) and its standard deviation of Tasar silkworm, *A. mylitta* (F1 and F2 Hybrid) were as follows. F1 Hybrid:  $31.00 \pm 1.08$  (S.D),  $30.00 \pm 1.47$  (S.D),  $30.31 \pm 0.75$  (S.D),  $31.38 \pm 1.52$  (S.D),  $29.52 \pm 0.81$  (S.D), and F2 Hybrid:  $28.58 \pm 0.94$  (S.D),  $29.06 \pm 1.19$  (S.D),  $29.66 \pm 0.87$  (S.D),  $29.76 \pm 0.96$  (S.D),  $30.90 \pm 1.07$  (S.D), for I, II, III, IV and V instars respectively. The instar-wise average Relative Humidity (%) and its standard deviation for F1 Hybrid were  $85.8 \pm 4.14$  (S.D),  $85.33 \pm 5.20$  (S.D),  $86.75 \pm 5.05$  (S.D),  $86.85 \pm 6.22$  (S.D) and  $87.2 \pm 7.15$  (S.D) and F2 Hybrid were  $89.6 \pm 3.28$  (S.D),  $91.57 \pm 2.93$  (S.D),  $91.85 \pm 0.69$  (S.D),  $92.14 \pm 0.69$  (S.D) and  $93.33 \pm 0.51$  (S.D) in I, II, III, IV and V instars respectively (**Table 2**).

In the present studies, the selection of parents was based on the synchronisation of emergence pattern of the Andhra local and Daba TV ecoraces of Tasar silkworm, *Antheraea mylitta* D (**Table 1**). The breeding trials was

done consecutively *i.e.*, F1 in the seed crop (July-August, first crop season) and F2 on commercial crop season (September October, second crop season) during which the temperature was  $28-31^{\circ}\text{C}$  and relative humidity was 88-93%. In an attempt to synchronise the moth emergence, the parental ecorace Andhra local, female was preserved in cool conditions, till the F1 male emerged during F2 Backcrossing, as it was showing the tendency of early emergence of 5-6 days, when compared to that of F1. The percentage of hatching was more in F1 than F2. *No. of male moths emerged is significantly greater than females in both F1 and F2*. The percentage mortality was greater in early instars *i.e.*, first to third instars due to diseases, pests and predators and a low mortality in fourth and fifth instars was seen in both F1 and F2 hybrid populations (**Table 3**).

The percent mortality has decreased from first to fifth instars, which is a significant observation. Though it was lesser in F2 when compared to F1 populations.

**Table 1: Date of emergence of parental and hybrid populations of tasar silkworm, *A.mylitta* D**

S. No.	Ecorace	Dates of Emergence	Dates of Coupling	Dates of Egg Laying	Total No. of Eggs	No. of eggs hatching
1	AL ♂	27-06-2017	27-06-2017	7-07-2017	192	185
2	DTV ♀	27-06-2017	27-06-2017			
3	F1 ♂	22-08-2017	25-08-2017	27-08-2017	165	140
4	AL ♀	22-08-2017	25-08-2017			

Table 2: Instar-wise Temperature (°C), Relative Humidity (%) of F1 and F2 hybrids of Tasar Silkworm *A. mylitta* D

Instar	Temperature (°C)		Relative Humidity (%)	
	F1	F2	F1	F2
I	31.00 ± 1.08	28.58 ± 0.94	85.8 ± 4.14	89.6 ± 3.28
II	30.00 ± 1.47	29.06 ± 1.19	85.33 ± 5.20	91.57 ± 2.93
III	30.31 ± 0.75	29.66 ± 0.87	86.75 ± 5.05	91.85 ± 0.69
IV	31.38 ± 1.52	29.76 ± 0.96	86.85 ± 6.22	92.14 ± 0.69
V	29.52 ± 0.81	30.90 ± 1.07	87.2 ± 7.15	93.33 0.51

Table 3: Rearing performance of F1 and F2 hybrids of Tasar silkworm, *A. mylitta* D (for 1 DFL = 200 eggs)

S. No.	Hybrid	No. of Dfls	No. of eggs	Mortality	% of Mortality
1	F1	1	185	90	48.64
2	F2	1	140	63	45

Table 4: Instar-wise Larval Period of F1 and F2 hybrids of Tasar Silkworm *A. mylitta*

Instar	Larval life Span		Mortality		Larval Weight (gm)		Larval Length (cm)	
	F1	F2	F1	F2	F1	F2	F1	F2
I	5	5	25	18	0.253 ± 0.16	0.312 ± 0.0	1.16 ± 0.32	1.17 ± 0.39
II	5	5	24	14	2.445 ± 0.36	2.526 ± 0.52	2.52 ± 0.17	2.76 ± 0.48
III	8	6	15	12	5.638 ± 0.94	5.415 ± 0.24	4.07 ± 0.4	4.425 ± 0.55
IV	9	11	14	10	9.348 ± 0.97	9.514 ± 1.26	6.16 ± 0.82	6.56 ± 1.11
V	15	16	12	09	26.43 ± 7.9	26.51 ± 7.52	10.14 ± 1.5	11.3 ± 1.26
Total	42	43	90	63				

- The larval life span was more or less the same in both F1 and F2
- The larval weight was more in F1 when compared to the F2 population
  - The larval length was greater in F2 than F1 in all the instars
  - The percent mortality has increased from F2 to F1 generation

The duration of development of first, second, third and fourth instars worms of F1 Hybrids took 5 days, 5 days, 8 days and 9 days respectively while the fifth instar took prolonged time (14-15 days) and F2 worms are took 5 days, 5 days, 6 days and 11 days respectively while the fifth instar took prolonged time {(15-16 days) (Table. 4)}.

In F1 generation it was observed that out of 200 (1dfls) taken, 185 eggs have been hatched, which is an important aspect of the present studies. The above results are in corroboration with the view that, for breeding, selection of the parent is the basic tool for further improved and standardized with backcross method, resulted in yielding better quality cocoons and cocoon yield in the commercial crop seasons. Silkworm hybrids have shown improved reeling performance [12] over pure races.

The present investigation, on breeding as one of the strategies for enhancing the productivity and quality of tasar raw silk, is aimed at improvement of Andhra local ecorace in order to bestow it with superior characters like survivability and fecundity of Daba TV in lines with preliminary studies [13].

Tasar culture is a forest based industry best suited to the economy and social structure of developing countries like India. Minimum investment is the most

significant feature of Tasar culture. State government should take most priority for raising and maintaining systematic Tasar host plantations, the state government of Telangana as already taken initiatives in this direction as Tasar Food Plants viz., *Terminalia arjuna* have been planted in its scheme of “Harithaharam” (Green Garlands).

The silkworm breeding is not only to enhancement of fecundity, continuance of quality cocoon production in variable climatic and geographic conditions leading to genetic improvement of traits either incorporating desirable genes and subsequently profitability of sericulture industry. In an attempt to overcome the problems faced by Andhra local ecorace, a comprehensive breeding program was suggested by earlier works [14, 15].

In the present breeding programme, which envisages evolution of not only a stable cocoon crop of tasar silkworm, *Antheraea mylitta* (Andhra local ecorace) with better egg - laying behaviour and uniform emergence but also at reducing larval and pupal mortality, leading to viable breed. The tasar silkworm, *Antheraea mylitta* D has wide genetic as well as phenotypic variability in its population; also a good material for the exploitation of heterosis, besides it has many constraints in silkworm hybridizations [16]. During

breeding, selection is the process of choosing the superior quality of parents, based on phenotypic traits, in the desired direction aiming for improvement in the genetic structure and productivity.

In a recent study based on genetic diversity, which revealed that, for the purposes of commercial exploitation and conservation, genetically divergent silkworm races can be used for developing hybrids with better silk productivity and adaptability [17].

The present investigation, on breeding as one of the strategies for enhancing the productivity and quality of tasar raw silk, is aimed at improvement of Andhra local ecorace in order to bestow it with superior characters like survivability and fecundity of Daba TV in lines with preliminary studies [13] further improved and standardized with backcross method, resulted in yielding better quality cocoons and cocoon yield in the commercial crop seasons.

#### CONCLUSION

The tropical Tasar silkworm, *A. mylitta*, Andhra local ecorace, embodied with unique phenotypic traits such as fecundity, voltinism, cocoon weight, silk ratio etc., is on the brink of extinction. It is the *need-of-the-hour* to develop hybrids by appropriate breeding techniques and contribute in its conservation.

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#### Competing interests

The authors declare that they have no competing interests.

#### Consent for publication

Not applicable.

#### Ethics approval and consent to participate

Not applicable

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