

EFFECT OF PHOTOPERIODIC CONDITIONS ON THE LARVAE AND COCOON OF TASAR SILKWORM (*Antheraea proylei* J.)

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Abstract

Environmental factors play critical role in the development and survival of insects and sericulture is the industry which totally rely on the silkworm. Photoperiodic induction acts as a sensitive index in Lepidopterans as well as in other insect groups. The present investigation was designed to study the impact of different environmental conditions viz. temperature, relative humidity and photoperiod on the laboratory cultured species of *Antheraea proylei* J. fed on *Quercus serrata* leaves. In an experimentation, equal (100 worms in each condition) number of larvae were divided into 3 groups: 1st group where larvae/cocoon were placed under 24 hour of light period, 2nd was under 24 hour dark and the 3rd was under normal conditions of 13 hrs Light:11 hrs Dark period with maintained temperature between 23⁰C to 26⁰C and humidity between 75-80%. It was observed that the body length, weight and life span varied in different photoperiodic conditions. The results showed that the preferable temperature range of 23-26 ⁰C and relative humidity of 75 to 80% was desired for the growth and survival of the larvae and cocoon with 24 hour light and 24 hour dark period of light. Similarly relative humidity of 75 to 80% was the desired range for rearing and survival performances which influences its productivity and quality. 24 hour dark condition has highest survival rate of 75% followed by normal condition 72% and the light condition of 24%. The light condition worm had shown lowest survival rate as they are found to be infected with a disease named Nucleopolyhedrosis virus and Baculovirus. The present investigation revealed that at optimum conditions of temperature, humidity and photoperiodism showed better performances as compared to light conditions.

Keywords: *Antheraea proylei* J., Lepidoptera, Photoperiod, Sericulture, Silkworm.

Introduction

Development of an insect requires suitable environmental condition like photoperiod, temperature and humidity. Number of scientist studied the sensitivity of silk worm against photoperiod and temperature. Photoperiodism act as regulator for various physiological processes of insect¹. It induced fluctuations in growth pattern seasonal morphological development and diapause.

Temperature also showed different development patterns during embryonic/larval stages. Sericulture is considered to an important agro based industries in India and is a second rank holder in silk production throughout the world. The principal source of wild silk/tasar silk which is also known as “golden fiber” is the *Antheraea* species belonging to family saturniidae. Being the source livelihood of poor people, different host plants are reared which helped in producing tasar silk of economic importance. The non tasar silk producing species are *Antheraea mylitta* (tasar of India), *Antheraea yamamai* (tasar of Japan), *Antheraea pernyi* (tasar of china) which fed on host plant *Tamerminalia shoreobusta*, *Tamerminalia arjuna* and *Tamerminalia tomentosa* during seed crop session of July- August and commercial crop Indian sericulture developed the stain *Antheraea proylei jolly* by crossing *Antheraea roylei* with a *Antheraea pernyi* of oak feeding silk worm. This new silk worm was also named as oak tasar or temperate tasar of economic importance². This species of oak tasar was established on along sub Himalayan belt rich in oak forest. But due to problems like moth emergence and oak foliation in spring, the oak tasar sericulture become restricted to Jammu and Kashmir, Uttarakhand and Manipur. Reports on the effect of photoperiodism and different temperature condition on *Antheraea proylei* J. was lacking pertaining to Himachal Pradesh. In order to fulfill the gap, present investigation was done with an attempt to study the effect of photoperiod on larval/cocoon characters of *Antheraea proylei*.

Materials & Methods

Disease free larvae of *Antheraea proylei* J. were arranged from Central Tasar Research and Training Institute, Extension Centre Chauntra, District Mandi (Himachal Pradesh) during the month of April to June 2022 in order to study the impact of different photoperiodic conditions on larvae as well as cocoon of Oak Tasar silkworm²⁻⁶. The appropriate temperature and relative humidity were maintained at $23 \pm 3^{\circ}\text{C}$ and $75 \pm 5\%$ up to adult stage. In the present study, three different conditions of 24 hour (complete light), 24 hour (complete dark) and normal outdoor condition (13L: 11D) were maintained. Rearing was done as per recommendation of Yadav and Jadhav (2014). Before rearing the cleaning, washing and disinfection of the apparatus with 2% formalin was done completely followed the guidelines of Krishnaswami.

Larvae formation: The larvae of *Antheraea proylei* J. were released on the two tender shoots (each shoot carries 100 worms) of the host plant (*Quercus serrata*) with the help of soft brush. The shoots bearing larvae were placed at two different photoperiodic conditions with 24 hour light and 24 hour dark cycles. The remaining 100 worms were released on the host plant in normal outdoor conditions with 13 hour light:11 hour dark cycles. The proper maintenance of temperature ($23 \pm 3^{\circ}\text{C}$) and humidity ($75 \pm 5\%$) conditions were maintained throughout the experimentation. The old shoots were replaced by fresh shoots at the interval of 12 hours until they reached the last larval stage (5th instar stage)⁷⁻⁹.

Cocoon formation: When the fifth instar was ready to make a cocoon, then at its first stage a scaffold made of silk strands was made between the leaves of feeding plant. A stalk or peduncle was produced to secure the cocoon to the leaf petiole or the twig. During cocoon formation, the

larvae were not disturbed so that they continue their spinning process. On distraction, they forgot to spin and the chances of their survival were decreased¹⁰.

Disease test: For the identification of the particular disease, disease test was performed for identification of the disease. With the help of mortar and pestle, the mixture of potassium hydroxide and water (2-5 gm of KOH and 10 ml of water in it) was prepared and used to detect the disease in larvae of silkworm under light microscope, compound microscope to identify the particular disease¹¹⁻¹⁴.

Results

Results obtained in relation to laboratory culture of *Antheraea proylei* J. at three different photoperiodic conditions viz. light, dark and normal outdoor conditions with respect to temperature and relative humidity.

Light conditions:- The data presented in table (1) indicates the length, width, weight, and color of the larvae in each instar stage. The results also indicated the effect of relative humidity, temperature and the conditions provided to worms.

INSTARS	LENGTH	WIDTH	WEIGHT	COLOUR
Ist instar	0.6cm	0.1cm	0.07gm	Blackish
IInd instar	1.8cm	0.3cm	0.15gm	Yellowish
IIIrd instar	3cm	0.8cm	1.40gm	Greenish
IVth instar	5cm	1.2cm	7.1gm	Greenish
Vth instar	6.5cm	1.7cm	23gm	Greenish

Table 1: The length, width, weight, and color of worm in each instar of light condition

When compared to the other conditions, the length weight, number of cocoon, and survival rate of light condition worms were the lowest. A sudden increase in the death rate was observed during 5th instar stage. To identify the reason for their deaths, the detailed examination under a microscope showed that the worms in light conditions were infected with a disease named Nucleopolyhydrosis virus and its other form Baculovirus.

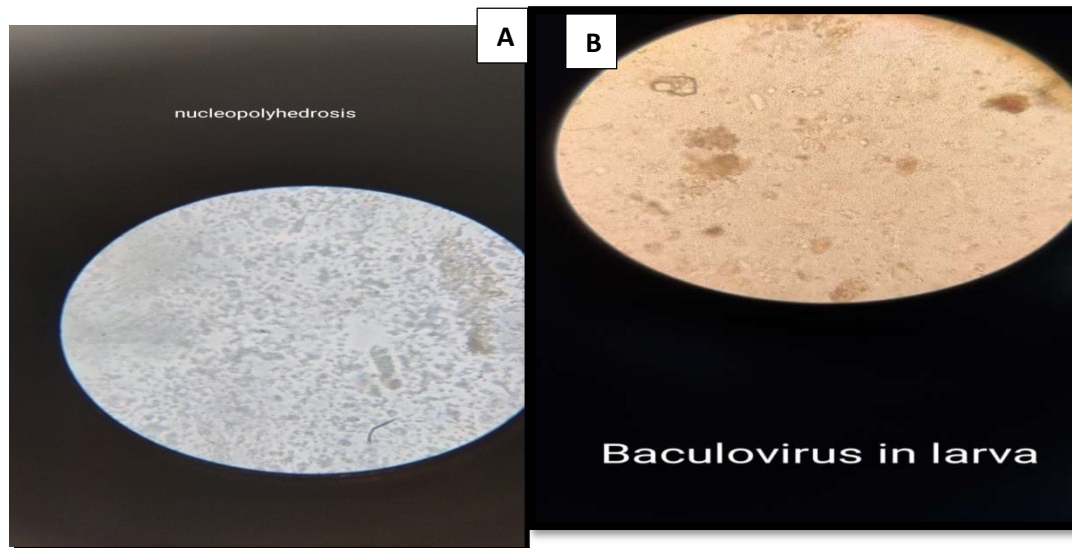


Fig 1: (A) Nucleopolyhedrosis virus and (B) Baculovirus in diseased larva (5th instar)

The mean life cycle of light condition worms was 70 which is longer as compared to dark and normal condition worms. The survival rate in this condition was (24%) (Fig. 2).

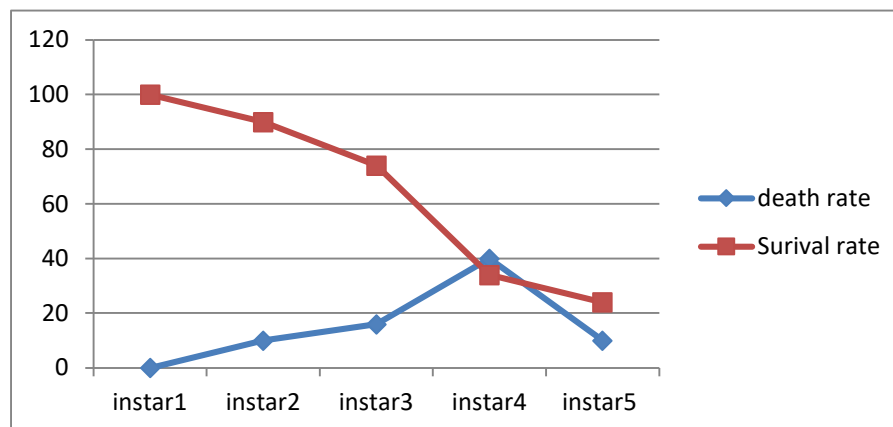


Fig 2: Graph showing the survival and death rate of light condition worms

Cocoon:- Light condition cocoons were less in number because they also got infected with disease. Only 24 cocoons were formed out of 34 larvae. The weight of cocoons was 5.34 ± 2 which was less as compared to cocoon of other conditions. The cocoon formation was 24% in light conditions (fig 3).



Fig 3: Larva died during cocoon formation

Dark condition:- Body length of dark condition worms was significantly faster under DD (Dark) than under LL (light) conditions. The cocoon formation started 2 days earlier under DD conditions than the LL conditions but time of formation was similar in both the treatments. The data presented in the table (2) indicated the length, width, weight and color of 24 hr dark condition worms. The life span of dark condition worms was 65 which is lesser as compared to the other conditions.

INSTARS	LENGTH	WIDTH	WEIGHT	COLOUR
Ist instar	0.6 cm	0.1cm	0.07 gm	Blackish
IInd instar	2cm	0.5cm	0.17gm	Yellowish
IIIrd instar	3.8cm	1 cm	1.6gm	Greenish
IV instar	5.9cm	1.4cm	9gm	Greenish
Vth instar	9cm	1.9cm	28gm	Greenish

Table 2: Length, width, weight and color of worm in each instar of dark condition.

The survival rate in this condition was higher (75%) than the other condition because they were protected from any disease and predator.

Cocoon:- The quantity of cocoon was higher as compared to other conditions. The cocoons were healthy and having weight 10.09 ± 2 gm (fig 4.6). The cocoon formation was 75% in dark conditions¹⁵.



FIG 4: Healthy cocoon of dark condition

Normal condition:- In normal conditions, larvae were exposed to 13 hour light and 11 hour dark photoperiodic cycle. Worms roam freely in the trees and feed their own. They grew under natural conditions and exposed to various environmental conditions. Body length increased faster than light condition worms but slower than the dark condition worms (table 3). Cocoon formation started one day earlier than the light condition worms. The survival rate in normal condition was 73% which was higher than the 24 hour light condition but lesser than the dark condition.

INSTARS	LENGTH	WIDTH	WEIGHT	COLOUR
Ist instar	0.6cm	0.1cm	0.07gm	Blackish
IInd instar	1.9cm	0.4cm	0.16gm	Yellowish
IIIth instar	3.4cm	0.9cm	1.5gm	Greenish
IVth instar	5.4cm	1.3cm	8gm	Greenish
Vth instar	7cm	1.8cm	25gm	Greenish

Table 3: Length, width, weight and color of worm in each instar of normal condition

Cocoon:- The larvae were healthy in normal condition but they were at risk of attack from predator and pest due to which only some larvae reach the cocoon formation which effected its quantity and quality. The weight of healthy cocoon is 12.9 ± 2 gm. which was higher than the other conditions. The cocoon formation was 73% in normal conditions.

Discussion:-

Our data revealed interesting patterns and specificity in uncharted territory. The formation of Tasar silk cocoons occurs rather autonomously on oak trees. The effect of photoperiod on the larvae of Oak Tasar silkworm (*Antheraea proylei* J.) was discussed below:-

Impact of Temperature:- Since temperature affects the pace of insect metabolism, it is thought to be primary regulator of insect development. The climatological elements have an impact on keeping the silkworm's economic features in top condition. The hatching percentage of eggs, longevity, growth and development all have been directly co-related with environmental conditions. Variations in the climatological variables cause out breaks of illness in silkworm.

According to Jolly, the ideal conditions for Tasar culture were a temperature range of 26°C to 30°C and a relative humidity of 80% to 85%. The experiment's findings have led us to assume that when the ideal ecological conditions are offered in a lab setting for the larval growth of Tasar worms, their qualitative and quantitative characteristic rise which was in accordance to our experimental conditions.

Silkworm is very delicate insect that feeds on variety of leaves (mulberry, oak, arjuna, castor etc.) and cannot survive in conditions of high temperature precipitation, humidity changes. Due to the fact that silkworms are cold blooded creatures, temperature directly affects a number of their physiological functions. Temperature is one of the abiotic elements that significantly affect silkworm productivity and growth. Early instar larvae are resistant to high temperatures and aid in boosting cocoon character and survival rates at their best. Temperature in late instar accelerates larval growth and shortens the larval stage. In contrast, growth might be sluggish and the larval stage lasted longer in colder climates. According to several researchers, the ideal temperature for optimum productivity is between 23°C – 27°C , while the ideal temperature for regular silkworm growth is between 22 – 27°C temperature below the 20°C and above 30°C have a direct impact on the physiology of silkworms, especially in the early instars, making them more prone to illness which further supports our investigation where temperature in range of 23°C to 26°C showed good result in the form, length, width, weight and cocoon quantity.

The growth and development of the poikilothermic insects was directly impacted by several environmental conditions. Understanding how insects adapt to their surroundings made easier by their consumption and usage of food. Through a variety of factors, food consumption and utilization directly affects the worm. The atmosphere's temperature and humidity levels at the time of rearing are the most crucial ones among them. Additionally, humidity has a direct impact on the

silkworm's physiological processes. The silk conversion rate reduces when the leaf temperature rose to 30 °C and fluctuations in humidity from 70-80% were reported. Studies on the relationship between food consumption, digestion, and body weight gain in silkworm showed that feed conversion efficiency indicators were higher under restricted feeding levels. It was established that the water content of the feed has a direct relationship with the ingestion, digestibility, and efficiency of conversion of ingested as well as digested food which supports our investigation where humidity 70-80% showed good result in length, weight and cocoon formation.

Mariswamy discovered that the temperature and humidity both plays a vital role in the development of disease. According to Anonymous the slight fluctuation in temperature during different stage of larvae development was more favourable for growth and development of pathogen. Similarly, in our investigation it has been observed that in 24 hour light conditions worms were infected with disease and caused pronounced effect on sericulture.

Impact of photoperiod on Oak Tasar silkworm:- According to Iqbal *et al.* (2008), the larvae kept in 24 hours light and dark provided with temperature $25 \pm 5^{\circ}\text{C}$ and humidity $70 \pm 10\%$ showed that dark condition expressed better result than the larvae in 24 hours light condition and normal condition in respect of length and weight of larvae which were in concordant to present investigation. Tobeed *et al.* (2008) worked on *Bombyx mori* where they found that the mean life cycle of *B. mori* was 62 days in dark and 68 days in light. Anonymous mentioned that the total life span of *Philosamia ricini* in dark and light condition was 53 and 74 respectively which were significantly different as compared to 65 in normal cycle. Similarly our result also revealed the life span of *Antheraea proylei* J. in dark was 65 days, light 70 days and in normal 68 days respectively which supports the above findings. According to Rahmathulla, silkworms have a propensity to crawl in the direction of low light due to their photosensitivity. Silkworms that were raised in constant light experienced a growth delay. Additionally, it causes pentamoult and reduces the weight of the larvae and the cocoon. In contrast, the larvae which were raised in total darkness and strong light experiences uneven growth and moulting. Larval duration was often longer during the light phase as compared to the dark phase. Similar results were observed in our experimentation where the larval growth period was more in light condition as compared to dark condition. In a study by Yadav and Jadhav, effectiveness of *Philosomia ricini* larvae maintained in normal, dark and light for 24 hours for ideal temperature of $25 \pm 2^{\circ}\text{C}$ and relative humidity of $75 \pm 5\%$. When compared to normal condition, larvae kept in dark setting displayed that they were noticeably better than those kept in light. Constant darkness did not affect the diurnal fluctuation in activity which favours our experimental work. discovered that the 24 hour dark condition (T3) showed heavier cocoon weight than the control normal condition (T1) followed by 24 hour light condition (T2) which supports our investigation.

Conclusion:-

For proper development and growth of silkworm larvae the production of host plant for particular host plant species of silkworm that provided leaves on which the worms feed, is important process

in sericulture industry. Also, effect of light and darkness plays a significant role in the development of silkworm larvae. The photoperiodic regimes has insignificant role in larval growth and other factors in *Antheraea pernyi* J. which created a need of hour in sericulture industry.

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Reference:-

1. Endo K, Kamata Y. 1985. Hormonal control of seasonal- morph determination in the small cooper butterfly, *Lycaena phlaeasdaimeo* Seitz. J Insect Physiol. 31:701-706,1985.
2. Endo K, Murakami Y. 1989. Photoperiodic control of the determination of three different seasonal phenomena of the swallowtail butterfly *Papilio xuthus* L. Zoolog Sci. 2:755-760
3. Khan, M.M. (2014). Effects of temperature and R.H. % on the commercial characters of silkworm (*Bombyx mori* L) cocoons in Anantapuramu district of A.P., India. Research Journal of Agriculture and Forestry Sciences. 2(11); 1-3,2014.
4. Yadav, V. and Jadhav, N. Impact of light and dark exposure on life cycle duration (days) of Eri silkworm, *Philosamia ricini* L. (Saturniidae : Lepidoptera). *Environment Conservation Journal*. 15(3); 81-83,2014.
5. Krishnaswami, S.J., Narsimhanna., Suryanarayan, S.K. and Kumaraj, S.. Manual of sericulture, silkworm rearing. FAO Agri. Services Bull., 15(2): 1-131,1973.
6. Renthlei, Z., Borah, B.K. and Trivedi, A.K. Photoperiod induced developmental effects on silkworm, (*Bombyx mori*). *Biological Rhythm Research*. 48 (1); 121-128,2016.
7. Yokoyama,T., Saita, S., Shimoda, M., Kobayashi, M., Mizoguchi, A. and Shiomi, K. Comparisons in temperature and photoperiodic dependent diapause induction between domestic and wild mulberry silkworms. *Scientific Reports*. 11, Article number (11): 8052,2021.
8. Kobayashi, The effects of photoperiod on the induction of egg diapause of Tropical races of the domestic silkworm, *Bombyx mori* and the wild silkworm *Bombyx mandarina*. Japan Agricultural Research Quarterly 23(3), 202-205,1990.
9. Peigler, R.S.Diverse Evidence that *Antheraea pernyi* (Lepidoptera : Saturniidae) is centerely of Sericultue Origin. Tropical Lepidoptera Research, 93-99,2012.
10. Iqbal, T., Inayatullah. M., Sadozai. A., Khan. I. A.Effect of light and dark exposure on the different parameters of silkworm *Bombyx mori*. (Bombycidae : Lepidoptera). Sarhad J. Agric.(24): 1, 2008.
11. Adams, J.R. AND McClintock, J.T. Nuclearpolyhedrosis virus of insects. In Atlas of invertebrate virus, 87-181 edited by J.R. Adams & J.R. Bonami.CRC press. Boca Raton, USA,1991.
12. Anonymous.The Textbook of Tropical Sericulture. Japanese Overseas Cooperation Volunteers, Tokyo, Japan, 458-459,1975.



13. Mariswamy, N., Studies on Pebrine disease of Muga and Eri silkworm. Annual Report, Central Muga and Eri Research Station, Titakar (Assam) for 1967-77, 1977.
14. Ramathulla, V.K., Management of climatic factors during silkworm rearing. The Textile Industry and Trade Journal, 25-26, 1999.
15. Deepali, Sharma M, kumar A, Kumar A, et.al, Studies on in-vitro evaluation of antimicrobial activity of silkworm cocoon extracts, Uttar Pradesh journal of zoology, 43(2): 43-49, 2022.