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Comparative Study Of Mulberry Silkworm (*Bombyx Mori L.*) Rearing In Parsendi (Chaki) Block Region Of Sitapur District And *In-Vitro* Studies In Lucknow Region.

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ABSTRACT-

Sericulture is considered agriculture and creates employment mainly in rural areas; hence, it does not need an explanation for its role and importance. Silk is the cultural tradition of India and the sericulture industry, wherein the distribution of currency is from rich to poor. It is providing nonstop profits to farmers as it is employment and income creating income industry, it is so entitled as the "*Kalpvriksha*" or "*Kamdhenu*" of the poor farmers (*B.K et.al*.,1994). In this study, two different eco-regions, the first in Parsendi and the second in Lucknow, observed food habits, length, width, and different larval stages of mulberry larvae and analysed mulberry silk cocoon length, cocoon weight, and cocoon width at different sites. We observed the life cycle of mulberry silk worm larvae at both sites. We find the larval length (Tech) and cocoon weight developing better in Parsendi sites than in Lucknow region. The life cycle of the mulberry silk moth in the Parsendi region takes 23 days, and that of the Lucknow region takes 25 days. We observe that in the Parsendi region, mulberry silk worm rearing is more stable than in the Lucknow region.

Keywords -*silk worm, sericulture, agriculture, bombyx morri***.**

INTRODUCTION:

Among various Agro-based industries in India, sericulture has its own significance. Sericulture is the art of rearing silkworms for commercial purposes. Mulberry leaves are used as silkworm feed, and they are also used for a variety of other purposes (Ghose *et.al*.,2017). Silk is the cultural tradition of India and the sericulture industry, wherein the distribution of currency is from rich to poor. (Vanitha C. *et al.,*2019). The Bombyx *mori* species of silkworm is most widely used in the commercial silk industries, and it is studied intensively by entomologists. It is providing nonstop profits to farmers as it is employment and income creating income industry, it is so entitled as the "*Kalpvriksha*" or "*Kamdhenu*" of the poor farmers (Sharnagat *et al*.,1994). India holds the third-highest ranking in the world, after China and Japan, as far as the production of mulberry silk is studied. In India, mulberry silk is mainly produced in Karnataka, Tamil Nadu, and Odisha, besides Maharashtra, West Bengal, Andhra Pradesh, and Uttar Pradesh. The mulberry silkworm is highly subtle, therefore unable to withstand the natural variation in humidity and temperature (Rahmathulla, 2012). suitable bivoltine races evolved were tested in the field for mid-February and mid-march, to mid-august and mid-September of Uttar Pradesh, India (A. Siddiqui, Singh *et al*., 2005). A lepidopteran model, *Bombyx mori*, of the family Bombycidae, has a rich repertoire of genetic information on mutation, thus affecting behaviour, morphology, and development. Silk can be obtained from the cocoon of the mulberry silk moth. Mulberry silkworms are

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monophagous, i.e., they feed only on mulberry leaves (*Morus alba* L.). Fibroin brins are conglutinated by sericin binder, which forms the silk fibres of the cocoon of the silk moth. Silk fibroin provides stiffness and strength due to its semi-crystalline structure and consists of a natural fibroin protein. Sericin, an amorphous protein polymer, acts as an adhesive to integrate the silk fibres and thus maintain the structural integrity of the fibres and cocoon (Ude *et al.*, 2014). Fibroin (silk filament) and sericin (silk gum) formed a multilayer composite material called a cocoon. Silk spinning is a natural selection process and forms a cocoon. The posterior region of the silkworm's gland produces aqueous solution, or silk. The silk spinning and developing into a cocoon is a natural selection process. The posterior region of the silkworm's gland produces an aqueous solution, known as silk, which is released through two spinnerets. The silk is a result of coagulated aqueous solution with silk gum (sericin) and coated and fastened together to form bave (twin filaments). Bave wraps around the silkworm body by gyrating its head, circular bending, and stretching its body to produce a compact cocoon in a programmed manner (Zhang *et al.,* 2013). Depending upon environmental conditions, the properties of the spun fibre, such as weight, thickness, color, and stiffness, vary through the silkworm's behaviour (i.e., processing) as well as through direct interaction with the material itself. At low temperatures, the rate of spinning is slow, while silkworms spin faster at high temperatures and fail to spin at lower temperatures. (Offord *et al.,* 2016). In the present study, two different eco-regions, Parsendi and Lucknow, observed food habits, length, width, and different larval stages of mulberry larvae and analyzed mulberry silk cocoon length, cocoon weight, and cocoon width at different sites. We observed the life cycle of mulberry silk worm larvae at both sites. In the year 2018-2019, the percentage production of four varieties of silk in India was as follows: Mulberry silk accounts for 71.50% (25,213 MT), Eri 19.40% (6839 MT), and Muga 0.66% (232 MT) of the provisional. Mulberry silk has contributed a huge part of the total raw silk production of 35,261 MT, making it the second largest producer next only to China. Tasar, Eri, and Muga have contributed 3,268 MT, 5,637 MT, and 170 MT, respectively. (Siddhapara & Surani, 2018).

Material and methods

Location of Rearing

Mulberry silk worm seeds and newly hatched larvae were collected from the Chaki silk farm, Parsendi, located in the district of Sitapur, Uttar Pradesh. An experiment was conducted during the first rearing crop (spring) season of 2020 at the Chaki Silk Farm, Parsendi, Sitapur, and lab rearing at the Department of Zoology, University of Lucknow, located at 27.5919° N, 80.8399° EN latitude, 26.7863° N, 80.8987° EN latitude, and E longitude, at an altitude of metres above MSL. In this region during the growing season, the average maximum and minimum were 28.4 and 23.5°C respectively. The annual precipitation data of the experimental site was found in the "Customized Rainfall Information System (CRIS)' of the India Meteorological Department (IMD, 2019). The geographical co-ordinates were also recorded at the collection site with the Geographical Positioning System (GPS).

2-Collection and rearing methods

Collection, preservation and identification:

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We collected disease-free laying eggs (B. mori) from Chaki Silk Farm, Parsendi, located in district Sitapur, Uttar Pradesh. by using various strategies (Gophane *et al*., 2017).

The silkworm larvae were carried from the Parsendi region using brush and collected in a soft plastic box, then brought to the laboratory in the Department of Zoology, University of Lucknow. Krishnaswami (1978) suggested a rearing method, that has been applied for rearing the silkworms. For rearing plastic trays of 36 cm length and 26 cm width were used in the laboratory, and to avoid moisture, the trays were covered with paraffin sheets. Cleaning of the rearing space by spreading lime and potassium permanganate to keep rearing enemies (ants, houseflies) away was done.

Newly hatched larvae were transferred into plastic trays using the shelf rearing method (Assistant, 2021) and fed with chopped growing mulberry twigs of approximately 0.5 to 1.0 sq. cm size, which were sprinkled over the newly hatched larvae, which crawled on the leaves and started feeding from the cut edges. The mulberry leaves were transferred into the cleaned rearing trays and spread over the larvae using feathers; chopsticks were used for detaching the larvae. For feeding the young larvae, regulated feed was provided, as was cleaning of the space, food spacing, and quantity of chopped mulberry leaves as per their feeding requirements. Chopped mulberry leaves were given to the larvae three times a day, at 9:00 a.m., 14:00 p.m., and 18:00 p.m., respectively. The second, third, fourth, and fifth instar larvae were fed with leaves from the second, third, fourth, and fifth rows of leaves from the top of the mulberry plant, respectively. During the moulting period, no disturbance was done to the larvae, and no feed was provided. Moulting completes in four stages: the first stage completes in 20 hours, the second in 24 hours, the third in 24 hours, and the fourth in 24 hours. The same process of rearing was performed at the Chaki silk farm at Parsendi. The quantity of food was increased as per the growth of larvae at both places. Larvae had shown a digesta-excreta relationship based on the dry or wet leaves of mulberry. Dry leaves were consumed more than moistened leaves and produced more excreta too (Alipanah *et al.,* 2020). The period between the hatching of the egg and the pre-pupa stage is termed the "larval period," whereas in the pre-pupal period, larvae undergo moulting to form pupae, and the emergence of adults from pupae is known as the "pupal period."

The length, width, and weight of cocoons were measured using a digital vernier calliper and recorded as 31.2 mm, &17.9 mm, and 0.38 g, respectively, at Chaki silkworm farm, Parsendi. The measurements of the cocoon using vernier calliper at our lab in Lucknow were 24.4 mm in length, 10.8 mm in width, and 0.28 g in weight, and they are white in colour. The emergence of adults from pupa was observed for their colour and longevity. Pupa length was also recorded, and adults after emergence were observed for their colour and longevity. The adult male and female moths can be easily differentiated on the basis of their shape and abdomen. The adult female's fecundity can be measured based on her egg-laying capacity or the number of eggs she lays during her life span.

Precautions taken during rearing silkworms

The silkworm larvae are prone to various diseases, so various precautions were taken during the rearing of silkworm larvae. Some important measures are the following:

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A disinfectant was used to sanitise the rearing room surroundings and rearing beds to prevent an outbreak of disease in newly hatched larvae and each instar larva in sericulture.

The silkworm larvae are prone to various diseases, so various precautions were taken during the rearing of silkworm larvae. Some important measures are the following: Rearing equipment and devices were sterilized and cleaned to maintain the appropriate rearing environment and sanitary conditions.

Washing hands and changing shoes were done before entering the rearing room to prevent the introduction of disease-causing bacteria. (Bajpai, 2015).

Mostly avoided touching with hand to food plants and silkworm. (Bambaniya *et.al* 2017).

Results & discussion

In the experiment performed at the two different environment the silk moth has shown much variance in the physical appearance as well as in the development and growth stages of the life cycle of mulberry silk. In our experiment we have drawn some inferences: -

Egg –It was observed that the fresh eggs laid by females were oval, ellipsoidal, with a slight dig in the centre of the egg at the dorsal and ventral sides, flat in shape, dull white in colour and smooth chorion at the time of oviposition. Later, before hatching, the chorion turns black in colour.

First instar: The freshly hatched larva (neonate) was dark brown or dull yellow. Under lab conditions at Lucknow, length and breadth vary from 10.9 mm to 15.5 mm, 1.7 mm to 2.3 mm, and 0.05 to 0.09 g, respectively, with an average of 13.2 mm and 2 mm, respectively, while the morphometrics of Parsendi region larvae are length 10.9 mm to 15.5 mm and width 1.7 mm to 2.3 mm, respectively, with an average length of 13.2 mm and a width of 2 mm Hence, it was observed that at early larval development, the average length of the larva showed some resemblance.

Second and third instar: The larval body had a brown-black head with a grey-brown body colour, with hairs arranged all over the body and on the second and fifth abdominal segments, a "C"-shaped marking and two round brown spots, respectively. The eighth abdominal segment had a horn-like projection, and the last segment was wider than other segments. The average length, width of the Lucknow region's 2nd instar larvae were 17.1 to 22.5 mm and 2.4 to 3 mm, while those of the Parsendi region had a length of 17.1 to 22.5 mm and an average length and width of 19.8 mm and 2.74 mm, respectively, at 25.7 degrees Celsius with an average percentage humidity of 75.5%. The average length and width of larvae in the Parsendi region were 19.8 mm and 2.85 mm, respectively, at an average temperature of 25.20 °C with an average percentage humidity of 82.5%.

The dimensions of length and width of third-instar larvae in the Lucknow region were 23.1 mm to 29.9 mm and 3.1 mm to 3.7 mm, with an average length of 26.54 mm and an average width of 3.38 mm at a temperature of 26.4 °F with an average percentage humidity of 67.2%. While in the Parsendi region, the measurements vary, i.e., the length and width of 3rd instar larvae were 24.4mm to 29.4mm and 3.6mm to 4 mm with an average length of 26.87 mm and a width of 3.82 mm at a temperature of 25.5 degrees Celsius with an average humidity of 80%.

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Fourth and fifth instar: The fourth instar larvae had setae all over the body and were pale greenish in colour. Under lab conditions in the Lucknow region, length and breadth vary from 30.1 mm to 37.15 mm and 3.9 mm to 4.5 mm, with a mean of 33.14 mm and 4.22 mm, respectively, at a temperature of 27 degrees Celsius with an average humidity percentage of 65.2%. At Parsendi region, the length and width of larva were 31.1mm to 38.5mm and 4.1 mm to 4.9mm, with an average length of 34.78 mm and a width of 4.52 mm, at a temperature of 26.2 degrees Celsius with an average humidity percentage of 75.4%, respectively. The fifth instar larva was pale yellow at the head, and its body was white in colour. Under lab conditions in the Lucknow region, length and breadth vary from 39.5 mm to 56.5 mm and 4.7 to 6.1 mm, respectively, with a mean of 47.5 mm and 5.45 mm, at a temperature of 27.1 °C and an average humidity percentage of 66.1%. At the Parsendi region, the length and width of larva were 41.5mm to 62.5mm and 5.2 mm to 7.2mm, with an average length of 51.71 mm and a width of 6.17 mm, at a temperature of 26.4 degrees Celsius with an average humidity percentage of 74.5%, respectively. Larvae undergo moulting four times through five larval stages.

Total larval period

The total larval period varied from 23 to 25 days from Parsendi to Lucknow place. The larval duration of growth, 23 + 0.77 days, was recorded as the indoor and outdoor larval development period range (Siddhapara & Surani, 2018).

Pre-pupa and Pupal Period

On reaching maturity, the larvae showed restlessness, stopped having their feed, and moved out and spun silky thread. Such worms became dull in colour, reduced in size, and initiated raising of their heads in search of a suitable place for cocoon formation. Under lab conditions in the Lucknow region, the pre–pupal and pupal periods ranged between 1 to 2 days and 13 to 14 days, while in the Parsendi region, the pre–pupal and pupal periods ranged between 2 to 3 days and 12 to 13 days.

Cocoon: Single threaded, dull white and oval shaped, cocoon with single or double layers of tough elastic protein around it was observed.

Pupa: A protective silk shell was used for the pupation. Pupa's body was divided into three different segments: head, thorax, and abdomen. Pupa was dark brown and spindly in shape; he also exhibited sexual dimorphism in size and abdomen prominently. The male pupa has a dot on the IX ventral segment, and the female has a small, fine line on the VIII and IX segments longitudinally.

Adults

A light brown to dark brown moth was male, whereas a grey to metallic brown moth was female. The abdomen of a male was narrow and slender, whereas the abdomen of a female was broad, large, and swollen. Male moths were usually smaller in size than female moths. In both the male and female moths, bipectinate, narrow antennae with absent anterior lateral hairs were present.

In our study, it was observed that the silkworm reared at Parsendi had shown an increase in length of 24.4mm–29.4mm in four-day larval stages (Table 1), while in the laboratory environment in our department at Lucknow there was a decrease in length and width of the

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larva and a difference of one day in the development. A decrease in larval feeding rate was also observed in the Lucknow region compared with the Parsendi region. The total number of days for the development of the larva at Parsendi was 23 days, whereas in the Lucknow region, the total larval period was 25 days. The development of the cocoon from the larva took 2-3 days, while in the Lucknow region, the larva spun the cocoon within 1-2 days. The emergence of the moth from its cocoon at Parsendi takes 13–14 days, but in the Lucknow region it takes 14–15 days. The number of days varies at both rearing places at all stages of larval, post-larval, pupal, and adult development due to changes in temperature, humidity, and also the feed (mulberry leaves from different regions) at both places. Fresh, healthy, disease-free, and pollution free leaves were fed to silkworms in the Parsendi region. Whereas in the Lucknow region, because of the limited number of mulberry trees and the pollution and dusty environment, mulberry leaves were not as nutritious as those from Parsendi for the larval and silkworm feed. Due to the change in temperature and humidity, the 3rd, 4th, and 5th instar larvae have shown decreases in length and width and also variation in the larval period, with a delay in the larval development period from Parsendi region to Lucknow region (tables 1 and 2). Hence, it can be concluded that the growth of the silkworm was dependent on the temperature and humidity of the surrounding area and region. Based on the environmental factors, in the Parsendi region, the growth of the larva also varies, as the larva from the 1^{st} instar to the 5^{th} instar has a maximum length of 10.9mm to 62.5mm with an average width of 3.87 mm at an average temperature of 23.7 degrees Celsius (Table 1 and Graph 1). While at Lucknow in the restricted environment, the length of the larva from the first to the fifth instar increased in length from 10.9 to 56.5 mm with an average width of 3.55 mm at an average temperature of 26.24 degrees Celsius (Table 2 and Graph 2). It was also observed that cocoon development varies with the difference in day delay between Lucknow and Parsendi. The images of different stages of the worm (A to H) were also collected, and a graph of developmental stages and the effect of humidity and temperature on the same has been prepared based on the observed and recorded data.

S. No	**Growth stage of instar larvae**	**Growing stage (hrs)**	**Feeding material**	**Average Length (mm)**	**Average Width (mm)**	**Average Temperature (^{O}C)**	**Average Humidity (%)**
1.	I	96	Newley Twig leaves	13.2	2	23.7	83
2.	II	96	Newley Twig leaves	19.8	2.85	25.2	82.5
3.	III	96	2^{nd} leaf from upper row	26.87	3.82	25.5	80

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4	IV	120	3rd leaf from upper row	34.78	4.52	26.2	75.4
5.	V	168	4th leaf from upper row	51.71	6.17	26.4	74.5

Table 1. Development, feed and growth of mulberry silk moth (*Bombyx mori L.*) at different temperature and humidity (Parsendi region).

- Total days of larval period 23.
- Total days larva converts to cocoon 2 to 3 days.
- Silk moth emerge from cocoon 12 to 13 days.

Table 2. The development, feed and growth of mulberry silk moth at different temperature and humidity (Lucknow region).

S. No.	Growth stage of instar larvae	Growing stage (hrs.)	Feeding material	Average length (mm)	Average width (mm)	Average temperature (^{O}C)	Average Humidity (%)
1.	I	96	Newley Twig leaves	13.2	2	25	78.7
2.	II	96	Newley Twig leaves	19.8	2.74	25.7	75.5
3.	III	120	2nd leaf from upper row	26.54	3.38	26.4	67.2
4	IV	120	3rd leaf from upper row	33.14	4.22	27	65.2
5.	V	168	4th leaf from upper row	47.35	5.45	27.1	66.1

- Total days of larval period 25.
- Total days larva converts to cocoon 1to 2 days.
- Silk moth emerge from cocoon 13 to 14 days.

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Figure 1- Outdoor rearing in Parsendi Region, Sitapur district, Uttar Pradesh.

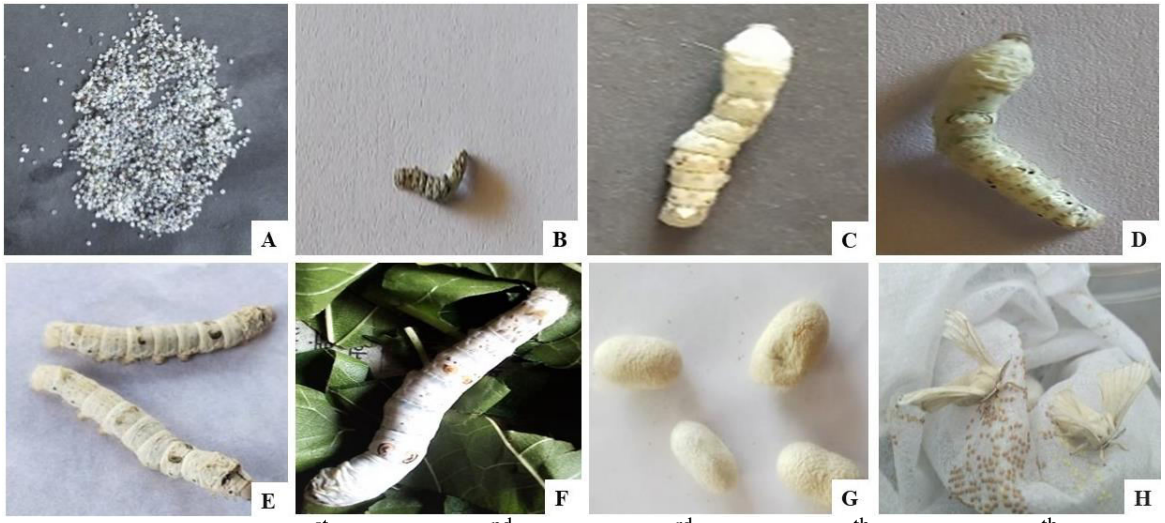
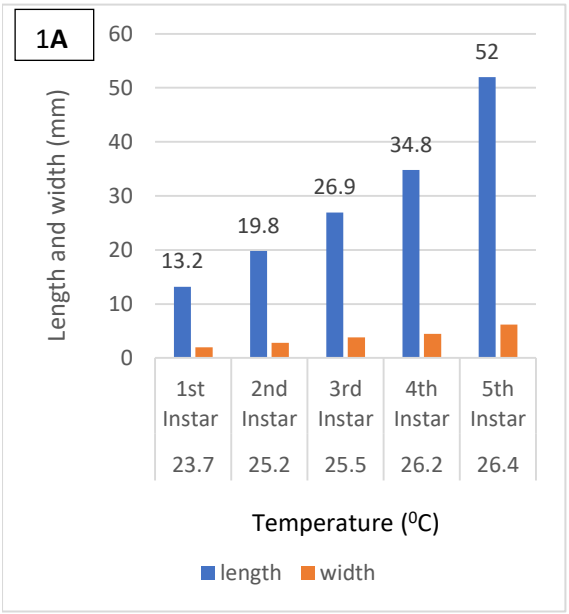
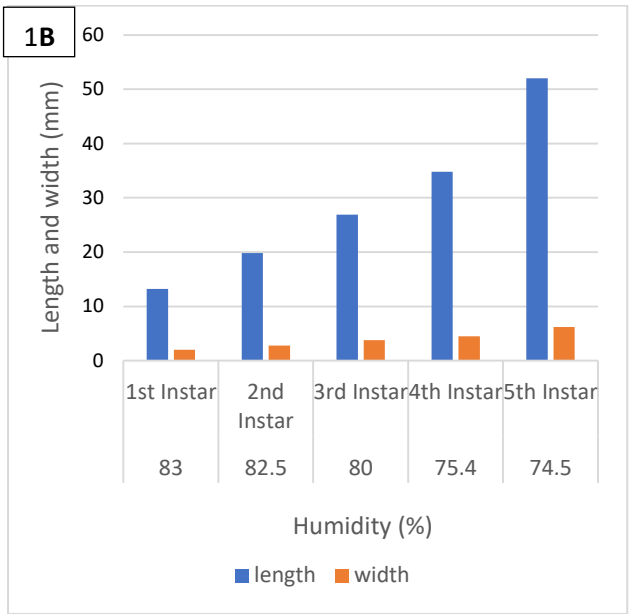


Figure 1. **A**= Egg, **B**=1st Instar, **C**=2nd Instar, **D**=3rd Instar, **E**=4th Instar, **F**= 5th Instar, **G**= Cocoon, **H**=Adult Moth.

Figure A to H: The images show developmental stages (A to H eggs, 1st, 2nd, 3rd, 4th, and 5th instar larvae, cocoons, and adult moths, respectively) of mulberry silkworm reared at Parsendi region, Sitapur district, Uttar Pradesh state, India. Graph showing the growth rate's dependability on environmental modalities such as humidity and temperature.





Graph 1A: Showing length and width at varying temperatures of *Bombyx mori* L. larvae reared at Parsendi (Chaki) of Sitapur district.

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Graph 1B: Showing length and width at varying humidity of *Bombyx mori* L. larvae reared at Parsendi (Chaki) of Sitapur district.

Figure 1- Indoor Lab rearing in Lucknow Region, Sitapur district, Uttar Pradesh.

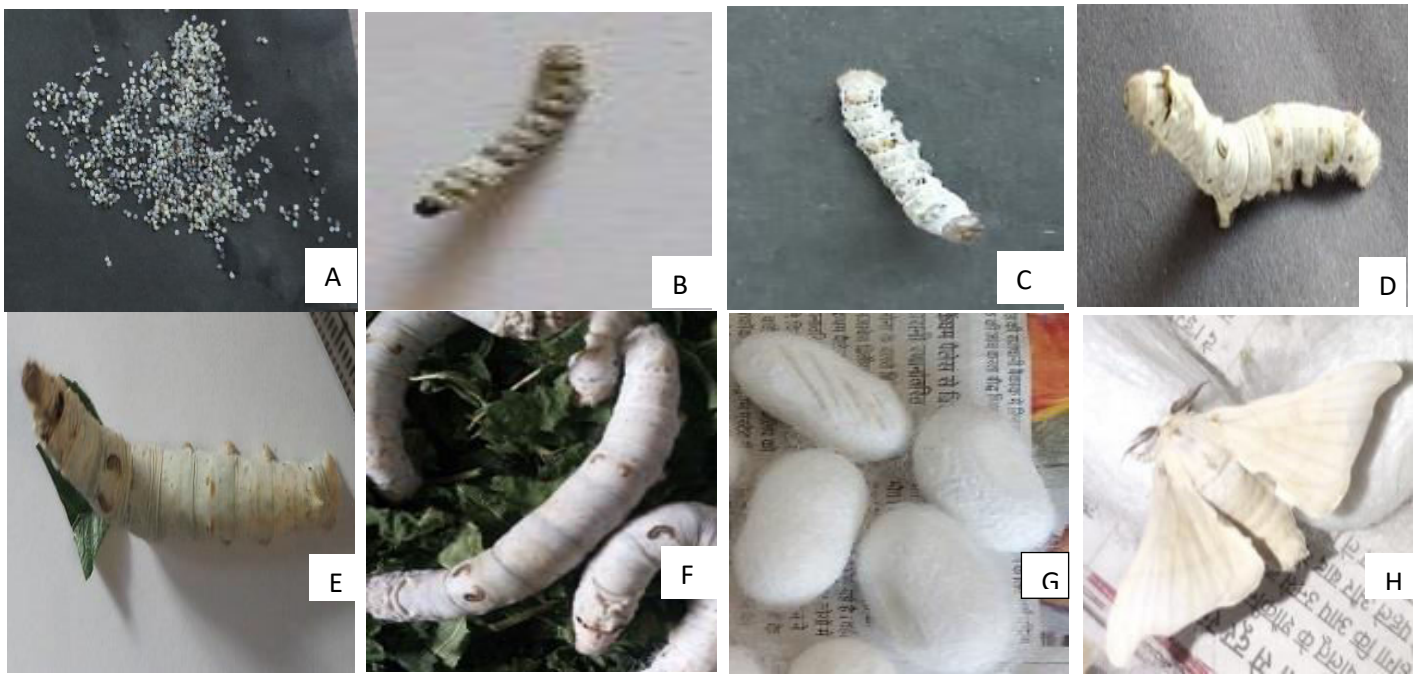

Figure 2. **A**= Egg, **B**=1st Instar, **C**=2nd Instar, **D**=3rd Instar, **E**=4th Instar, **F**= 5th Instar, **G**= Cocoon, **H**=Adult Moth.

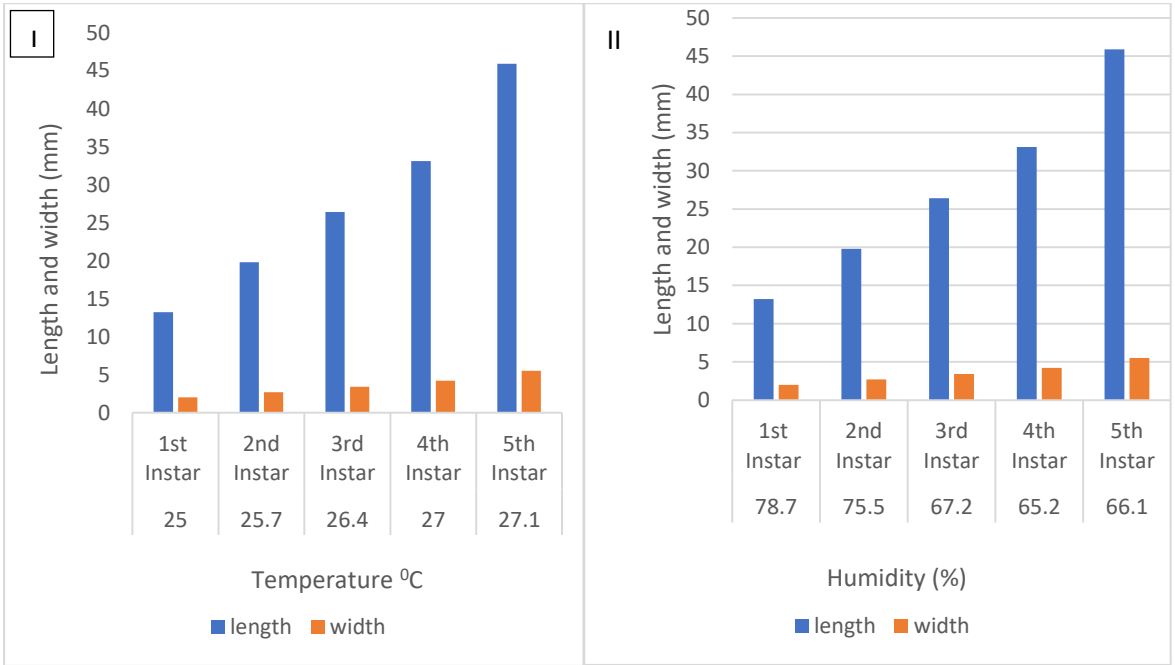

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Figure 2- A to H, and graph I &II: - The images showing developmental stages (A to H-egg, 1st,2nd,3rd,4th,5th instar larva, cocoon and adult moth, respectively) of mulberry silkworm reared at Department of Zoology at University of Lucknow, Lucknow district of Uttar Pradesh state, India. Graph showing the growth rate dependability on environmental modalities such as humidity and temperature.

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