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Effect of temperature on the egg laying capability, fertility, and incubation period of eggs in mulberry silkworm (*Bombyx mori* L.)

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Abstract
By exposing 4th and 5th instar larvae of inbred silkworm lines, the effects of temperature changes on fecundity, incubation period, and fertility of the silkworm egg lines were studied 2022 during autumn and spring at 10°, 14°, 18°, 26°, 34°, and 38°C. In contrast to the maximum fecundity rate of 386 eggs per female and the incubation duration of 9.46 days at 26˚C, the minimum level of fecundity was 249 eggs per female and the incubation period of eggs decreased significantly to 21.66 days at 10˚C. Fecundity and incubation time decreased significantly over 26˚C. Temperature has been shown to negatively impact fecundity, incubation, and fertility in inbred silkworm lines. The ideal temperature and humidity for raising silkworm lines to produce seed cocoons is 26±1˚C with 80±5% humidity and 12±1 hours of light per day. This combination can be used for hybridization and the production of more eggs and a shorter incubation period for eggs with higher fertility. Studies revealed that fluctuations in humidity and temperature during larval rearing led to a high frequency of unfertilized eggs and a low fecundity rate. According to the study, temperature and humidity changes can be avoided to improve fecundity, incubation, and fertility.

Keywords: Temperature, silkworm, fecundity, fertility

Introduction

The agricultural practice known as sericulture is primarily the cultivation of mulberry trees (*Morus* sp. pl.) in order to produce leaves that are fed to silkworms (*Bombyx mori* L.), who are raised to produce silk. The commerce of silk played a significant role in the earliest stages of human history, enabling the first wave of globalization that connected Europe and Asia via the so-called "Silk Road" approximately 2,000 years ago (Giora *et al*, 2022) [4]. These days, the production of silk is distributed across around 60 nations and accounts for 0.2% of global textile production. The nations that produce the most silk are China, India, Thailand, Vietnam, Uzbekistan, and Brazil. A class of naturally occurring structural proteins known as "silk" is created and spun into long, fibrous filaments by many Arthropoda, including spiders and lepidoptera. The domestic silkworm *Bombyx mori* L produces the most well-known and extensively researched form of silk (Zhou *et al*, 2020) [23].

Since the commencement of silk production, the material has mostly been used for textile and medical purposes. This is because medical research has shown that silk's biocompatibility may be effectively utilized during surgery (van Turnhou *et al*., 2018) [19]. Male and female larvae of pure strains are raised in a specialized environment to establish the parental lines that are then crossed among them in order to produce commercial silkworm eggs; the most common parental lines are found in Chinese and Japanese strains (Joseph Raj *et al*, 2019) [6]. In general, hybrids and polyhybrids are more productive than pure strains and more resilient to biotic and abiotic stress, however, since air temperature and relative humidity have a significant impact on larval metabolism, controlling environmental parameters is essential for the best development of reared larvae (Rahmathulla *et al*., 2012) [13].

The *Bombyx mori* L silkworm is a multivoltine and monofagus domesticated species. Silkworms use behavioral or physiological mechanisms to regulate their body temperature to some extent. Silkworms have an ecological range that indicates their tolerance limit, just like other insects (Manjunatha *et al*, 2010) [10]. Neuroendocrine cells produce hormones that regulate different developmental programs in silkworms. The temperature difference in the surrounding environment has a significant impact on the function of neurosecretory cells. As a result, it is necessary to understand how temperature changes affect growth and development. Thus, the laboratory's temperature stress test is a great tool for determining the ideal temperature range for sericulture. India produces relatively few silkworm eggs in comparison to other nations that practice sericulture, and it lags the potential of silkworm races (Andadari L *et al*, 2022) [2].

To improve farmer returns and raw silk productivity, sericulture's commercial seed production operations must be optimized. the difficult process of creating silkworm breeds that can lay eggs in the right temperature conditions. A review of the literature indicates that no research has been done in the Khammam district about the fecundity, fertility, and incubation duration of silkworm eggs. The goal of the current study was to determine how temperature variations affected the number of silkworm eggs produced, their fertility, and how long they took to hatch. Finding the reason for low yield and low egg fertility across all sericulture stations would be aided by the study.

Methodology

Six series of tests were created to see how different temperatures affected *Bombyx mori's* performance. For conducting the studies, temperature regimes such as 10°, 14°, 18°, 26°, 34°, and 38°C were selected. In the initial set of trials, disease-free lays (DFLs) from laboratory-reared silk moths were continuously moved to a BOD incubator that was kept at 10±1˚C (one of the experimental temperature regimes) in the lab. The control for the tests was set under optimal settings, which included 26±1˚C temperature, 80±5% relative humidity, and 12±1 hours of light per day.

The DFLs were repeatedly moved to the BOD incubator after being cleaned with 2% formalin for 15 minutes in order to improve the adhesiveness of the eggs on the card and surface disinfections. Thirty normal egg laying (three batches of ten laying in each batch) were taken for each replicate in order to determine the fecundity. For every experiment, three duplicates were conducted. The amount of time needed for incubation before larvae hatch was determined for each set of experiments individually in order to track how temperature affected the incubation duration of silkworm eggs. There were three copies manufactured, with ten lying in each copy. By calculating the mean value of the collected data, the average amount of time that eggs needed to incubate until larvae hatched was determined. For each batch in the study, the average hatching of 10 layings was used to calculate the representative hatchability percentage per laying. For every replication, thirty laying (three batches of ten laying in each batch) were counted. For every experiment, three duplicates were conducted.

$$\text{Hatchability (or) fertility \%} = \frac{\text{No. of hatched eggs}}{\text{No. of fertilized eggs}} \times$$

One-way ANOVA was used to statistically assess the data for all experimental parameters.

Results and Discussion

According to the data shown Table 1, the ability of *Bombyx mori* to lay eggs rose as temperature rose from 14˚ to 26˚C, but beyond 26˚C, fecundity began to diminish as temperature rose up to 34˚C.At 14˚C, the least number of eggs laid by a female moth was measured as 249, while at 26˚C, the maximum number of eggs laid by a female was 386 (Figure1). When the temperature increased from 14˚ to 18˚C, there was a noticeable difference in the trend of rising fecundity; however, the trend of growing fecundity remained constant until it reached 26˚C. The capacity to lay eggs decreased gradually and only to 34˚C above 26˚C.

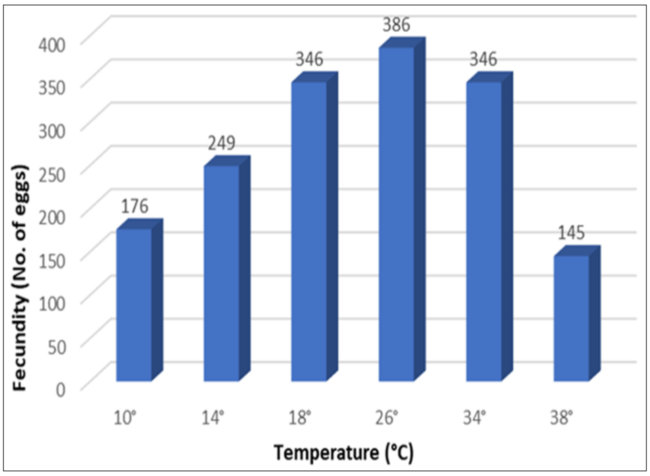

Fig 1: Temperature's impact on *Bombyx mori's* Fecundity

According to S.V. Seshagiri *et al* (2009) [16], fecundity is a genetic trait whose expression in the genotype of an insect species such as the mulberry silkworm is directly correlated with certain physiological and ecological parameters (Rahmathulla *et al*, 2012) [13]. Moth fecundity developed from pupae derived from chilled eggs (Abdurrahman *et al*, 2008) [1], and it has been observed that refrigerated pupae exhibit a dramatic drop in silkworm egg-laying capability.

Table 1: Temperature's impact on *Bombyx mori*'s Fecundity

Temperature (°C)	10°	14°	18°	26°	34°	38°	F=ratio n_1= 3 n_2= 8
Fecundity (No. of eggs)	176	249	346	386	346	145	248*
SD±	±5.34	±6.34	±6.88	±7.71	±5.43	±4.34	

Each value represents mean ±SD of three replicates, *P<0.01

But when feeding *Bombyx mori* larvae on mulberry leaves treated with ascorbic acid, there was a noticeable increase in fertility (Muzamil *et al*, 2023) [11]. While (Hasan *et al*, 2023) [5] proposed that the length of mating in *Bombyx mori* influences egg production, (Zhang *et al*, X 2022) [22] recognized that the quality of mulberry leaf was the primary determinant for good fecundity in *Bombyx mori*. Silkworm fecundity improved when Bombyx mori pupae were heated (Wang *et al*, 2023) [20], and rearing temperature positively correlated with fecundity in *Bombyx mori* (Ruth *et al*, 2019) [15]. Given that silkworm larvae consume the least amount of mulberry leaf at very low temperature regimes and that temperature and metabolic rate are the primary causes of low fecundity at temperature, it can be assumed that the cause of poor fecundity at very low temperature regimes may be due to content scarcity and essential oogenesis.

The data shown Table-2 unequivocally show that temperature regime modification had a significant impact on the time that *Bombyx mori* eggs needed to incubate. The incubation period of eggs dropped significantly from 21.66 days at 10˚C to the shortest duration of 6.56 days at 38˚C with the temperature increase from 10˚C to 38˚C (Figure 2). The incubation period of eggs decreased sharply from 21.66 to 15.16 days with an increase in temperature from 10˚C to 14˚C; however, the rate of decrease in incubation period duration was moderate with an increase in temperature at higher regimes. Egg incubation took place for 9.46 days at 26˚C.

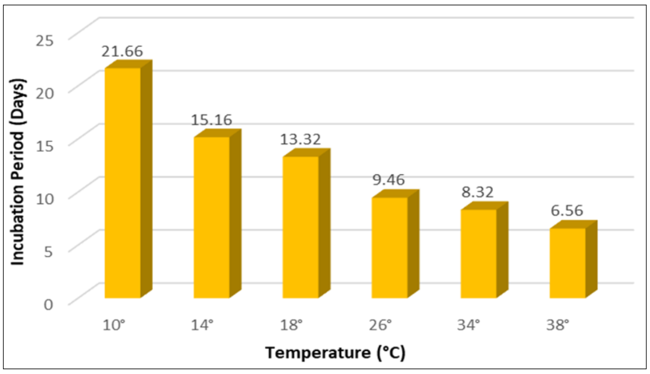

Fig 2: Effect of temperature on the incubation period (days) of Bombyx mori

The length of time that *Antheraea assama* takes to incubate is significantly impacted by heat acclimation (Rao K P *et al*,

1997) [14]. In a similar vein, *Bombyx mori's* incubation period was influenced by the length of mating (Kumari *et al*, 2011) [8]. Juvenile hormone activity had a significant impact on how long *Bombyx mori* eggs took to incubate (Xu Q *et al*, 2012) [21]. Low egg recovery in various settings may result from genetic variation among silkworm breeds. A similar conclusion has been published by Rahman *et al* (2008) [12]. It can be deduced that in extremely low temperatures, the development of eggs is slowed because energy resources are not mobilized as quickly to meet the basic requirements for egg development.

Table 2: Effect of temperature on the incubation period (days) of *Bombyx mori*

Temperature (°C)	**10°**	**14°**	**18°**	**26°**	**34°**	**38°**	**F=ratio n_1= 5 n_2= 12**
Incubation Period (Days)	21.66	15.16	13.32	9.46	8.32	6.56	297.22*
SD±	±0.46	±0.60	±0.64	±0.26	±0.16	±0.32	

Each value represents mean ±SD of three replicates., *P<0.01

The data collected (Table-3) makes it evident that temperature change had a significant impact on the percentage of hatchability of *Bombyx mori* eggs. The hatchability significantly increased from 45.15% at 10˚C to the greatest level of 92.69% at 26˚C as the temperature rose from 10˚C to 26˚C. The hatching percentage significantly decreased with further temperature rise above 26˚C, reaching 73.69% at 38˚C. At 10˚C, the minimum hatchability was found to be 45.15% (**f**igure 3). Activities are limited in their ability to survive and grow according to the degree of their genetic makeup. The percentage of hatching was significantly reduced when the length of time *Bombyx mori* eggs were refrigerated increased (Shen *et al*, 2023) [17].

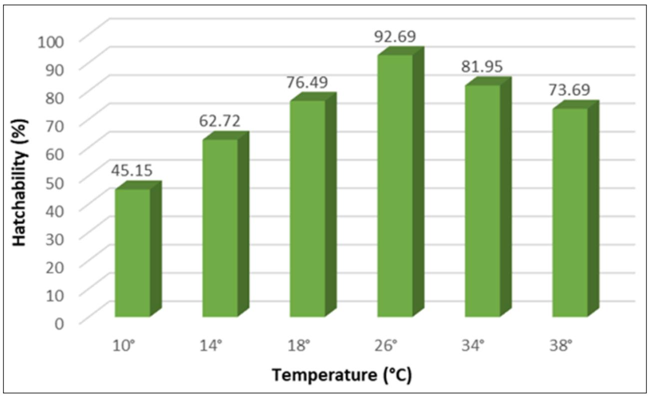

Fig 3: Impact of temperature on the hatchability (%) of Bombyx mori

Early-stage embryos are less likely to hatch when refrigerated (Khan *et al*, 2013) [7], although older eggs are better suited for long-term cold storage (Mahdavi *et al*, 2013) [9]. The percentage of *Bombyx mori* eggs that hatch is not significantly affected by the ascorbic acid treatment. While treating *Bombyx mori* eggs with HCL produced the best hatchability eggs, there were no appreciable changes in the percentage of hatching between multiple and single mating (Gai *et al*, 2020) [3]. (Suzuki *et al*, 2014) [18].

Table 3: Impact of temperature on the hatchability (%) of *Bombyx mori*

Temperature (°C)	10°	14°	18°	26°	34°	38°	F=ratio n_1= 5 n_2= 12
Hatchability (%)	45.15	62.72	76.49	92.69	81.95	73.69	344.93*
SD±	±1.34	±2.48	±2.41	±2.71	±2.08	±2.06	

N. Sd = Not Survived *P<0.01 Each value represents mean ±SD of three replicates.

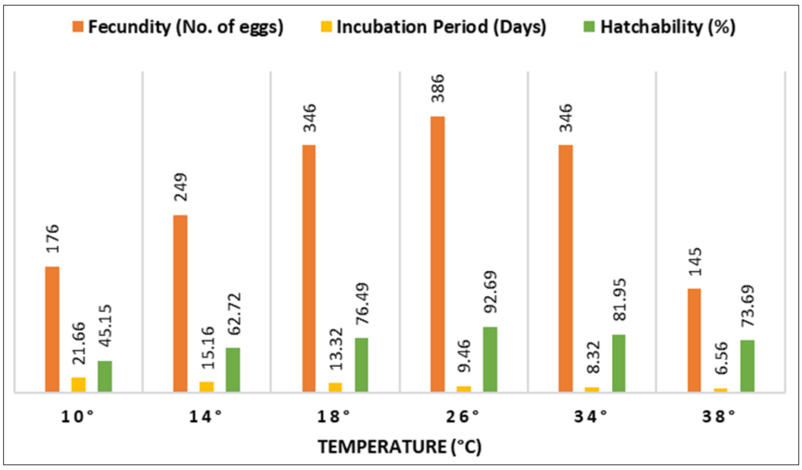

Fig 4: Graphical representation of temperature effect

The percentage of *Bombyx mori* eggs that hatched was influenced by ecological conditions (Rahmathulla *et al*, 2012) [13]. It is assumed that the activity of the enzymes involved in the development of eggs resulting in low hatching percent and the mobilization of necessary contents for the correct development of fertilized eggs. However, at temperatures as high as 38˚C, the eggs' accelerated metabolism may waste energy resources at the expense of other resources needed for the development of the eggs, which would explain the low hatch rate.

Conclusion

The current study's findings thus suggest that temperature change had a significant impact on *Bombyx mori*'s various developmental stages. The study also recommends that when choosing silkworm lines for additional research for reelers and farmers, other factors influencing seed cocoon production, such as fecundity, egg incubation time, and hatchability percentage, should be assessed in addition to commercial features using the evaluation index method (EIM).

Competing interests
The authors declare that they have no conflict of interest

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