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Impact of mating duration on reproductive and biochemical parameters of mulberry silkworm (*Bombyx mori* L.)

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Abstract

The study was undertaken to assess the impact of different mating durations (1-5 hours) on some reproductive and Biochemical parameters of the silkworm *B. mori* L. The healthy seed cocoons of inbred silkworm races viz; CSR2 and CSR4 were obtained from the Silkworm Germplasm Bank, CoTS, SKUAST-K, Mirgund. The selected cocoons were cut open for sex determination, the male and female pupae were segregated and maintained separately under ideal temperature at $25\pm 1^{\circ}\text{C}$ and relative humidity of 75-80% till moth formation. The male and female silk moths were utilized as experimental material and allowed to copulate as per the experimental plan. The study revealed that mating duration has significant impact on the pre-oviposition duration, oviposition duration, fecundity (number of eggs laid/laying), fertility (viable egg percentage), egg retention percentage, egg weight and hatching percentage in silkworm *B. mori* L. The pre-oviposition and oviposition durations decreased with increase in mating duration. Fecundity and fertility increased with increase in mating duration and maximum fecundity and fertility of 532 and 97% was recorded at 5 hours mating duration. However, egg retention percentage decreased, while egg weight increased with increase in mating duration. Specifically, the hatching percentage was found to increase with longer mating durations and highest hatching percentage of 97% was recorded at 5 hours mating duration. In case of biochemical parameters viz; carbohydrate and protein content of silkworm egg, no significant variations were observed across the treatments. The study highlights the importance of optimizing mating duration for reproductive success, making it a valuable factor in silkworm breeding programs aimed at maximizing viable egg production.

Keywords: *Bombyx mori* L., biochemical, duration, mating, mulberry, reproductive, silkworm

Introduction

Silkworm *Bombyx mori* L. is an economically important insect known for silk production. Silk is one of the bio-resources for human life and has occupied a place of pride from times immemorial. There have been sustained efforts to improve silk quality and productivity. Successful rearing of silkworm and cocoon production depends on several factors. Among these factors seed, breed and feed are of vital importance. Quality silkworm seed is the basic and vital input to harvest a successful cocoon crop (Singh and Saratchandra, 2004) [22]. Quality of silkworm seed is determined by many factors of significant importance wherein mating duration and fertility of eggs play an important role (Ullal and Narasimhanna, 1981) [25]. Two important phases of reproduction viz; mating and egg laying are very crucial in insects especially in silk moth as it is of commercial importance.

Mating is an essential behavior in the life cycle of living organisms for perpetuation of population. A number of intrinsic and extrinsic factors of significant importance are involved in successful mating and egg deposition by an adult silk moth. Besides biochemical, physiological and environmental factors, attraction of reproductively competent male and female moth for mating, duration and frequency of mating, age of moth at the time of mating are of significant importance. The success of mating depends on silkworm genotype, the overall environmental conditions, food and nutrition, physiological, reproductive, health status of participating male and female moths, their age and ultimately mating duration affects it considerably (Singh and Tripathi, 1995; Singh and Saratchandra, 2004) [23, 22].

The mating duration in silk moth plays a crucial role in their reproductive performance, egg fertility, hatchability and overall progeny. Reproductive success directly impacts silk production, making it essential to optimize mating conditions for higher egg yield. Mating in silk moth is not a mere process obligatory either for Oviposition but inseminating the female is essential prerequisite factor which has a profound influence on the reproductive behavior, physiology and further development of the female moth. The post-mating effect of male on females include induction of oogenesis (Pickford *et al.*, 1969) [16], enhancement of fecundity (Danthanarayana and Gu, 1991) [2], suppression of remating, stimulation of oviposition (Watanabe, 1988) [26], and acceleration of egg laying (Herndon and Wolfner, 1995; Soller *et al.*, 1997) [7, 24]. This finally results in a sound impact on rearing performance in the next generation. In most of the insects egg laying can take place irrespective of mating. Mating is a decisive factor which not only influence the total number of eggs but also important in the inducement of the regular oviposition (Ridley, 1990) [18]. It is generally recognised that the male factors or the oviposition stimulating substance derived from the male reproductive glands and transferred to the females during copulation induce brisk oviposition behaviour (Fugo and Arisawa, 1992) [5]. However, for successful oviposition participating male and female partners should be reproductively competent, mating should be accomplished at the appropriate time and mating should ensure transfer of sperm and the viscous heterogeneous secretions including male factor into the reproductive tract of the adult female. Punitham *et al.* (1987) [17] have reported that mating duration upto 6 hours increased the total egg output and reduced pre-oviposition period but subsequent increase in mating duration resulted in negative effects. Mating has a profound impact on the fertility and oviposition, the mated moths lay mostly fertilized eggs (which produce viable progeny) and a very small quantity of unfertilized and dead eggs (Krishnaswamy *et al.*, 1973) [9]. Understanding the relationship between mating duration and reproductive outcomes is vital for improving silkworm breeding practices and enhancing the productivity of seed or eggs. The study delves into the effects of different mating durations on key reproductive parameters such as the fecundity (number of eggs laid/laying), fertility (the percentage of eggs that successfully hatch into larvae) and hatching percentage of eggs of silk moth. Furthermore, the research investigates the influence of mating duration on the biochemical composition of seed.

Materials and Methods

The healthy seed cocoons of inbred silkworm races viz; CSR2 and CSR4 were obtained from the Silkworm Germplasm Bank. The selected cocoons were cut open for sex determination, the male and female pupae were segregated and maintained separately at a room temperature of 25 ± 1 °C and relative humidity of 75-80% till moth formation. The males and females were utilized as the experimental material. Healthy male and female moths were selected for mating replication wise as per the treatment plan. Four replications were maintained for each treatment, with 15 couplings in each replication. The moths in different groups were allowed to mate for the specific duration as per

treatment plan after which moths were decoupled manually. Following the decoupling of mated moths, the female moths of each treated batch were collected individually and transferred to egg card sheets for oviposition within individual cellules. Dark conditions with appropriate temperature and relative humidity (25 ± 1 °C) and 75-80% were maintained till the complete oviposition was achieved. The data regarding fecundity, Fertility and Hatching percentage were recorded for each treatments and replications.

Estimation of total Carbohydrates

Total carbohydrate was estimated by Anthrone Method (Dubois *et al.*, 1956) [4]. Requisite quantity of eggs were homogenized in 5 ml of 80% ethanol and centrifuged at 2,000 rpm for 5 min, the precipitate was washed with 5 ml of 80% ethanol. Combined supernatant was evaporated under reduced pressure. The residue was dissolved in 1 ml of distilled water. The homogenate was centrifuged at 3000 rpm for 10-15 minutes. 1 ml of supernatant was drawn and transferred to another test tube. 4 ml of Anthrone reagent was added and boiled exactly for 8 minutes and cooled rapidly in running water. Optical density (OD) was taken at 630 nm against blank solution.

Quantitative Protein estimation

The quantitative analysis of total proteins present in the eggs was performed by Lowry's method (Lowry *et al.*, 1951) [12]. To 1 ml sample, 0.5 ml solution C was added and incubated at room temperature for 10 min. Then 0.5 ml of Folin's Ciocalteu reagent was added followed by thorough mixing. After incubation for 30 m at room temperature, absorbance of the color developed was measured at 660 nm using Spectrophotometer (Thermo Scientific, NanoDrop).

Results

Effect of mating duration on Reproductive parameters of silkworm *B. mori* L.

Pre-oviposition and oviposition duration

The study revealed that the pre-oviposition and oviposition duration decreased with increase in mating duration from 1 to 5 hours. The Highest pre-oviposition and oviposition duration was recorded at 1 hour mating duration and the lowest pre-oviposition and oviposition duration was recorded at 5 hour mating duration (Table-1).

Table 1: Effect of different mating durations on Pre-oviposition and Oviposition durations (hours) of silk moth *B. mori* L.

Parameter Treatment	Pre-oviposition duration	Oviposition duration
T ₁ : 1 hour	7.25	26.00
T ₂ : 2 hours	6.23	25.00
T ₃ : 3 hours	5.25	24.00
T ₄ : 4 hours	4.35	22.00
T ₅ : 5 hours	4.17	21.00
CD ($p < 0.05$)	0.146	1.242

T₃: 3 hours mating duration was taken as control

Fecundity (Number of eggs laid/laying) and Fertility (viable egg percentage)

Fecundity and fertility significantly increased with increase in mating duration. The highest fecundity (532) and fertility (97%) was recorded at 5 hour mating duration (Table-2).

Table 2: Effect of different mating durations on Fecundity (Number of eggs laid/laying) and Fertility (%) of silk moth *B. mori* L.

Parameter Treatment	Fecundity (Number of eggs laid/laying)	Fertility (viable egg percentage)
T ₁ : 1 hour	523.00	90.50
T ₂ : 2 hours	525.00	91.50
T ₃ : 3 hours	529.00	95.25
T ₄ : 4 hours	530.00	96.50
T ₅ : 5 hours	532.00	97.00
CD ($p < 0.05$)	1.456	1.914

T₃: 3 hours mating duration was taken as control.

Egg Retention percentage and Egg weight (gram/laying)

Similarly the Egg retention percentage decreased with increase in mating duration. Maximum egg retention percentage of 4.20% was recorded at T₁ followed by T₂ (3.80%), T₃ (3.02%), T₄ (2.80%) and lowest egg retention of 2.40% was recorded at T₅ (Table-3). With respect to different mating durations highest egg weight was observed at 5 hour (0.319 g/laying) and the lowest egg weight of 0.313 was observed at 1 hour (Table-3).

Table 3: Effect of different mating durations on Egg retention percentage and Egg weight (g/laying) of silk moth *B. mori* L.

Parameter Treatment	Egg Retention percentage	Egg Weight (g/laying)
T ₁ : 1 hour	4.20	0.313
T ₂ : 2 hours	3.80	0.315
T ₃ : 3 hours	3.02	0.317
T ₄ : 4 hours	2.80	0.318
T ₅ : 5 hours	2.40	0.319
CD ($p < 0.05$)	0.111	0.001

T₃: 3 hours mating duration was taken as control.

Hatching percentage

Hatching percentage also increases with increase in mating duration (Table 4). Highest hatching percentage of 97.00% was recorded at 5 hour mating duration and lowest hatching percentage of 90.50% was recorded at 1 hour duration.

Table 4: Effect of different mating durations on Hatching Percentage and of silk moth *B. mori* eggs

Parameter Treatment	Hatching percentage
T ₁ : 1 hour	90.50
T ₂ : 2 hours	92.50
T ₃ : 3 hours	95.50
T ₄ : 4 hours	96.50
T ₅ : 5 hours	97.00
CD ($p < 0.05$)	2.151

T₃: 3 hours mating duration was taken as control.

Effect of mating duration on Biochemical constituents of silkworm eggs

Carbohydrate content (mg/ml)

The results with respect to carbohydrate content in silk moth *B. mori* eggs at different mating duration from 1 to 5 hours (Table-5) revealed that there was no significant variation among the treatments. Highest carbohydrate content of 1.30 mg/ml was recorded at T₃ followed by T₅ (1.29 mg/ml), T₄ (1.27 mg/ml), T₂ (1.25 mg/ml) and lowest carbohydrate content was recorded at T₁ (1.24 mg/ml), which were statistically non-significant.

Protein Content (ug/ml)

The data with respect to protein content of silk moth *B. mori* eggs at different mating duration from 1 to 5 hours (Table-5) indicated that there was no significant differences among the treatments. Lowest protein content was recorded at T₅ (2.44 ug/ml). Conversely, highest protein content was recorded at T₃ (2.62 ug/ml) followed by T₁ (2.53 ug/ml), T₂ (2.52 ug/ml), T₄ (2.48ug/ml) respectively, which were statistically non-significant.

Table 5: Effect of different mating durations on Carbohydrate and Protein content of silk moth *B. mori* eggs

Parameter Treatment	Carbohydrate content (mg/ml)	Protein content (ug/ml)
T ₁ : 1 hour	1.24	2.53
T ₂ : 2 hours	1.25	2.52
T ₃ : 3 hours	1.30	2.62
T ₄ : 4 hours	1.27	2.48
T ₅ : 5 hours	1.29	2.44
CD ($p < 0.05$)	NS	NS

T₃: 3 hours mating duration was taken as control.

Discussion

The results obtained during the present study are in conformity with the findings of Punitham *et al.* (1987) [17] who have reported that pre-oviposition duration decreases with increase in mating durations in silk moth *B. mori* L. Omkar *et al.* (2006) [15] have reported that with increase in mating duration pre-oviposition duration decreases in *Cheilomenes sexmaculata* and *Coelophora saucia*. The decrease in pre-oviposition duration is probably indicative of the fact that prolonged mating triggers females to expedite egg production and oviposition. This could be a result of either increased sperm content, increased stimulation indicative of male potential or increased access to certain contents of male ejaculates, which could be nutritional or stimulatory in nature. Similarly, Narayanan *et al.* (1964) [14] have reported that increase in mating duration reduces pre-oviposition duration. of Manoharan (1983) [13] and Gowda (1988) [6] who have reported that the speed of oviposition is altered significantly, faster in the longer durations and slower in the moths mated for shorter durations. Leopold (1976) [11] reported in many insects that Oviposition-Stimulating Substances (OSS) derived from the male reproductive tract are transferred to the females during mating have the ability to accelerate the oviposition behavior. Saheb *et al.* (2005) [19] who have reported that duration of pairing has an inverse correlation with oviposition duration i.e., with increase in mating duration oviposition duration decreases.

Saikia *et al.* (2019) [20] reported that fecundity increases with increase in mating duration in muga silk moth *A. assama*. Punitham *et al.* (1987) [17] reported that increase in mating duration not only reduces pre-oviposition duration but also increases the egg output. Bhattacharyya *et al.* (2016) [1] reported that fertility is directly proportional to coupling duration and maximum at 5 hours coupling duration. Female silk moths store sperm in their spermatheca after mating. Sperm can remain viable for several days and fertilize eggs over multiple ovipositions. A longer mating durations can lead to a larger amount of sperm being stored, which might result in higher fertility. Similarly Kumar (2023) [10] also has reported that with the increase in mating durations from 1 to 5 hours resulted in increase in fertility in silk moth *B. mori* L.

Dharavath *et al.* (2024) ^[3] reported in muga silk moth *A. assamensis* that egg retention in the abdomen is significantly higher in mechanically coupled moths than in naturally coupled moths. The higher egg retention is due to the impact of mechanical mating which lasts for short duration, than natural mating which lasts for longer duration in muga silk moth. If the mating duration is too short, not all the eggs are released from the ovaries. The release of eggs into the reproductive tract might be delayed or incomplete, leading to egg retention. Saikia *et al.* (2019) ^[20] reported that the fecundity of muga silk moth in terms of number and weight of eggs had an increasing trend with prolonged mating duration. A longer mating duration may allow for more sperm to be transferred and stored, which contributes to an increase in egg weight. Saikia *et al.* (2023) ^[21] also revealed that the weight of eggs in gram per laying increases with increase in mating duration. Punitham *et al.* (1987) ^[17] revealed that an increase in mating duration of silk moth *B. mori* L. from 3 to 9 hours enhanced hatching percentage of eggs from 83 to 97%. Saikia *et al.* (2023) ^[21] have reported that mating duration had significant effect on hatching percentage of eggs of muga silk moth. Similarly, Jadav and Gajare (1978) ^[8] reported that 4 hours of mating duration resulted in significant increase of hatching percentage of eggs of silkworm *B. mori* L.

Conclusion

The results of the study demonstrated that mating duration had significant impact on reproductive parameters viz: pre-oviposition duration, oviposition duration, fecundity, fertility. The mating duration in silk moth *B. mori* L. plays a crucial role in reproductive success and production of viable eggs. Major finding of the study indicate that prolonged mating duration significantly enhances fertilization rate, leading to a higher percentage of viable eggs that hatch successfully. The study highlights the importance of optimizing mating duration to maximize fertile egg production and consequently, the productivity with implications for silkworm seed industry and for commercial sericulture. The results obtained from the study demonstrated that a longer mating period enhances reproductive success, making it a valuable factor for its application in silkworm breeding programs aimed at maximizing viable egg production.

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Competing interest

The authors declare no conflict of interest in the publication of this manuscript.

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