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Impact of Coupling Duration on Fecundity and Fertility of Muga Silkworm (*Antheraea assamensis* Helfer) During Grainage Operation

Mahasankar Majumdar [a++*], Vikram Kumar [b++], Lopamudra Guha [c#], Abhishek Singh [d++], K. Indirakumar [e++] and Kartik Neog [c†]

[a] Central Silk Board, MESSO, P4 Unit, Mendipathar, Meghalaya, India.
[b] Central Silk Board, MESSO, P3 Unit, Rompara, Meghalaya, India.
[c] Central Silk Board, MESSO, Guwahati, Assam, India.
[d] Central Silk Board, MESSO, P3 Unit, Nongpoh, Meghalaya, India.
[e] Central Silk Board, MESSO, P4 Unit, Tura, Meghalaya, India.

Authors' contributions

This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.

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ABSTRACT

Quality seed production is considered the backbone of the sericulture industry. Eggs are the primary and fundamental component of Muga culture, so the production of quality eggs is imperative. For successful production of cocoon crops, an adequate quantity of good-quality eggs is

[++]Scientist – C;
[#]Scientist – D;
[†] Director;
**Corresponding author: E-mail: maha.majumdar@gmail.com;*

essential. In the case of silk moth mating, the time varies from 5 to 8 hours, which is generally termed as the coupling period. The temporal aspects of mating in terms of duration may also impact the number of eggs laid, the pattern of egg laying, and their viability. The present investigation intends to find out whether the duration of the coupling period has any relation with the fecundity and fertility of silk moths. The study was conducted with the Muga Silkworm (*Antheraea assamensis* Helfer) in Baisakhi and Bhodia seed crops. The experiment reveals that total fecundity almost remains the same above 5 hours of coupling duration, but fertility is directly proportional to the coupling duration and reaches a maximum (85%) at 7-8 hours of coupling duration. It is also observed that coupling duration of more than 8 hours also affects the fecundity of Muga silk moths, but fertility remains the same as the control batch or the 7-8 hour batch.

Keywords: Fertility; fecundity; coupling; muga; egg.

1. INTRODUCTION

Seed is the backbone of the sericulture industry. The quality of silkworm seed may be defined as the one where the layings are entirely free from diseases, have a higher number of viable eggs, give uniform hatching, and assure a stable crop. The Muga silkworm (*Antheraea assamensis* Helfer) is multivoltine and has several crops in a year conducted outdoors, facing all the vagaries of nature. During summer, the temperature rises from 35 to 38˚C with fluctuations in temperature and humidity that interfere with Muga seed production for Kotia commercial crops. These results in the emergence of crippled moths, poor coupling aptitude, reduced egg-laying capacity, unfertilized eggs, poor embryonic development, desiccation of eggs, and leads to hatching failure [1-3]. The timely supply of an adequate quantity of good-quality, disease-free eggs to the sericulturists is crucial for the successful harvest of cocoon crops. This task assumes tremendous significance in topical sericulture activities, and practices are adapted to suit the conditions and conveniences of the rearers [4-6]. Since egg production of the silkworm is managed by seed producers, various processes such as procuring quality cocoons, moth emergence, mating, egg laying, preservation, and hatching of eggs are all important for maximizing viable egg production [7-11] Tazima, 1962; Kovalev, 1960; Ayuzawa et al., 1973.To sense the precise location and olfactory cues of the female moth the antennae of the male moth bend downwards during the mating period. Mating lasts for 10-12 hours but can continue up to 24 hours into the next day if undisturbed. Similar mating behavior has been reported in other wild silk moths (Kuang-Ming & Ta-Yuan, 1958; Singh &Debaraj, 2011; Singh et al., 2011a,b,c). Copulation by one male moth is sufficient for fertilization of the female moth. Male moths are utilized for a second mating when there is a shortage of fresh male moths. In natural conditions, male moths fly long distances in search of females, and female moths also fly, particularly after mating, to lay eggs on the leaves and branches of food plants. However, they usually do not fly during the daytime. The moths do not lay all eggs in one place but scatter them in batches to ensure filial survivability. Coupled moths detach with slight mechanical disturbance. The lifespan of adult moths is 7-10 days.

2. MATERIALS AND METHODS

The present study was carried out at the CSB, MESSO, P-4unit Mendipathar, North Garo Hills, Meghalaya, P-3 Unit Rompara, P-3 Unit Nongpoh, and P-4 Unit Tura from 2021 to 2023 to evaluate the effect of coupling duration in two different seed crop seasons, namely Baisakhi (April-May) and Bhodia (August-September). The details of the methodology adopted during the time of investigation are given below.

Experimental Design: Selected seed cocoons were harvested on the 8th day after the mounting of spinning silkworms. The cocoons were gently shaken to verify the number of live and dead pupae. All the cocoons were tested, and the percentage of pupation was calculated. Defective and deformed cocoons from the batches were removed, and the cocoons were retained for conducting the grainage operation and experiments, as explained below.

GrainageOperation: The selected 100 'Bhorpok' cocoons were placed on a bamboo mat and covered with a net. Three replications of each treatment (100 cocoons in each replication) were preserved separately in a cocooning room, where recommended temperature and relative humidity were maintained at 25°C ± 5°C and 75% ± 5%, respectively.

Fig. 1. Study area

Moth Emergence: On the expected day of emergence, it started at 3 pm onwards, and moths were allowed to emerge. After the moths emerged, mechanical coupling was conducted for different time slots. Mechanical coupling started at 6 am on the next day after emergence.

Coupling and Decoupling: After completion of emergence, the recommended ratio of male and female moths (3:1) was maintained in each time slot batch. In this experiment, each replication and time combination allowed for 10 female moths and 30 male moths. After coupling, decoupling was done as per the treatment, i.e., giving the coupling duration of 1 hour, 2 hours, 3 hours, 4 hours, 5 hours, 6 hours, 7 hours, 8 hours and 9 hours for each treatment. Then the females were subjected to oviposition and tied in *'Kharika,'* and the males were disposed of. At the time of coupling, decoupling, and oviposition, a temperature of 25°C ± 5°C and a relative humidity of 80% to 85% were maintained.

Treatment Details: Emerged females and males were kept for coupling for different durations.

T1: 1-hour time is given for coupling (6 am to 7 am)
T2: 2-hour time is given for coupling (6 am to 8 am)
T3: 3-hour time is given for coupling (6 am to 9 am)
T4: 4-hour time is given for coupling (6 am to 10 am)
T5: 5-hour time is given for coupling (6 am to 11 am)
T6: 6-hour time is given for coupling (6 am to 12 noon)
T7: 7-hour time is given for coupling (6 am to 1 pm)
T8: 8-hour time is given for coupling (6 am to 2 pm)
T9: 9-hour time is given for coupling (6 am to 3 pm)

(Control) T7: 7-hour time is given for coupling (6 am to 1pm)

Oviposition: After decoupling the females, females from separate treatments were individually kept for oviposition in an Oviposition room. Each moth was tied in a single *Kharika*, and the room was maintained in dark conditions. The moths were kept undisturbed for 72 hours in that room. Temperature and relative humidity were maintained at 25°C ± 5°C and 75% to 80%, respectively. These procedures were maintained in two selected different seed crop seasons.

Fig. 2. 7& 8 Hourscoupling shows better performance

Fig. 3. 1-5 Hours coupling shows less performance

Calculation of Fecundity: All treatments were maintained separately, and females were allowed to deposit the eggs. Eggs from different treatments were recorded. Eggs deposited by females in T7 were considered as controls. Each egg-laying female was recorded replication-wise for all treatments as mentioned above. Data obtained were statistically analyzed to calculate the fecundity of different treatments.

Calculation of Fertilized and Unfertilized Eggs: Fertilized (viable) eggs were also calculated along with unfertilized (unviable or depressed) eggs in different treatments to determine the number and percentage of fertilized and unfertilized eggs at each treatment. This assessment was conducted to better understand the percentage of fertilized eggs in terms of quantity from an economic perspective.

3. RESULTS AND DISCUSSION

The present study was undertaken to know the impact of different coupling durations on the reproductive performance in two different seasons i.e. Baisakhi and Bhodia in three consecutive years (2021-2023).

4. DISCUSSION

The following bar diagram can help us to vividly discuss the fertilization and fecundity of Muga Silkworm Baisakhi and Bhodia seed crops respectively.

The present study was undertaken to investigate the effect of different coupling durations of Muga Silkworm (*Antheraea assamensis* H) and its impact on the fertility and fecundity of different seed crop seasons. The study was conducted with 3 replicates per year of Muga silkworms, all of which showed that different types of coupling duration had a significant effect on total fecundity. Total fertility is much lower when coupling duration is between 1-5 hours than when it is 6-8 hours. In this study, it was observed that coupling durations of 7 hours and 8 hours showed better performance in terms of total fecundity, i.e., 148 nos. & 151 nos. in Baisakhi Crop and 165 nos. & 168 nos. in Bhodia Crops respectively (Table 1 & Table 2). However, the duration of coupling had a significant effect on the fertility of eggs. In the Baisakhi crop, 31.48% of eggs were fertilized in T1, followed by T2 (34.23%), T3 (44.83%), T4 (56.41%), T5 (67.16%), T6 (78.17%), T7 (85.14%), T8 (86.09%), and T9 (83.97%) [Table-1]. These

results indicate that the percentage of fertility significantly increased from T7 onwards. Similar results were found in the case of the Bhodia crop. In this crop, 29.82% of eggs were fertilized in T1, followed by T2 (31.90%), T3 (40.50%), T4 (52.07%), T5 (62.04%), T6 (69.48%), T7 (81.21%), T8 (82.14%), and T9 (81.02%) [Table-2]. These results indicate that the percentage of fertility significantly increased from T7 onwards. Therefore, 7-8 hours of coupling (T7 & T8) is necessary to ensure proper fertilization of Muga silkworm eggs. It is also observed that more than 8 hours coupling also affect fecundity. Inside the cocoon, the pupa transforms into a moth during metamorphosis. Moths emerge from the cocoon during the evening and continue until the next morning (Kakati &Benchamin, 2000). Male and female moths are easily identified by their colour, wings, size of the abdomen, and size of antennae (Singh et al., 2013). The body length of mothsis 3.5 cm (female) and 3 cm (male). Generally, male moths are more active than female moths. Male moths couple with female moths in the evening, and during coupling, they prefer a dark environment. Moths have two pairs of wings, the forewing and the hind wing (Arora & Gupta, 1979). In males, antennae are larger than in female moths. The antennae consist of two regions: the scape and pedicel (which contains sense organs and Johnston organ). Moths have three pairs of jointed legs, situated on the abdomen. After 8 to 10 hours of coupling, the female moth lays eggs on the *Kharika* (made of straw, known as “Sang Kher” in Assamese) (Sarmah et al., 2010). The

Table1. Number of total eggs laid, number of fertilized and unfertilized eggs in different treatments at Baisakhi Crop

Treatment	Number of total eggs	Number of fertilized eggs		Number of unfertilized eggs	
		Mean no	Mean%	Mean no	Mean%
T1	108	34	31.48	74	68.52
T2	111	38	34.23	73	65.77
T3	116	52	44.83	64	55.17
T4	117	66	56.41	51	43.59
T5	134	90	67.16	44	32.84
T6	142	111	78.17	31	21.83
T7	148	126	85.14	22	14.86
T8	151	130	86.09	21	13.91
T9	131	110	83.97	21	16.03
SE(m)	1.186	1.528		1.036	
C.D.	3.552	4.574		3.103	

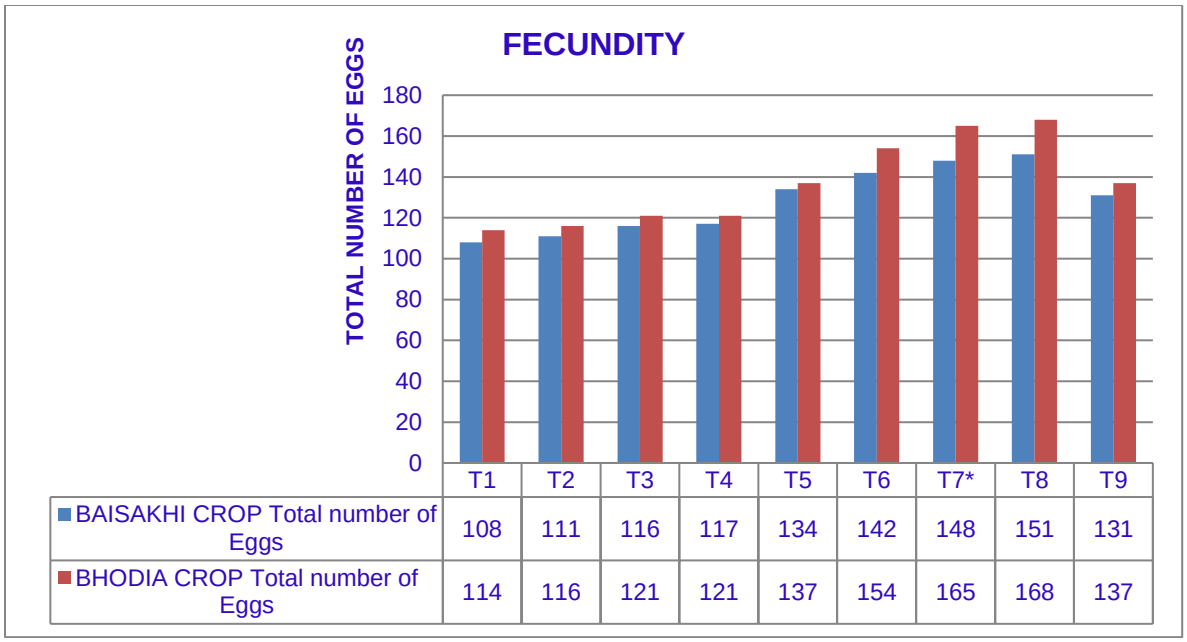
Note: T1 toT9 stated as Treatment details where T7 is control

Table2.Number of total eggs laid, number of fertilized and unfertilized eggs in different treatments at Bhodia Crop

Treatment	Number of total eggs	Number of fertilized eggs		Number of unfertilized eggs	
		Mean no	Mean%	Mean no	Mean%
T1	114	34	29.82	80	70.18
T2	116	37	31.90	79	68.10
T3	121	49	40.50	72	59.50
T4	121	63	52.07	58	47.93
T5	137	85	62.04	52	37.96
T6	154	107	69.48	47	30.52
T7	165	134	81.21	31	18.79
T8	168	138	82.14	30	17.86
T9	137	111	81.02	26	18.98
SE(m)	1.774	1.700		1.633	
C.D.	5.313	5.089		4.889	

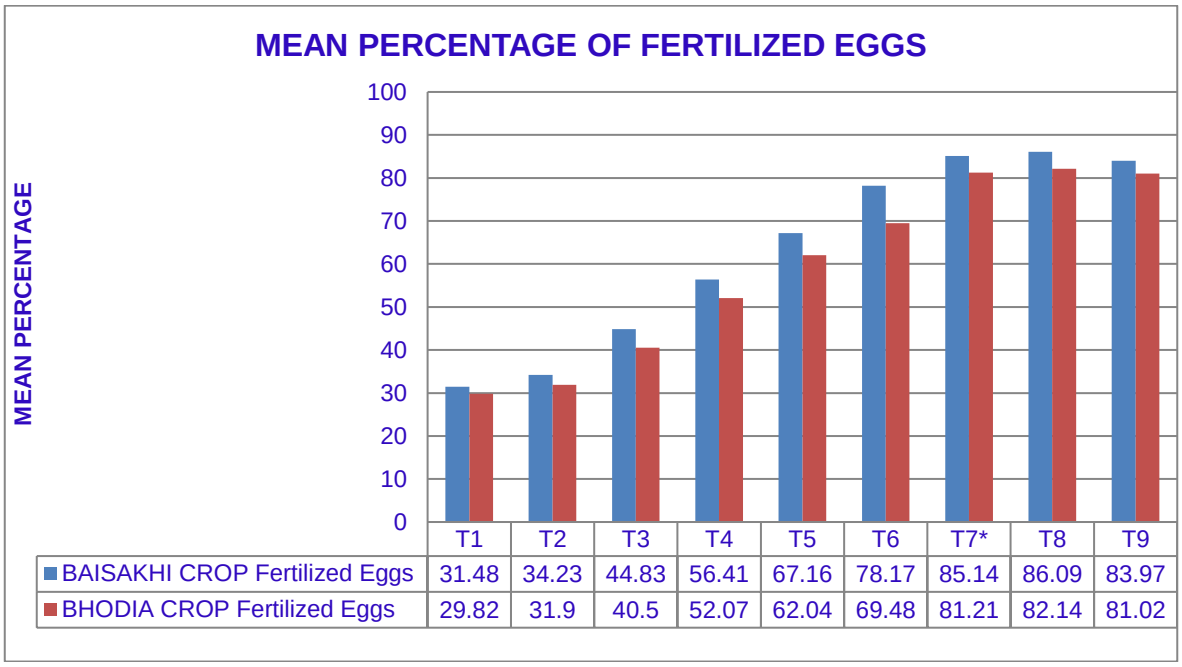
Note: T1 toT9 stated as Treatment details where T7 is control

female moth can lays eggs for up to 6 days, but those laid in the first three days are optimal for rearing purposes. During this stage, they do not consume food, and eventually, they die within 7 to 10 days (Jolly et al., 1975). It was also observed that 1-5 hours of coupling is not sufficient to fertilize all the eggs in certain cases. In this study, it is observed that the timing of emergence and mating has a significant impact on the reproductive success of *A. assamensis*. The timing of mating of coupling duration is important for the number and viability of eggs laid. Understanding these temporal aspects of reproduction could be crucial for developing effective survival stratigies for the mono-race of A. assamensis. Hence, through this study an effort is made to predict the role of actual coupling duration without affecting the chance of fertilization of eggs.



	T1	T2	T3	T4	T5	T6	T7*	T8	T9
BAISAKHI CROP Total number of Eggs	108	111	116	117	134	142	148	151	131
BHODIA CROP Total number of Eggs	114	116	121	121	137	154	165	168	137

Plate 1. Fecundity of muga moth



	T1	T2	T3	T4	T5	T6	T7*	T8	T9
BAISAKHI CROP Fertilized Eggs	31.48	34.23	44.83	56.41	67.16	78.17	85.14	86.09	83.97
BHODIA CROP Fertilized Eggs	29.82	31.9	40.5	52.07	62.04	69.48	81.21	82.14	81.02

Plate 2. Mean percentage fertilized muga eggs

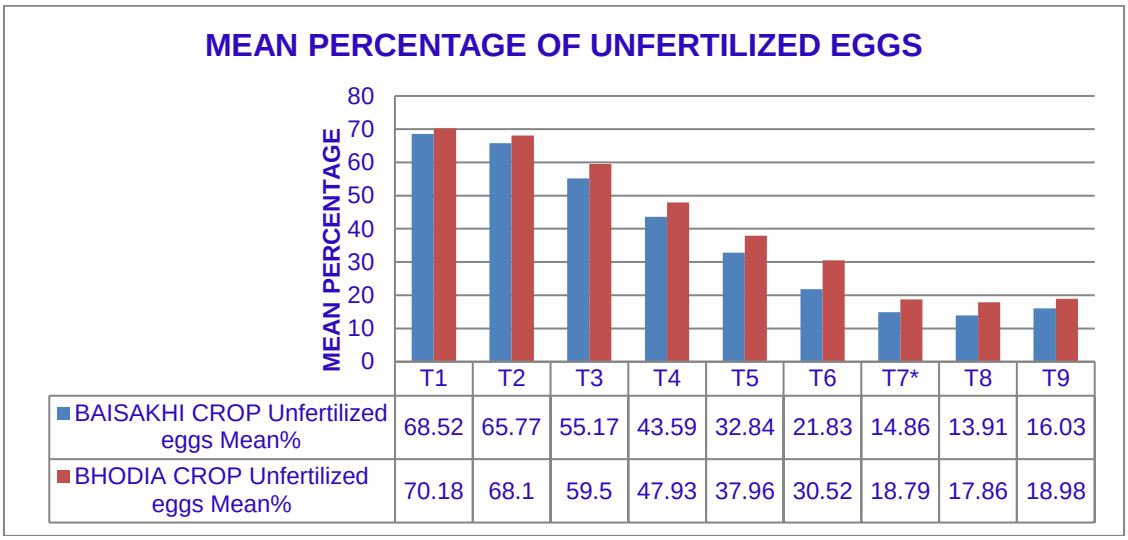


Plate 3. Mean percentage Unfertilized Muga Eggs

5. CONCLUSION

Our study underscores the critical impact of coupling duration on the fecundity and fertility of Muga silkworms. Optimal coupling periods of 7-8 hours significantly enhance fertility, ensuring a higher percentage of viable eggs. These findings provide valuable insights for sericulture practices, emphasizing the importance of precise grainage protocols to maximize egg quality and production. This study explores how variations in oviposition and time limited fecundity of female moths can impact egg production in seed production centres. By understanding these complexities, we can optimize the factors that influence egg production and gain insights into reproductive success in economically important moths. This knowledge could prove invaluable in improving grainage yields and meeting the growing demand for muga seeds in future.

COMPETING INTERESTS

Authors have declared that they have no known competing financial interests OR non-financial interests OR personal relationships that could have appeared to influence the work reported in this paper.

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