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Phenology of Eri silkworm *Samia ricini* (Donovan) in Lower Assam and its impact on upscaling eri seed production

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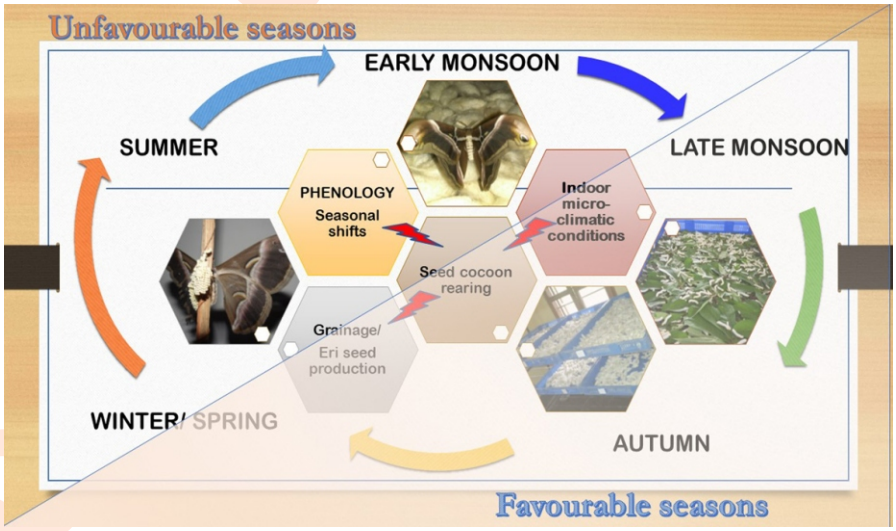
Abstract

Aim: A phenology study on the seed cocoon rearing performance of *Samia ricini* (Donovan) in different seasons of Lower Assam and subsequent production of disease-free laying was undertaken.

Methodology: In each season, four batches of seed cocoon rearing were conducted on eri silkworms of Borduar race (n=100 dfls) which were reared only on the castor, the primary host plant, to eliminate the variability due to different host plants. The data on different seed cocoon rearing and reproductive parameters were recorded for every rearing and pooled season-wise for comparison.



Results: The highest seed cocoon yield per dfl was recorded during autumn (215.92±6.24 nos) followed by winter/spring and late monsoon seasons. During autumn, significantly higher ERR% (90.69±1.01) was observed. Grainage operations conducted on the seed cocoons of various seasons revealed ideal dfl to cocoon ratio during autumn (1:3.5) and late monsoon (1:4.2). The percentage of moth emergence was significantly high in autumn (92.27± 2.90), followed by late monsoon (89.07± 5.38).

Interpretation: Results evidently indicated that late monsoon and autumn seasons spanning from September to November months can be utilized effectively for large scale eri silkworm seed production in Assam.

Key words: Eri silkworm, Phenology, Reproductive performance, *Samia ricini*, Seed cocoon rearing

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Introduction

The North-Eastern India is the home tract for several wild sericigenous insects (Boro and Borah, 2020; Hani and Das, 2019; Kakati and Chutia, 2009). Silk moths have been bred continuously for silk for over five thousand years, and their wild counterparts gradually became extinct (Dandin and Prabhu, 2023). The domesticated eri silk moth, *Samia ricini*, was derived from *Samia canningi* (Hutton) thousand years ago in Northeast India (Peigler and Luikham, 2013). Eri silk has been confounded in some literature with silks of *Samia cynthia* (Drury) from North-Eastern China and *Samia pryeri* (Butler) from Japan *Samia cynthia* was introduced in Europe during 1850 and in the United States in 1861, but is presently extinct in the United States (Peigler and Naumann, 2003). There are twenty-six ecoraces of *Samia ricini* distributed in different parts of North-east India (Debraj *et al.,* 2001; Singh and Ahmed, 2017). To conserve the existing eri silkworm eco races, seed production sector needs to be strengthened and proper gene bank maintenance is inevitable. Eri silkworm rearing is a traditional practice in Assam and eri silk is considered the pride of Assam and rearing of eri silkworm is a heritage and prerogative for the tribals of North-east India since time immemorial (Hani and Das, 2019; Tulu *et al.,* 2022).

Apart from silk, eri pupa is also relished as a delicacy due to its high nutritional value and enriched protein (Gangopadhyay *et al.,* 2022). The Brahmaputra valley of Assam and its neighbouring states together produce more than 90% of eri cocoons (Brahma *et al.,* 2019). On the other hand, to promote expansion of ericulture in non-traditional states strengthening of eri silkworm seed production in the traditional states becomes essential (Swathiga *et al.,* 2019). In recent years, the eri culture is becoming popular in non-traditional states *viz.* Andhra Pradesh, Tamil Nadu, Orissa, West Bengal, Sikkim, Jharkhand, Bihar, Punjab and Uttar Pradesh (Sarmah *et al.,* 2012; Tamta and Mahajan, 2021). In North-east India, the production of superior quality seed cocoons is challenging due to impending climate change and global warming that induces seasonal shifts and extreme climatic fluctuations. Also, each favourable season for commercial eri rearing demands seed cocoon during unfavourable season for egg production (Elumalai *et al.,* 2021; Lalitha *et al.,* 2017). Phenology is the study of relationships between environmental conditions and biological processes of silkworm development. Silkworm growth and developmental processes are influenced by their physiology determined by several factors such as metabolic rate, enzymes activities, nutrient conversion, digestion, assimilations, excretion, nervous stimulation, hormonal actions, etc.

Temperature and humidity also fluctuate widely, not only season to season but also within the day itself during any season. With the rise in temperature, the metabolic activities of the worms increase, while they retard during low temperature. Therefore, at high optimum temperature and relative humidity, the growth of larva is rapid and consequently the duration of the larval period is shortened. On the other hand, growth is slow at low temperature with prolonged larval duration. Therefore, studies on phenology of eri silkworm across the prevailing seasons in Lower Assam can provide scientific basis for shifting the seed cocoon crop schedules to achieve timely supply of superior quality eri seeds. In the present study, an attempt was made to evaluate the phenology of eri silkworm (Borduar race), seed cocoon rearing and its reproductive performance under the influence of different seasons as experienced in Lower Assam during 2019-20. The study can envisage a model to reframe the prevailing brushing schedules of eri silkworm seed crop and upscale eri seed production for large-scale commercial crops.

Materials and Methods

The study was conducted in Kamrup District of Lower Assam during summer (April- June 2019), early monsoon (July-August, 2019), late monsoon (September, 2019), autumn (October to November, 2019) and winter/ spring (December to March 2020). In each season, four batches of seed cocoon rearing were conducted on disease free layings of Borduar race of eri silkworms produced from EBSF, Topatoli. Eri silkworm seed cocoon rearing was conducted in selected adopted seed rearer (ASR) field and its respective grainage was conducted at Eri Basic Seed Farm of Central Silk Board at Topatoli, Kamrup District Assam. Though indoor rearing, the seed cocoon rearing in different ASRs field at Lower Assam lacked infrastructure for manipulating the temperature and relative humidity conditions and hence, there was a profound influence in the phenological phases of growth and development of eri silkworm.

The rearing was conducted only on castor, the primary host plant, to eliminate variability due to different host plants. Seed cocoon rearing was conducted on castor leaves in shelf method as per standard methods (Singh and Ahmed, 2017; Dulumani, 2015). The eri silkworm larvae were fed twice during first instar, thrice during second and third instar, four times during fourth and fifth instar. The first three instar worms were fed with tender leaves whereas the fourth and fifth instar worms were fed with mature leaves. The mature worms were hand picked from the rearing trays and transferred to mountage for spinning. The matured seed cocoons were harvested after completion of spinning and pupa formation. Data on different seed cocoon rearing parameters such as developmental duration, percent mortality due to uzifly infestation and other diseases were recorded. The effective rate of rearing (ERR) was calculated by finding the percentage of cocoon harvested with respect to number of worms brushed. The seed cocoon yield per dfl was recorded for every rearing and pooled season-wise for comparison.

The harvested healthy seed cocoons were procured from ASRs field and kept for emergence in plastic trays in a single layer at eri basic seed farm. The plastic trays were arranged in stands following three tier system in a properly cross ventilated grainage hall. After emergence, the adults were allowed to expand and harden their wings. Emerged valid male and female moths were kept in moth cages for coupling. Natural mating occurred within 2-

3 hrs of moth emergence. After 6 hrs of coupling, the fertilized female moths were placed in traditional ovipositional device, 'Kharika' and allowed to lay egg. The egg laying was carried out for 2 days and the mother moths were subjected to examination (Fujiwara *et al.,* 1984) for pebrine detection.

The dfls of the entire batch were harvested after individual mother moth examination and surface sterilized. The grainage parameters such as moth emergence, fecundity and cocoon to dfl recovery was recorded. The cocoon: dfl ratio was calculated by finding the ratio of number of seed cocoon processed to the number of dfls harvested. Data was subjected to analysis of variance (ANOVA) to test the significance of seed cocoon rearing performance across the seasons. The pooled data of 4 batches of seed cocoon rearing and their respective grainage trials conducted during the specific season is expressed as mean ± standard error. Multiple comparison of means was made depending on F-ratio and the critical difference (CD) values based on student-t criteria at 5% and 1% level of significance following the standard methods (Gomez and Gomez, 1984).

Results and Discussion

The pooled mean of duration of phenological phases recorded during seed cocoon rearing, temperature range and relative humidity recorded during the season and respective reproductive performance in grainage operations of four batches of eri silkworm seed production at each season are presented in Table 1 and 2. The experimental findings of the present study, revealed significantly higher hatching percentage during autumn (84.25± 2.78), followed by late monsoon (82.75± 3.12). The lowest hatching percentage (72.75±2.17) was recorded during summer. This is due to the deposition of unfertilized eggs and low coupling efficacy during summer affected by high temperature ranging from 32-37^{0}C. Higher coupling was observed with the highest hatching percentage during September to November.

The data on duration of different phenological phases recorded during seed cocoon rearing revealed that the egg incubation period (10.25± 0.75 days) and larval period (25.00 ± 1.47 days) were significantly longer in winter/spring and shortest during summer (6.75± 0.25 days and16.25± 0.25 days). The early and late monsoon seasons were on par for all the phenophases. The larval duration for rearings spanning between April to September months ranged between 16-17 days and were found statistically on par. Nevertheless, mortality due to other diseases were high during monsoon and summer seasons. The percent larval mortality during summer (11.52±2.24) was significantly high due to uzi fly. Pebrine was controlled in all seasons by proper disinfection and intermittent examination. However, incidence of other diseases like flacherie, CPV were noticed and significantly higher larval mortality (%) was recorded during late monsoon (30.42±2.15) followed by early monsoon (28.22±5.82) and summer (24.14±6.29). The lowest larval mortality was noticed during autumn followed by winter/spring season. The total developmental duration during summer and monsoon seasons were found on par. However, significantly extended duration of development was noticed during autumn (25.00±0.82 days) and winter/ spring (28.00±1.47 days). The life cycle of eri silkworm is increased due to the lesser day threshold temperature in winter (Singh and Benchamin, 2002). The developmental rate of silkworm embryo was directly influenced by temperature and relative humidity (Rahmathulla, 2012). Due to their poikilothermic nature, silkworms' body temperatures are directly influenced by the ambient temperature. The rise in ambient temperature, accelerate larval growth and shortens larval period, while low temperatures, lengthens the larval period and delays the growth.

The physiology of silkworm is directly affected by the temperature during rearing, including digestion, blood flow and breathing. Similarly, humidity helps to decrease the length of growing period of silkworm by accelerating the activity of physiological function. The pH of heamolymph is low at high humidity (80-90%) than at low humidity 60% condition. Expiration of CO_2 increases with rise in humidity. On the contrary, low humidity prolongs the length of the growing period of larva (Das *et al.,* 2021; Teronpi *et al.,* 2020). The combined effect of both temperature and humidity regulates the growth of the silkworms. Temperature and humidity are complementary to each other. Humidity directly influences the physiology of silkworm larva, while indirectly influences the rate of withering of leaves in larval beds. Numerous substantial researches on how temperature and relative humidity affect the biological and commercial characteristics of cocoons have been reported earlier (Sugai and Takashashi, 1981; Sahu *et al.*, 2006; Vaidya *et al.*, 2014).

The optimum temperature range (25-30^{0}C) and relative humidity (80-90%) experienced during autumn season was conducive for the seed cocoon rearing. Effective rate of rearing is the best indicator of survivability of silkworm during the crop. Significantly, highest ERR (%) was recorded in autumn (90.69±1.01), followed by winter/ spring (85.35± 4.30). The ERR% observed in summer, early monsoon and late monsoon were statistically on par and lower as compared to autumn and winter. Earlier studies on different strains of *Samia ricini* revealed higher ERR% (92-95) in autumn and spring. Autumn and spring were considered better for all six strains studied in terms of ERR than winter and summer (Sharma and Kalita, 2017). Teronpi *et al.* (2020) reported that autumn season showed better performance than spring season in respect of weight of full-grown matured larvae and ERR. Summer season recorded less productivity due to low rate of feeding, while winter revealed low productivity performance due to low food conversion efficiency.

The highest seed cocoon yield per dfl was recorded during autumn (215.92±6.24nos) followed by winter/ spring and late monsoon seasons. The lowest seed cocoon yield was recorded during early monsoon (128.43± 29.89) and summer (147.94±15.34). The critical factors that reduced the seed cocoon yield per dfl during early monsoon and summer were low hatching percentage, mortality due to uzi infestation, diseases and impact

Table 1: Phenophases of Eri silkworm seed cocoon rearing in different seasons

Seasons (Months)	Temp range (°C)	RH range (%)	Rearing parameters					Mortality (%)	
			Hatching (%)	Incubation period	Larval period	Pupal period	Total Duration (Egg –adult emergence)	Uzi	Others
Summer (Apr- Jun)	26-37	69-82	72.75±2.17	6.75±0.25	16.25±0.25	15.50±0.29	38.75±0.50	11.52±2.24	24.14±6.29
Early Monsoon (July- Aug)	26-34	76-85	78.25±1.35	7.25±0.25	16.25±0.25	16.25±0.48	39.50±0.63	8.12±2.95	28.22±5.82
Late Monsoon (Sept)	25-32	82-90	82.75±3.12	7.50±0.29	16.75±0.25	17.25±0.48	41.50±0.50	2.37±0.39	30.42±2.15
Autumn (Oct-Nov)	25-30	80-90	84.25±2.78	8.75±1.18	25.00±0.82	22.00±2.16	55.75±2.53	0.00	9.31±1.01
Winter/ Spring (Dec- Mar)	16-23	65-92	74.25±0.48	10.25±0.75	28.00±1.47	31.50±0.65	69.75±2.50	0.86±0.15	13.75±4.32
CD value			5.61*	1.36*	1.66**	2.38**	3.51**	3.55**	9.42**

Each value represents pooled data of 4 batches of seed cocoon rearing trials conducted during the specific season as mean ±S.D. ** Significant at 1% level; * Significant at 5% level

Table 2: Eri silkworm grainage parameters and respective seed cocoon rearing parameters of different seasons

Seasons (Months)	ERR(%)	Seed cocoon yield per dfl	Valid moth emergence (%)	Coupling percentage	Fecundity	Cocoon to dfl recovery
Summer (Apr- Jun)	64.34±7.14	147.94±15.34	70.44±3.21	24.17 ± 0.31	263.25±14.07	7.08±0.71
Early Monsoon (July- Aug)	63.66±7.99	128.43±29.89	77.38±5.69	27.12 ± 2.34	284.25±11.27	8.50±1.08
Late Monsoon (Sept)	67.20±2.15	151.15±4.17	89.07±5.38	38.38 ± 0.77	310.75±6.56	4.26±0.45
Autumn (Oct-Nov)	90.69±1.01	215.92±6.24	92.27±2.90	41.29 ± 2.08	339.75±13.08	3.49±0.48
Winter/ Spring (Dec- Mar)	85.35±4.30	173.14±6.37	79.16±5.31	27.06 ± 4.29	281.75±6.97	6.06±0.77
CD value	11.24**	33.37*	9.88*	4.44*	29.84*	1.57*

Each value represents pooled data of 4 batches of seed cocoonrearing trials conducted during the specific season as mean ±S.D. ** Significant at 1% level; * Significant at 5% level

of high temperature during spinning. The important grainage parameters that determine the ideal seed production process are the moth emergence percentage, effective coupling, fecundity and eventually the seed cocoon: dfl recovery. The percentage of valid moth emergence with effective coupling was significantly high in autumn season (92.27±2.90) followed by late monsoon (89.07±5.38) and winter/spring (79.16±5.31). Non-emergence of moth from seed cocoons is one of the vital parameters that can hamper the success of eri silkworm seed production. The present results revealed ideal valid moth emergence from seed cocoons during autumn (92.27±2.90%), and late monsoon (89.07±5.38 %) as compared to all other seasons. Fluctuation of temperature and humidity has significant influence on grainage characters, such as irregular emergence of moth, occurrence of cripple moth and unhealthy moths (Elumalai *et al.,* 2021). The coupling percentage was found significantly better in autumn (41.29 ±2.08) and late monsoon (38.38 ± 0.77) as compared to other seasons. Evidently, the coupling percentage was lower in

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summer (24.17± 0.31) and early monsoon (27.12 ± 2.34). During summer and early monsoon seasons spanning from April to August, high fluctuations in temperature (26-37^{0}C) and humidity (69 to 85%) was reported. In a previous study, Hussain *et al.* (2011) demonstrated that fluctuations in rearing temperature and humidity adversely affects the egg production and egg fertility in silkworm moths. The perusal data revealed significantly higher fecundity during autumn (339.75±13.08), followed by late monsoon (310.75±6.56). The lowest fecundity was recorded during summer (263.25± 14.07) and early monsoon (284.25± 11.27). The results are in agreement with the trends shown by Sharma and Kalita, (2017) on rearing performance of six strains of eri silk worm in four different seasons.

The cocoon to dfl ratio determines the economic feasibility and acts as an indicator for success and failure of grainage operations. The ideal cocoon to dfl ratio was found during autumn (1:3.5) and late monsoon (1:4.3). Poor dfl recovery was recorded during early monsoon (1:8), followed by summer (1:7). Generally, the valid moth emergence and coupling percent are positively correlated. Invalid moth emergence noticed during summer and late monsoon increased the cocoon to dfl ratio, leading to low seed yield. The phenology of eri silkworm and seed cocoon performance were primarily controlled by the ambient microclimatic conditions influenced by temperature and relative humidity. The present study clearly indicated that autumn and late monsoon seasons spanning from September to Mid- November months showed significantly high seed production performance. Previous studies (Lalitha *et al.,* 2017, Dulumani, 2015, Sarkar *et al.,* 2010, Kumar and Elangovan, 2010; Debraj *et al.,* 2003) have reported favourable grainage characters during autumn season. Seed cocoon rearing temperature, relative humidity, host leaf nutrition (Sarmah *et al.,* 2015; Hazarika *et al.,* 2003) and adoption of standard rearing practices (Basumatary *et al.,* 2003) were identified as other critical factors determining the grainage performance. Summer months are unsuitable for eri silkworm seed production due to less number of moth emergence with low coupling recovery, low fecundity and low hatchability of eggs.

Higher proportion of non-emerged cocoons, emergence of crippled moths and adverse effects on ovulation and oviposition is mainly attributed to high temperature experienced during spinning (Lalitha *et al.,* 2020; Das *et al.,* 2021). Among all the silkworms, eri silkworm is hardy and comparatively more tolerant to temperature and humidity fluctuations (Thangavelu *et al.,* 1986; Prasad and Saha, 1992). However, due to global warming, the summer months are gradually extending for more period in Lower Assam and also becoming critical with prolonged high temperature, which is found detrimental to the development of eri silkworms. Proper care advocation during feeding, moulting, larval maturation and spinning may prevent crop loss. Manipulation of microclimatic conditions to combat adverse effect of high temperature and humidity during rearing and grainage operations is one of the feasible options. It may be concluded from the present study on phenology of eri silkworm that upscaling eri silkworm seed production should be encouraged and scheduled during autumn and late monsoon season and can be avoided during summer at ASRs field in Lower Assam climatic conditions. Seed cocoon rearing during winter/ spring season can provide good quality seeds for the upcoming summer season. But eri seed rearing in summer should be handled in controlled conditions carefully to obtain optimum results. The current study also depicts the realistic field performance of ASRs in Lower assam across the season from where seed cocoons are procured in large quantities for eri silkworm seed production. These findings have implications for prior planning for pre-seed and seed crop of eri silkworm to ensure successive commercial crop rearing massively for enhancement of raw silk production.

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