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Significance of Cocoon Refrigeration on the Larval Performance of Multivoltine Mulberry Silkworm (*Bombyx mori* Linn.)

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Abstract: *Bombyx mori* has an economic importance because of the commercial values of its silk that is called the queen of textiles. The silk is preferred over all other types of fibers due to its remarkable properties like water absorbency, heat resistance, dyeing efficiency and luster. The present study reveals the effect of cocoon refrigeration on the larval performance i.e., larval weight, larval duration and survival of larvae. The cocoons were consigned to low temperature at 5°C at 0, 2, 4, 6 and 8 days of prerefrigeration and refrigerated for 0, 5, 10, 15, 20, 25 and 30 days. The maximum larval weight was noticed in case of 0 day prerefrigerated-0day refrigerated cocoons. The survival of larvae was noticed maximum in case of 6 dayprerefrigerated-0day refrigerated cocoons and the maximum larval duration was noticed in case of 2 day pre-refrigerated-30 day refrigerated cocoons. At low temperature, the insects will get acclimatized.

Key words: Prerefrigeration period · Refrigeration period · Larval weight · Larval duration · Survival of larvae

INTRODUCTION

Silkworm is one of the most important domesticated insects, which produces luxuriant silk thread in the form of cocoon by consuming mulberry leaves during larval period. The mulberry silkworm *Bombyx mori* L. is very delicate, highly sensitive to environmental fluctuations and unable to survive extreme natural fluctuation in temperature and humidity because of their long years of domestication since 5000 years. Thus the adaptability to environmental conditions in the silkworm is quite different from those of wild silkworm and other insects. The larval duration, larval weight and survival of larvae are the most important factor which influences the production of cocoon on commercial scale. The efforts are being made to evolve new technologies that are cost effective, labour saving and eco friendly. In order to increase the production of silk, efforts have been made to study the effect of temperature [1], relative humidity [2], photoperiod [3], artificial diet [4], X-rays [5], magnetization of eggs [6, 7], magnetization of larvae [8], magnetization of cocoon [9], incubation period of eggs [10], long term preservation of cocoon [11], global warming [12], 20- hydroxy ecdysone hormone [13] etc on the performance of *B.mori*. Rise in temperature increase various physiological fluctuations and with a fall in temperature, the physiological activities are decreased. The variation in the environmental conditions during the last decade emphasizes the need of management of the temperature for sustainable production. The present study is an attempt to study the effect of refrigeration of cocoon on the larval performances.

MATERIALS AND METHODS

Seed Cocoon: The seed cocoon (pupa enclosed in silken case) of multivoltine mulberry silkworm *Bombyx mori* nistari, a native of west Bengal in India, was taken in the present study. The seed cocoon (pupa enclosed in silken case), obtained from the silkworm grainage Behraich, Directorate of Sericulture Uttar Pradesh and were maintained in the plywood trays (23×20×5 cm) under the ideal rearing condition in the silkworm laboratory [14] Deen Dayal Upadhyaya Gorakhpur University, Gorakhpur. The temperature, relative humidity and photoperiod were maintained at 26±1°C, 80±5% RH and 12±1 hours light a day respectively till the emergence of moth from the seed cocoon. The moths emerged generally in the morning at

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around 4 am. The trays, in which seed cocoon were kept, were suddenly illuminated by light in the morning at 4 O'clock on 9th and 10th day of spinning. The newly emerged moth, from seed cocoons, were quickly picked up and kept sex-wise in separate trays to avoid copulation. The male moths were smaller and more active than the female moths. The whole grainage operation was performed [14, 15].

Rearing of Larvae: After two consecutive days of hatching, the silkworm larvae were collected with the help of bird's feather and reared to maintain a stock culture in the silkworm laboratory at 26±1°C, 80±5% RH and 12±1 hour light a day. Four feeding of the small pieces of fresh and clean leaves of *Morus alba* were given to the larvae and care was taken that food always remained in excess in the rearing trays. After completion of fifth instars, the ripe worms ceased feeding and ready for spinning. Now small mountages were provided to the ripe worm. The ripe worms soon begin the mounting which was completed within three days. Thus sufficient number of cocoons was obtained.

Experimental Designs: The seed cocoon was obtained from the silkworm grainage and was maintained in the laboratory. The moths emerged were allowed to mate. After mating the female moths were allowed for egg laying. The eggs laid were transferred to the BOD incubator for hatching. After hatching the larvae were collected and reared to maintain a stock culture in the laboratory. After completion of fifth instars, the mountages were provided to the ripe worms for the formation of cocoon. Thus sufficient no. of cocoons was obtained which were used for further experiments to the refrigeration of cocoon.

Refrigeration of Cocoon: The cocoons obtained from the laboratory were refrigerated for different duration at varying conditions of pre-refrigerated periods of cocoons. The 'pre-refrigeration' period refers to be the duration between the completion of the cocoon formation and beginning time of the refrigeration of experimental cocoon. The zero day (0 day) refrigeration of cocoons refers to be the control (no refrigeration of cocoon). During the pre refrigeration period the cocoons were kept in BOD incubator maintained at optimum condition of temperature, relative humidity and photoperiod at 26±1° C, 80±5% RH and 12 hours dim light a day respectively For the refrigeration of cocoon 630 cocoons were consigned to low temperature at 5°C at '0 day' prerefrigeration period (one of the prerefrigeration conditions i.e. control). The cocoons were refrigerated for 0,5,10,15,20,25 and 30 days. For this purpose a group of 90 cocoons (30 cocoons in each of the three batches.) were released at once without any refrigeration (0 day pre refrigeration and 0 day of refrigeration) of cocoon which was taken as control. Further the rest of 540 cocoons were consigned at 5°C inside the refrigerator. After this a second group of 90 cocoons (three batches of 30 cocoons in each batch) were cold treated for 5 day refrigeration and were released from refrigeration accordingly in the groups of cocoons as in previous cases, after 10,15, 20,25 and 30 days of transferred chronically to BOD incubator maintained at optimum conditions of rearing. Three replicates of each experiment were made.

Like the above experimental designing at 0 day of pre refrigeration period, the similar series experiments were performed for the refrigeration of cocoons at 2,4,6 and 8 day of pre refrigeration periods of the cocoon. The moth emerged commonly in the morning at around 4:00 AM. The newly emerged moths were kept sex wise in separate trays to avoid copulation within the same group. Further, three batches each containing 5 good males and 5 good females were made and they were allowed to mate. After 4 hours of mating, the paired moths were decoupled manually. Further the gravid females were allowed to lay eggs on the sheet of paper. The egg laying moths were covered by open plastic cellules to prevent the intermixing of egg masses deposited by different female moths. After 24 hours of egg layings, the female moths were individually examined for their disease freeness. The disease free laying (DFLs) thus prepared, were washed with 2 % formalin for 15 minute.To increase the adhesiveness of eggs on cards and surface disinfections. Thereafter the egg sheets, with eggs laid on were thoroughly washed with running water to remove formalin the eggs were dried in BOD incubators maintained in the laboratory. After two consecutive days of hatching, the silkworm larvae were collected with the help of brush and reared to maintain a stock culture in the silkworm laboratory at 26±1°C, 80±5% RH and 12±1 hours light a day.

Larval weight-For the determination of larval weight 30 larvae (three batches of 10 larvae in each batch) were recorded. Three replicates of each experiment were made; the larval weight was taken on the day when fifth instar larvae stop feeding.

Larval duration-For the determination of larval duration 90 larvae (three batches of 30, larvae in each batch) was taken for observation. The time required from the hatching of larvae to the third day of spinning by the fifth instar larvae was considered as larval duration.

Survival of larvae-For determining the survival of larvae 90 larvae (three batches of 30, 3rd instar larvae in each batch) were taken under the observation. The number of larvae which attained the pupal stage was counted for the calculation of the survival of larvae as following.

$$\text{Percent survival of larvae} = \frac{\text{No of larvae pupated}}{\text{No of 3}^{rd}\text{ instar larvae taken for observation}} \times 100$$

RESULTS

Larval weight: The data obtained from table-1 clearly indicates that the duration of cold storage and pre refrigeration period of silkworm cocoon have considerable impact on the weight gained by the larvae of silkworm larvae. At all the durations (0, 2, 4, 6 and 8 days) of prerefrigeration, the weight gained by the larvae of refrigerated cocoons declined considerably with the increasing duration of refrigeration. At the prerefrigeration periods of 0, 2, 4, 6 and 8 days, the larval weight decreased from 1.700±0.007 to 1.180±0.011 g, 1.645±0.018 to 0.956±0.043 g, 1.581±0.023 to 1.101±0.045 g, 1.540±0.036 to 1.198±0.014 g and 1.490±0.047 to 1.280±0.026 g, respectively with the increase in the refrigeration period from 0 to 30 days. The larval weight was (1.700±0.007 g) maximum in case of untreated cocoons and minimum (0.956±0.043 g) in case of 2 day prerefrigerated-30 day refrigerated cocoons. Two-way ANOVA indicates that both the duration of refrigeration and prerefrigeration significantly ($P<0.01$) influenced the weight of silkworm larvae.

Larval duration: The data obtained from table -2 clearly indicates that both the refrigeration period and prerefrigeration period of cocoon influenced the larval duration of silkworm. At all the conditions of prerefrigeration periods (0, 2, 4, 6 and 8 days) the time required from 1st instars to fifth instars larvae has been noticed to be increased considerably with the increasing duration (up to 30 days) of cold storage of cocoons. In all the conditions of cold storage i.e. 0, 2, 4, 6 and 8 days, the larval duration increased from 25.01±0.300 to 27.90±0.291 days, 24.60±0.256 to 29.72±0.283 days, 24.34±0.326 to 28.89±0.264 days, 24.10±0.048 to 28.23±0.121 days and 23.60±0.216 to 27.44±0.280 days respectively with the increase in the refrigeration period from 0 to 30 days. Maximum (29.72±0.283) larval duration was noticed in 2 days prerefrigerated – 30 days refrigerated cocoons while it was minimum (23.60±0.216) in case of non-refrigerated cocoons. The rate of increase in the larval duration was

Table 1: Effect of the refrigeration of cocoon on the weight (g) of *Bombyx mori* larvae.

Prerefrigeration Period (days)	Refrigeration Period (days) 0	5	10	15	20	25	30	F_1-ratio n_1=6
0	1.700±0.007	1.521±0.012	1.455±0.022	1.380±0.011	1.347±0.021	1.286±0.018	1.180±0.011	7.2313*
2	1.645±0.018	1.463±0.027	1.421±0.013	1.338±0.029	1.306±0.011	1.173±0.015	0.956±0.043	
4	1.581±0.023	1.411±0.011	1.386±0.011	1.299±0.005	1.246±0.013	1.101±0.045	N.Sd	
6	1.540±0.036	1.375±0.014	1.346±0.021	1.271±0.009	1.198±0.014	N.Sd	N.Sd	
8	1.490±0.047	1.326±0.020	1.280±0.026	N.Sd	N.Sd	N.Sd	N.Sd	

F_2-ratio= 6.3942* n_2=4

*P<0.01 N.Sd = Not Survived

Each value represents mean± S.E of three replicates.

Table 2: Effect of the refrigeration of cocoon on the duration (days) of *Bombyx mori* larvae.

Prerefrigeration Period(days)	Refrigeration Period (days) 0	5	10	15	20	25	30	F_1-ratio n_1=6
0	25.01±0.300	25.19±0.459	25.36±0.320	26.07±0.313	26.62±0.270	27.51±0.247	27.90±0.291	1.7143*
2	24.60±0.326	25.37±0.315	25.70±0.334	26.41±0.300	27.26±0.463	28.01±0.302	29.72±0.283	
4	24.34±0.326	25.62±0.230	26.16±0.460	27.06±0.520	27.90±0.291	28.89±0.264	N.Sd	
6	24.10±0.048	25.92±0.282	26.60±0.277	27.39±0.577	28.23±0.121	N.Sd	N.Sd	
8	23.60±0.216	26.26±0.051	27.44±0.280	N.Sd	N.Sd	N.Sd	N.Sd	

F_2-ratio= 3.5757* n_2=4

*P< 0.01 N.Sd=Not survived

Each value represents mean ± S.E. of three replicates.

Table 3: Effect of the refrigeration of cocoon on the survival (%) of *Bombyx mori* larvae.

Prerefrigeration Period (days)	Refrigeration Period (days)							F_1-ratio
	0	5	10	15	20	25	30	n_1=6
0	79.99±1.925	76.29±0.978	73.32±1.113	71.10±0.643	68.88±1.110	65.92±1.334	59.62±1.337	6.6897*
2	82.21±1.281	74.43±1.284	71.47±1.615	69.25±0.973	66.29±0.978	61.47±1.481	52.21±2.940	
4	84.81±2.892	71.10±2.313	68.14±1.957	66.28±1.612	64.07±1.334	54.06±2.063	N.Sd	
6	89.99±1.925	69.25±2.892	66.29±2.670	64.07±2.590	60.36±0.978	N.Sd	N.Sd	
8	81.10±2.939	67.03±2.063	65.18±1.957	N.Sd	N.Sd	N.Sd	N.Sd	

F_2-ratio= 5.4813* n_2=4

*p<0.01 N.Sd=Not Survived

Each value represents mean± S.E of three replicates.

almost of similar trend in all the cases of the prerefrigeration of cocoon. In 4, 6 and 8 days of prerefrigerated conditions, the pupae did not survived after 25, 20 and 10 days of refrigeration period. Two way ANOVA indicates that both the duration of refrigeration and prerefrigeration have significant (P<0.01) influence on the larval duration.

Survival of Larvae: The data presented in table 3 clearly indicates that both, the prerefrigeration period and the duration of refrigeration have very low degree of influence on the survival of larvae. For all the prerefrigeration periods i.e. 0, 2, 4, 6 and 8 days, the survival of larvae, obtained from the refrigerated cocoons, decreased with the increasing duration of refrigeration from 0 to 30 days. In all the conditions of prerefrigeration period i.e.0, 2, 4, 6 and 8 days, the survivility percentage of larvae decreased slowly from 79.99±1.925 to 59.62±1.337%, 82.21±1.281 to 52.21±2.940%, 84.81±2.892 to 54.06±2.063%, 89.99±1.925 to 60.36±0.978% and obtained from the refrigerated cocoons, decreased with the increasing duration of refrigeration. The declining trend of the survival percentage with the increasing refrigeration period was common in all the cases. Two way ANOVA indicates that both the duration of refrigeration and prerefrigeration have significant (P<0.01) influence on the survival of larvae.

The cold storage duration and prerefrigeration period both caused considerable impact on the larval performances. It is concluded that the cold storage of *B. mori* cocoon, up to 10 days, has almost no negative impact with regard to the larval performances. Thus if required, the cocoons of *B.mori* nistari can be refrigerated up to 10 days for the purpose of commercial rearing.

DISCUSSION

The duration of cold storage and the prerefrigeration period both have considerable impact on the weight gained by *B. mori* larvae. The larval weight of silkworm has been reported to vary in accordance with the variation in the varieties of same host plant, used for feeding of *B. mori* [16]. The larval weight of *B. mori* has been noticed to be affected by the starvation of larvae [17]. Photoperiod influence larval weight [3]. Relative humidity influences weight of *B. mori* larvae [18]. Refrigeration of cocoon influence larval weight [19]. Refrigeration of eggs at blue pigmentation stage of *B. mori* influence larval weight [20]. Refrigeration of silkworm eggs at blue eye spot influence weight of silkworm larvae [21]. Refrigeration duration of *B. mori* eggs influences weight of silkworm larvae [22]. Seasonal variation influence larval weight [23]. The Artificial diet influence larval weight [4]. X-rays influence larval weight [5]. The magnetization of eggs influence larval weight [6]. Cocoon magnetization influence larval weight [7]. Effect of magnetization on larval weight of *B. mori* L [8]. Cocoon magnetization influence larval weight of *B. mori* [9]. Nutrition influence weight of *B. mori* larvae [24-25]. Protein and fiber constituent influence weight of silkworm larvae [26]. Herbal tonic influence larval weight [27]. Antibiotics influence the larval weight of *B. mori* [28].

The larval duration is an important economic parameter to measure the relative growth rates. It is well known that an ideal race is one which has a shorter larval duration resulting in low leaf consumption and avoidance of disease [29]. Temperature affects the physiological process of larval body resulting in marked seasonal variations, therefore, larval duration varied significantly due to seasonal changes [13]. Larval duration of bivoltine silkworm was more in winter but the span was comparatively less in dry, wet and summer season [14]. When the silkworm larvae were exposed to 24 hours light/day, the larval span was prolonged, resulting in good production of cocoon [15]. An increase in the temperature resulted in increased metabolic activity which ultimately decreased the larval duration [23]. The exogenous application of juvenoids prolonged the larval period [30]. The application of manta at the concentrations of 2.5 ppm. caused delay in the feeding period which resulted in the prolonged larval duration

[31]. The juvenile hormone secreted by corpora allata is responsible for preventing the metamorphosis [32]. Decrease in temperature enhances the moulting duration in silkworm [33].

The prerefrigeration period and the duration of refrigeration have very low degree of influence on the survival of larvae.Temperature variation influenced the physiological ability of insect, therefore, larval duration varied significantly due to seasonal changes [34]. The average temperature between 23.9 to 25.8°C along with 90.9% relative humidity has been proved to be favorable for the best survival of *B. mori* larvae [35]. The exposure to high temperature during the later developmental stage considerably reduced the survival rate [36]. The activity of Ache decreased significantly in head portion of the larvae after cold stress. The possible reason behind this may be that due to cold stress some inhibitory compounds are likely to be produced in larval body which leads to death [37]. Rearing of silkworm larvae at lower levels of RH resulted in lower fecundity, hatchability, pupation and higher larval mortality [38]. Varying sets of temperature and humidity affect both quantity and quality of silkworm as 40% mortality of larvae was recorded at 25°C. [39]. The air current of 1.0m/sec during 5^{th} age rearing reduces the larval mortality [40]. The prerefrigeration and refrigeration periods significantly influenced the larval performance. The refrigeration of cocoons possibly causes certain biochemical and physiological alterations which influenced the larval performances of *B. mori* larvae.

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