Biological Forum – An International Journal **16(8): 349-356(2024)**

ISSN No. (Print): 0975-1130
ISSN No. (Online): 2249-3239

Optimizing Preservation Duration of Mulberry Silkworm Crossbreed Eggs in Cold Storage

Y. Surjalata Devi[1*], Anil Kumar Verma[1], Chandrakanth Nalavadi[2], Anowar Hossain[3] and Biplab Pramanick[1]
[1]Central Sericultural Research Training Institute, Berhampore (West Bengal), India.
[2]Central Sericultural Research Training Institute, Mysore (Karnataka), India.
[3]ICAR-National Rice Research Institute, Cuttack (Odisha), India.

(Corresponding author: Y. Surjalata Devi)*
(Received: 01 June 2024; Revised: 03 July 2024; Accepted: 23 July 2024; Published: 14 August 2024)
(Published by Research Trend)

ABSTRACT: This study aimed to evaluate the impact of extended cold storage on the hatching percentage and rearing performance of hybrid silkworm eggs (*Bombyx mori* L.). Disease-Free Egg Layings (DFLS) of different multi × bi hybrids were subjected to cold storage at 2.5°C for varying durations. The results indicated that up to 75 days of storage, the hatching percentage remained above 90%. Notably, 21Y × (B.Con.1 × B.Con.4) exhibited 88% hatching rate even at 85 days, alongside 12Y × (B.Con.1 × B.Con.4), which demonstrated stable and consistent hatching performance across different storage periods. Performance across all breeds was optimal at 65 days of cold storage, with significantly better quantitative traits compared to normal cold storage conditions. Traits such as single cocoon weight (SCW), single shell weight (SSW), effective rate of rearing by weight, and pupation rate showed significant at the 1% level at 65 days of storage. These findings suggest that controlled cold storage can effectively maintain high hatching rates and rearing performance, providing a potential solution for stabilizing seed supply in sericulture.

Keywords: Multi × Bi, Cold storage, Disease free Layings, Hatching percentage, Sericulture.

INTRODUCTION

Sericulture is the cultivation of silkworms for the production of silk, primarily involving the rearing of various silkworm species, including the Mulberry silkworm (*Bombyx mori*), Tasar silkworm (*Antheraea mylitta*), Muga silkworm (*Antheraea assamensis*), and Eri silkworm (*Samia ricini*). India is the second-largest producer of silk in the world, contributing 13% of global silk production, following China, which accounts for 80%. Among the four types of silk produced in India, mulberry silk dominates with a 70% share, while Vanya silk, which includes Tasar, Eri, and Muga, accounts for the remaining 30%. The primary regions for mulberry sericulture in India are Karnataka, Andhra Pradesh, Tamil Nadu, West Bengal, and Jammu & Kashmir (Shirota, 2000).

In West Bengal sericulture is practiced both in the plains and hilly regions where plains experience hot and humid summers with temperatures ranging from 23 to 43°C and humidity levels between 62 and 97%, while winters are relatively cold and dry, with temperatures varying between 6 and 23°C and humidity levels of 30 to 90%. These climatic variations affect both mulberry plant growth and silkworm rearing efficiency. A total of five distinct crop schedules are followed by Seri-farmers, such as Agrahayani (November), Falguni (February), Baishakhi (April), Shravani (June–July), and Bhaduri/Aswina (August/September). The first two crops are considered favourable, Baishakhi is classified as semi-favorable, while the last two are unfavourable for silkworm rearing (Bindroo *et al.*, 2012; Kabita *et al.*, 2023). While bivoltine silkworms can be reared in the hilly regions, sericulture in the plains is primarily dominated by multivoltine breeds, mainly Nistari. However, considering the mild winter and spring climate, there is potential for hybrid rearing of Multi × Bi or Bi × Bi silkworms during the favorable seasons of spring (February–March) and autumn (November–December), when temperatures range between 23 and 28°C with 70–90% relative humidity. During the Baishakhi (April) season, only Multi × Bi hybrids can be reared successfully.

Despite the availability of favorable seasons for hybrid silkworm rearing, commercial seed production remains a significant challenge. One major issue is that parent rearing for autumn seed crops during August–September is difficult due to high humidity and temperatures, leading to high mortality rates and poor seed production (Abhilash *et al.*, 2021). Additionally, the silkworm *Bombyx mori* L. experiences embryonic diapause, allowing bivoltine eggs to be preserved under hibernation schedules through low-temperature storage for controlled hatching. However, multivoltine eggs lack diapause and cannot be stored beyond 20 days, limiting their usability (Horie *et al.*, 2000; Tanaka, 1964). In contrast, bivoltine silkworm eggs, which undergo diapause, can be effectively cold-stored for extended durations ranging from 1 to 10 months through the application of controlled chilling and hibernation protocols (Narasimhanna, 1998). Another challenge is the fluctuating demand for Disease-Free Egg Layings (DFLs), which creates an imbalance in production, leading to shortages during peak seasons and oversupply during slack seasons. This results in price fluctuations and inefficiencies in seed production and utilization (Hoque and Hasmi 2023).

To address these challenges, refrigeration techniques for extending the storage of hybrid silkworm eggs could help ensure a steady supply of Disease-Free Egg Layings (DFLS) even during unfavourable seasons. Developing a systematic and reliable method for preserving silkworm eggs for extended periods would enable better management of demand and supply fluctuations, ultimately benefiting farmers and improving the efficiency of silk production (Iizuka *et al.*, 2008; Mochida *et al.*, 2006). Sericulture also offers valuable career and business opportunities, especially for rural and semi-urban populations. Its growth can boost rural development by reducing poverty, generating income, and curbing urban migration. As a sector, it also promotes entrepreneurship, which is essential for economic growth and job creation (Savithri *et al.*, 2024).

For the efficient and productive rearing of silkworm, the hatching time must coincide with the availability of food and suitability of environmental conditions. For the regular supply of silkworm seeds, the refrigeration of silkworm eggs has been practised since long. Although, bivoltine breeds/hybrids can be cold stored upto 10 months without change in their rearing parameters, multi/ multi bivoltine hybrids can't be stored for longer duration due to their non hibernating character. This leads to time-bound rearing of cross breeds with multivoltine race as mother parents, failing which the DFLs go waste. Despites advancements in sericulture, challenges remain in optimizing hybrid silkworm rearing, developing climate-resilient breeds, and improving seed production efficiency. Limited research exists on enhancing parent stock survival, extending the storage of non-diapause eggs, and addressing fluctuations in Disease-Free Layings (DFL) demand. Additionally, cost-effective and scalable refrigeration techniques for hybrid silkworm egg preservation need further exploration. Optimizing of preservation of multi × bi is thus essential for enhancing the sustainability and productivity of sericulture in West Bengal.

MATERIALS AND METHODS

The present study was conducted in Central Sericulture Research and Training Institutes (CSRTI), Berhampore, West Bengal, India in the year of 2018. Total four crossbreeds (Multi × Bi) were selected for the experiment included N(P) × (SK6 × SK7), M6DPC × (SK6 × SK7), 12Y × (B.Con.1 × B.Con.4), and 21Y × (B.Con.1 × B.Con.4). The study involved the preparation and cold storage of Multi × Bi Disease-Free Layings (DFLS) at least three months beyond their normal preservation time 20 days.

After 85-90% egg laying by female moths, the egg sheets were dipped in 2% formalin solution for 10–15 minutes for surface sterilization, followed by drying in the shade and kept at room temperature for 42-44 hours. The eggs were preserved at 5°C for 20 days, with an intermediate step at 15°C for two days, a process known as single-step refrigeration. For double-step refrigeration, the DFLs were transferred from 5°C to 15°C for two days after completing the maximum safe preservation period, allowing the embryos to advance to the C1 and C2 developmental stages. The eggs were subsequently then stored at 2.5°C for a maximum of 80 days, totalling over three months. To prevent cold injury and thermal shock, eggs were transitioned through 15°C for 2–3 hours during both preservation and release (Table 1).

Hatching tests were conducted at five-day intervals after release from 2.5°C storage to evaluate hatchability and incubation duration. A total of three DFLS of each hybrid were released in three replications, ensuring passage through an intermediate temperature of 15°C for 2–3 hours before incubation at 25±1°C with 70–80% relative humidity. The eggs were incubated until they reached the blue-black stage of embryonic development and then subjected to black boxing for 48 hours. The hatching percentage was calculated individually for each DFLS. Subsequently, the rearing was conducted after 65 days cold stored DFLS of each hybrid and a normal 20 days cold stored DFLS. The data were recorded for different quantitative traits such as Pupation rate, cocoon yield per 10,000 larvae (number and weight), cocoon weight, cocoon shell weight, and cocoon shell percentage. These traits are critical for determining the fitness, survival, and economic viability of silk production (Rao *et al.*, 2006). The recorded data were analysed using R Studio (version 4.2.2).

RESULT AND DISCUSSION

Performance of silkworm hybrids at varied cold storage period. The performance of different silkworm hybrid DFLs was evaluated under both ideal and long-term cold storage conditions. Key traits such as hatching percentage and hatchability were measured to assess the impact of storage duration.

Hatching at Ideal storage/standard protocol. At standard cold storage conditions (5°C for 20 days), the hatching percentage was highest for the hybrid N(P) × (SK6 × SK7), recording a 96.16% hatchability rate (Table 2). Other hybrids also demonstrated high initial hatchability rates, including 94.96% for M6DPC × (SK6 × SK7), 99.96% for 12Y × (B. Con1 × B. Con4), and 98.16% for 21Y × (B. Con1 × B. Con4). These results indicate that the standard cold storage conditions are optimal for preserving the hatchability of both multivoltine and Multi × Bi hybrid silkworm Disease-Free Layings (DFLs).

Effects of Extended Cold Storage

N(P) × (SK6 × SK7). When the preservation duration is increased to 25 days (20 days in 5°C and 5 days in 2.5°C), the hatching% obtained is 89.26% followed by 85.38% for 30 days eggs storage. When the eggs are kept in the cold storage for 45-60 days, the percentage of hatching is observed to be 96 to 92%, which indicates a 4% decline in the total number of larvae emergences. Again, after 25 days of extra keeping the eggs at 2.5 °C compared to ideal cold storage duration, a slight increase is observed (96.74%). A decrease of 90.55%-78.33% was exhibited from 70-85 days of storage, and at 90 days, a sharp decline up to 13% was observed (Table 2). This hatching rate of N(P) × (SK6 × SK7) (orange line) (Fig. 1) shows the steepest decline, indicating a lower tolerance to prolonged cold storage compared to other hybrids. Cold storing the eggs beyond this specific period leads to a decrease in the hatched larvae percentage. (Manjula & Hurkadli 1993).

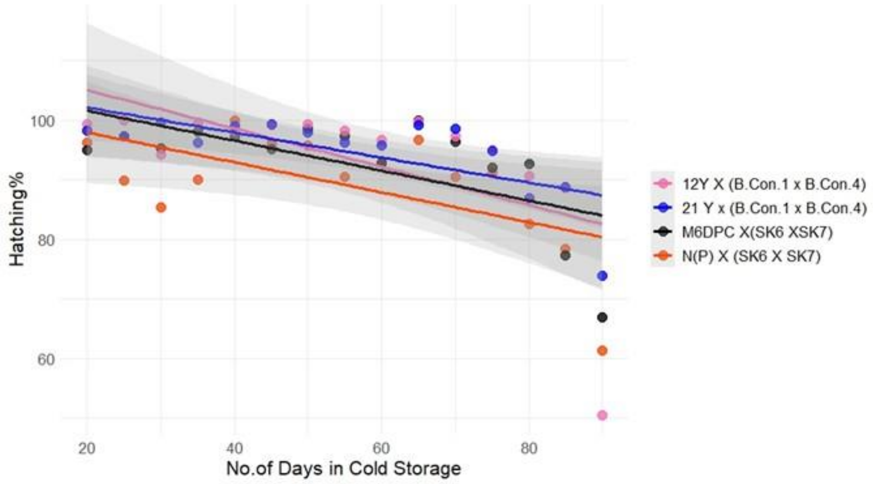

Fig. 1. Effect of Cold Storage Duration on Hatching Percentage in Different Silkworm Hybrids.

M6DPC × (SK6 × SK7). Initial hatching percentage of the cross-breeds M6DPC × (SK6 × SK7) is 94.96 (Table 2), which is the lowest among the hybrids. The black regression line follows a moderate declining trend (Fig. 1) except for 65 days where the black point is depicted higher than the other days' allotted scatter point of this hybrid. It suggests achieving an increasing hatching rate of almost 99.05%, and this data trend also followed the remaining three hybrids. The number of incubation days, which means the eggs attained blue black stage, body pigmentation in 6 days at 25°C up to 65 days, and as the number of days of cold storage increases from 70-90 days, the incubation days also increase from 6 to 7 days for all hybrids. This crossbreed demonstrates a mid-range tolerance to cold storage, with a stable but noticeable decline (Nagraj *et al.*, 1984). This hybrid initially has a lower hatching percentage than 12Y × (BCON1 × BCON4), but their lines intersect at day 70, it implies that different silkworm hybrids have varying thresholds for cold-induced embryonic stress, affecting their viability at different storage durations (Nagaraju & Goldsmith 2002).

12Y × (BCON1 × BCON4). 12Y × (BCON1 × BCON4) shows highest hatching percentage (99.30) at ideal cold storage condition at 5ºC for 20 days than other hybrids which may indicate the bivoltine male component enhance the metabolic activity of eggs which promote uniform larvae emergence at optimal environmental condition. This cross-breed shows moderate decline but remains above 90% in most cases. It maintains the highest hatching rate (25 day is 99.52%, 40 day is 98.17%, 60 day is 96.66%, and 80 day is 90.68%) throughout the storage period upto day 80 and shows highest peak at 65 day (99.78%) which suggesting higher compatibility to longer cold storage conditions. Similar kind of finding was mention by Tayade *et al.* (1987).

21Y × (BCON1 × BCON4). When this cross breed was subjected to longer cold storage beyond 20 days up to 70 days, their hatching was still maintained greater than 95%, with variable hatching. After 70 days, there was a steady decline in the hatching percentage with 86.83% on 80 days cold stored, 88.75% on 85 days, and 73.95% on 90 days. It was also observed that the incubation period was increased after 65 days of cold storage. In the present study, it is observed that these hybrid layings of 40 hours of oviposition may be cold stored for 70-75 days without any loss in yield attributing character. The laying of 40-42 hours of the oviposition performs better concerning their yield parameter and by following the double step refrigeration method. The result is supported by Sarker *et al*. (2016), in which the hatching of the multi × bivoltine dfls was postponed safely up to 85, 75 and 65 days for the layings of 24–30, 36-40 and 44-50 hours of oviposition, respectively. Few studies (Sarkar, 2014; NSSO, 2017) have also explored the methods to extend the preservation period of multi × bi DFLS up to 100 days without affecting the hatching and the cocoon yield at farmer level. But exact maximum days preservation when the hatching percentage remains more than 90% is not reported.

Statistical Analysis. To visually interpret the effect of cold storage duration on egg viability across different silkworm breeds, a scatter plot with regression lines was constructed. This graphical approach allows for a clear comparison of hatching percentages over varying egg-releasing times (cold storage duration) among the breeds. By plotting each data point and fitting breed-specific regression lines, the figure provides insight into both the trend and variability of hatching performance under cold storage conditions. The inclusion of confidence intervals further enhances the visualization by reflecting the statistical reliability of each trend. This enables a straightforward assessment of breed-specific tolerance or sensitivity to prolonged storage, which is crucial for optimizing storage protocols and selecting breeds with better post-storage viability for commercial rearing.

Rapid climatic changes around the world have made it difficult to rear silkworms throughout the year. As a result, producing hybrid Disease-Free Layings (DFLs) during unfavorable seasons has become a major challenge. DFLS of bi × bi can mitigate the favourable seasons by preservation in cold storage for use in the next favourable season. In contrast, the preservation of multi × bi or non-diapausing eggs remains a significant challenge, as their viability typically declines after 15 to 20 days. Rajanna *et al*. (2009) conducted a study on double step refrigeration to meet the demand of commercial non-diapause eggs of the farmers in Indian conditions par with the control up to 80 days in all the combinations of PM × CSR2 and N × NB4D2 to delay hatching.

The graph (Fig. 1) represents the effect of the total time duration to keep multivoltine or multi × bi DFLS in cold storage on the hatching percentage of different cross-breeds. The x-axis denotes the number of days in cold storage, while the y-axis represents the hatching percentage. Each point represents an observed hatching percentage at a specific time point. The trend lines, accompanied by confidence intervals, depict the decline in hatching rate over time for different crosses. The regression line of all four silkworm hybrids exhibits a declining trend in hatching percentage as cold storage duration increases. The observed decline in hatching percentages extended refrigeration periods have been shown to adversely affect egg viability, leading to reduced hatchability, suggesting that prolonged cold storage negatively impacts embryonic development, resulting in lower hatching (Hussain *et al.*, 2011).

In this plot (Fig. 1), the shaded regions around the trend lines represent the 95% confidence intervals of the regression models. These intervals indicate the range within which the true hatching percentage is likely to fall, given a certain number of days in cold storage. Blue and Pink regression lines at the start have narrow Cis, which indicate higher precision and less variability in hatching percentage at early storage duration. The Black and Orange lines have wider Cis, especially at later stages, which implies greater uncertainty in hatching rates for these crosses as storage time increases. There are Some Cis overlaps between different crosses, suggesting that their differences may not be statistically significant in certain storage periods.

In the scatter regression plot (Fig. 1), the blue regression line of 21Y × (BCON1 × BCON4) shows a gradual decline, but slightly steeper than the pink line of 12Y × (BCON1 X BCON4), and initial hatching percentage is 98.16, which is very close to the black regression line. The scatter point of this regression line falls very close to and similar data trend to 12Y but with a slightly steeper decline, indicating shared common bivoltine foundation cross (BFC1) as male component and mild sensitivity to prolonged cold storage time at 2.5°C. It has exhibit lowest decline in hatching rate (21Y is 73.95%, 12Y is 50.53%, M6DPC is 66.96%, and Nistari is 61.38% at day 90 than other hybrids which suggest that 21Y possesses a higher resilience to prolonged cold storage. Also, the intersection between blue (21Y) and pink (12Y) regression lines displayed (Fig. 1) which suggests that the relationship between cold storage duration and hatching percentage varies across different silkworm hybrids and that their relative performance changes at a 45 day. This highlights the differential response of bivoltine hybrids to storage duration and the importance of selecting hybrids with better cold storage resilience for sericulture practices (Bhat *et al.,* 2002). Previous studies have also shown that bivoltine hybrids generally exhibit moderate resistance to cold stress, but specific genetic backgrounds can enhance this trait. The findings of Rajanna *et al*. (2011) also suggest that hatchability, early-stage mortality, cocoon yield, and cocoon characteristics showed no significant differences in batches preserved for 50 days using double-step refrigeration, when compared to the 20-day control group.

Also, to understand the impact on quantitative traits, rearing performance was evaluated for these four cross-breeds after 20 days release of eggs, which was taken as a control, and 65 days (20 days at 5°C and 45 days at 2.5°C) consignment, which was used as treatment (Table 3). The performance of all these hybrids with compare to their control were observe significantly difference for the traits of SCW(0.015 g, $P< 0.01$), SSW(0.005 g, $P< 0.01$), SR(0.37%, $P< 0.01$), ERR by weight(0.184 kg, $P< 0.01$), and pupation rate(0.175%, $P< 0.01$) except hatching% and ERR by number which were non-significant. The single Cocoon weight for N(P) × (SK6 × SK7), M6DPC × (SK6 × SK7), 12Y × (B.Con.1 × B.Con.4), and 21Y × (B.Con.1 × B.Con.4) when reared after 20 days consignment are 1.610g, 1.624g, 1.788g and 1.609g whereas when it is brushed after 65 days cold storage, they are 1.578g, 1.542g, 1.665g and 1.589g respectively where the cocoon weight of treated DFLS are same or little less than control. The same data trend was followed for single shell weight and shell percentage (Govindan and Naranaswamy 1986). The number of Cocoon per 10,000 larvae reduces 6.1% for hybrids of Nistari plain, 5.45% for M6DPC hybrids, 2.36% for 12Y hybrids, and 1.74% for 21Y as compared to their control hybrids. Similarly, the treatment crossbreed performance reduces almost 1-2% in the case of effective rate of rearing by weight(kg) and pupation rate (%). Studies by Benchamin & Jolly (1987); Rahmathulla (2012) have shown that extended cold storage increases oxidative stress in silkworm embryos, leading to delayed metabolic activation, reduced pupation rates, and lower silk yield. This finding was supported by (Jingade *et al.,* 2015). Optimizing storage durations is crucial to maintaining egg viability and sericulture productivity. Hybrid-specific responses to cold stress highlight the need for tailored preservation protocols.

The observed decline in hatching percentage along with rearing parameters such as ERR by number, ERR by weight, SSW, SCW with prolonged cold storage aligns with findings in silkworm cryopreservation studies. Extended cold storage negatively impacts embryonic viability due to physiological stress, desiccation, and metabolic changes (Howe *et al.,* 1967). By double step refrigeration, multi × bi hybrid DFLS can be preserved up to 75 days with more than 90% hatching rate and attributed a sharp decline at 80, 85, and 90 days with respectively, 90%, 80%, and 60% hatching. These results indicate that short- to mid-term cold storage is feasible; longer durations may lead to considerable losses (Venkatesh *et al*., 2004). The hatching percentage of 12Y × (B.Con.1 × B.Con.4) and 21Y × (B.Con.1 × B.Con.4), these two crossbreeds steadily reduce and maintain higher hatching rates at days 65-70, possibly due to genetic resilience against prolonged storage conditions. Their stability for performance of the traits of SCW, SSW, and Pupation rate suggests potential for improved silk production with minimal losses. M6DPC × (SK6 × SK7) may be stored for up to 60 days without significant loss in hatching percentage, but prolonged storage beyond this point results in considerable declines. Thus, it might be viable for moderate storage durations. The lower performance of N(P) × (SK6 × SK7) indicates that this cross is more sensitive to longer cold storage duration, and it should be stored for shorter periods (<40 days) to minimize losses. This could be attributed to weaker cold adaptation mechanisms or increased embryonic mortality (Subramanya & Murkami 1994). For silkworm breeding programs, selecting cold-resistant hybrids like 12Y × (B.Con.1 × B.Con.4) and 21Y × (B.Con.1 × B.Con.4) could enhance larval availability and silk yield. Further research on optimizing storage conditions or genetic interventions could help improve hatching success in sensitive crosses (Verma & Chuahan 1996).

Table 1: The schedule followed for the preservation disease free laying of the Muti × Bi hybrids.

Standard preservation schedule							
TEMPERATURE	25°C	15°C	5°C	15°C	2.5°C	15°C	25°C
HOURS/DAYS	42-44Hours	2-3Hours	20 Days	-	-	-	Incubation
Preservation schedule followed for double step refrigeration							
TEMPERATURE	25°C	15°C	5°C	15°C	2.5°C	15°C	25°C
HOURS/DAYS	42-44Hours	2-3Hours	20 Days	2 Days	80Days	2-3 Hours	Incubation

Table 2: Hatching percentage of Multi × Bi hybrids after different days in cold storage.

BREEDS	DOL	DOC	TNDC	DOR	NDI	DOHT	HA%
Breed A	20/5/2018	22/5/2018	20 days	13/6/2018	6	22/6/2018	96.16
Breed B							94.96
Breed C							99.30
Breed D							98.16
Breed A	20/5/2018	22/5/2018	25 Days	18/6/2018	6	27/6/2018	89.86
Breed B							97.23
Breed C							99.05
Breed D							97.28
Breed A	20/5/2018	22/5/2018	30 Days	24/6/2018	6	1/7/2018	85.38
Breed B							95.25
Breed C							94.25
Breed D							99.65
Breed A	20/5/2018	22/5/2018	35 Days	29/6/2018	6	7/7/2018	90.02
Breed B							98.02
Breed C							99.52
Breed D							96.17
Breed A	20/5/2018	22/5/2018	40 Days	4/7/2018	6	12/7/2018	99.50
Breed B							97.24
Breed C							98.17
Breed D							99.03
Breed A	20/5/2018	22/5/2018	45 Days	9/7/2018	6	17/7/2018	96.02
Breed B							95.12
Breed C							99.13
Breed D							99.27
Breed A	20/5/2018	22/5/2018	50 Days	13/7/2018	6	21/7/2018	95.69
Breed B							98.51
Breed C							99.32
Breed D							97.86
Breed A	20/5/2018	22/5/2018	55 Days	18/7/2018	6	26/7/2018	90.41
Breed B							97.35
Breed C							98.21

Breed D							96.20
Breed A	20/5/2018	22/5/2018	60 Days	23/7/2018	6	31/7/2018	92.81
Breed B							92.76
Breed C							96.66
Breed D							95.76
Breed A	20/5/2018	22/5/2018	65 Days	28/7/2018	6	5/8/2018	96.74
Breed B							99.45
Breed C							99.78
Breed D							99.16
Breed A	20/5/2018	22/5/2018	70 Days	2/8/2018	7	11/8/2018	90.55
Breed B							96.31
Breed C							97.28
Breed D							98.54
Breed A	20/5/2018	22/5/2018	75 Days	7/8/2018	7	15/8/2018	91.14
Breed B							91.95
Breed C							95.03
Breed D							94.76
Breed A	20/5/2018	22/5/2018	80 Days	12/8/2018	7	21/8/2018	82.58
Breed B							92.58
Breed C							90.68
Breed D							86.83
Breed A	20/5/2018	22/5/2018	85 Days	17/8/2018	7	26/8/2018	78.33
Breed B							77.31
Breed C							88.61
Breed D							88.75
Breed A	20/5/2018	22/5/2018	90 Days	22/8/2018	7	31/8/2018	61.38
Breed B							66.96
Breed C							50.53
Breed D							73.95

N.B: DOL- Date of Laying, DOC- date of Consignment, TNDC- Total Number of Days in Cold Storage, DOR- Date of Egg Release, NDI- Number of Days in Incubation, DOHT- Date of hatching test, HA%- hatching Percentage, Breed A- N(P) × (SK6 × SK7), Breed B-M6DPC × (SK6 × SK7), Breed C- 12Y × (B.Con.1 × B.Con.4), Breed D- 21 Y × (B.Con.1 × B.Con.4)

Table 3: Rearing performance of hybrids after 65 days (Treatment) and 20 days (Control) preservation in cold storage.

Cross-Breeds	TD (days)	HA%	SCW(g)	SSW(g)	SR	ERRN	ERRW	PR
N(P) × (SK6 × SK7)-T	65	96.74	1.578	0.228	14.45	8733	12.86	78.26
N(P) × (SK6 × SK7)-C	20	99.00	1.610	0.236	14.89	9300	13.76	79.79
M6DPC × (SK6 × SK7)-T	65	100.0	1.542	0.239	15.49	8667	13.33	70.52
M6DPC × (SK6 × SK7)-C	20	97.25	1.624	0.243	14.96	9167	14.03	74.77
12Y × (Bcon1 × Bcon4)-T	65	99.78	1.665	0.28	16.81	9167	13.66	78.33
12Y × (Bcon1 × Bcon4)-C	20	98.20	1.788	0.298	16.71	9389	14.05	81.77
21Y × (B.Con.1 × B.Con.4)-T	65	99.16	1.589	0.261	16.42	9400	13.06	78.54
21Y × (B.Con.1 × B.Con.4)-C	20	98.56	1.609	0.265	16.45	9567	14.00	80.47
CD		NS	0.015^{**}	0.005^{**}	0.37**	NS	0.184**	0.175**

N.B: TD- Total Number of Days in Cold Storage, HA%- hatching percentage, SCW- Single Cocoon weight, SSW-Single Shell Weight, SR- Shell Percentage, ERRN- Effective Rate of Rearing by Number, ERRW- Effective Rate of Rearing by weight(kg), PR- pupation Rate.

CONCLUSIONS

The present investigation provides critical insights into the development of extended cold storage protocols for the preservation of mulberry silkworm eggs, aiming to ensure a continuous and reliable supply of Disease-Free Layings (DFLs) to silkworm rearers. Among the hybrids evaluated, 12Y × (B.Con.1 × B.Con.4) and 21Y × (B.Con.1 × B.Con.4) exhibited superior viability during extended storage, whereas N(P) × (SK6 × SK7) demonstrated the greatest decline in egg viability. Based on the experimental results, it is concluded that cold storage duration can be extended by an additional 45 days beyond the conventional 20-day protocol achieving up to 65 days of storage without causing a significant reduction in hatching percentage.

FUTURE SCOPE

The implementation of double-step refrigeration for multi × bi hybrids Disease-Free Layings (DFLs) in sericulture presents significant potential for advancements in storage, transportation, and quality maintenance which can extend the shelf life of DFLs ensuring uniformity in larval. It can also help in maintaining the demand of cross breed layings efficiently by preparing the DFLs utilizing good quality seed cocoons, so that the farmers will get sufficient even during unfavourable season along with the preceding favourable season when there is high demand of seed cocoons.

Author contributions: Ms. Y Surjalata Devi and Anil Kumar Verma conceived and designed the experimental work; Y Surjalata Devi and Biplab Pramanick performed the experimental rearing and collected the initial data; Chandrakanth Nalavadi and Anowar Hossain edited the draft and performed statistical analysis. All authors read and approved the final manuscript.

Acknowledgement. I would like to thank all the scientists and staff of Silkworm Breeding and Genetics Division, CSRTI, Berhampore, West Bengal for providing support in the experimental rearing work and cold storage of the DFLS. Also, acknowledge Central Silk Board for providing platform for carrying out the experimental work with ease.
Conflict of Interest. None.

REFERENCES

A leaflet of (SSTL, Kodathi) NSSO, Central Silk Broad, title "Long term preservation technology for cross breed (PM × CSR2) eggs" (2017).

Abhilash, P. L., Sarkar, S., Ghosh, M. K. and Nair, K. S. (2021). Constraints and challenges of silkworm seed production in North West Bengal. *Journal of Entomology and Zoology Studies, 9*(5), 377-379.

Benchamin, K. V. and Jolly, M. S. (1987). Principles of silkworm rearing. *Proceedings of the Workshop on Sericulture in Tropics*, 63-108.

Bhat, M., Devi, S., Chanotra, Z., Ariba, K., Amita, K., Rakesh, K., Abdul, A. and Azam, M. (2002). Cold Shock Response in Biological Traits of the Silkworm, *Bombyx mori*. *Current Journal of Applied Science and Technology,* 35-44.

Bindroo B. B, Suresh K., Saha A. K., Chattopadhyay G. K. and Lakshmi H. (2012) Silkworm hybrids and cropping schedule in West Bengal. *Indian Silk Vol. 3* (51 old).

Govindan, R. and Naranaswamy, T. K. (1986) Influence of Refrigeration of eggs of multivoltine silkworm, *Bombyx mori* L. at eye spot stage on rearing performance. *Sericologia, 26*(2), 151-155.

Hoque, M. A. and Hasmi, M. R. K. (2023). Growth and development of sericulture production and mulberry plants cultivation in West Bengal: An overview. *International Journal of Humanities Social Science and Management, 3*(3), 389-398.

Horie, Y., Kanda, T. and Mochida, Y. (2000). Sorbitol as an arrester of embryonic development in diapausing eggs of silkworm, *Bombyx mori*. *Journal of Insect Physiology, 46*(9), 1009-1016.

Howe, R. W. (1967). Temperature effects on embryonic development in insects. *Annual Review of Entomology,* (12), 15–42.

Hussain, M., Khan, S. A., Naeem, M. and Nasir, M. F. (2011). Effect of Rearing Temperature and Humidity on Fecundity and Fertility of Silkworm, *Bombyx mori* L. (Lepidoptera: Bombycidae). *Pakistan Journal of Zoology, 43*(5).

Iizuka, T., Mase, K., Okada, E. and Yamamoto, T. (2008). Development of a long-term storage method for diapause eggs in some hybrid races of *Bombyx mori*. *Journal of Insect Biotechnology and Sericology, 77*(2), 67-70.

Jingade, A. H., Lekha, G., Srinivasa, B. G., Manjula, A. and Sivaprasad, V. (2015). A feasibility study towards cryopreservation of silkworm eggs: response of non-diapause silkworm eggs to low temperature. *Cryo letters, 36*(1), 19–24.

Kabita, S., Parvin, S. and Parvin, J. (2023). A comparative study on rearing performances of three breeds of mulberry silkworm, *Bombyx mori* in Sujapur, Malda, West Bengal, India in the Spring season. *International Journal of Entomology Research, 8*(7), 13-17.

Manjula, A. and Hurkadli, H. K. (1993). Effect of cold storage of multivoltine and multi x bivoltine silkworm eggs, *Bombyx mori* L at low temperature on hatching performance. *Sericologia, 33*(4), 633–640.

Mochida, Y., Takemura, Y., Matsumoto, M., Kanekatsu, R. and Kiguchi, K. (2006). Long-term preservation of silkworm eggs at a low temperature and its effect on their progenies. *Journal of Insect Biotechnology and Sericology, 75*(2), 89-93.

Nagaraju, J. and Goldsmith, M. R. (2002). Silkworm genomics – Progress and prospects. *Current Science, 83*(4), 415-425.

Nagraj, C. S., Dutta, R. K. and Etsuji Sugai (1984). Effect of refrigeration and the degree of parthenogenic induction in *Bombyx mori* L. *Indian Journal of Sericulture, 32*(3&4) 253-255.

Narasimhanna, M. N. (1988). Manuals of egg production. Published by Central Silk Board. Govt.of India.119.

Rahmathulla, V. K. (2012). Management of climatic factors for successful silkworm (*Bombyx mori*) crop and higher silk production: A review. *Psyche: A Journal of Entomology*, 1–12.

Rajanna, K. L., Raju, P. J., Prabhakar, C. J. and Kamble C. K. (2009). Studies on long term preservation of non-diapause eggs of silkworm, Bombyx mori L. *Indian Journal of Sericulture, 48,* 156-161.

Rajanna, K. L, Reddy, G. B., Harlapur, V. K. and Basavaraja, H. K. (2011). Development of new cold storage preservation technology for cross breed (PM × CSR2) eggs of Silkworm, *Bomby Mori* L. *Sericologia, 51*(1), 77-90.

Rao, C., Seshagiri, S., Ramesh, C., Ibrahim, B. K., Nagaraju, H. and Chandrashekaraiah (2006). Evaluation of genetic potential of the polyvoltine silkworm (*Bombyx mori* L.) germplasm and identification of parents for breeding programme. *Journal of Zhejiang University. Science, B, 7*(3), 215.

Savithri, G., Sujathamma, P., Rani, A. S., K .S. Shanthi Sree and M. Swetha Priya (2024). Sericulture – An Entrepreneurial Spark. *Biological Forum – An International Journal, 16*(6), 164-168.

Sarkar, S. (2014). in thesis entitled "Studies on Qualitative and Quantitative Characters of Silkworm (*Bombyx mori* L.) For Improving the Production of Disease Free Layings".

Sarker, S., Chaterjee, H. and Verma, A. (2016). Standardization of preservation schedule for multibivoltine layings of *Bombyx mori* L., a potential bio resource, in the tropics of eastern India. *Uttar Pradesh Journal of Zoology, 36*(2), 99–104.

Shirota, T. (2000). Influences of refrigeration humidity on the weight and hatchability of silkworm eggs Bombyx mori. *Reports of the Silk Science Research Institute (Japan),* (48).

Subramanya, G. and Murkami, H. (1994). Cold storage and its impact on silkworm egg viability. *Sericulture Science Journal, 30*(2), 102-108.

Tanaka, Y. (1964). Studies on the preservation of silkworm eggs: II. Refrigeration of eggs at blue body stage. *Bulletin of the Sericultural Experiment Station of Japan, 19*(3), 201-209.

Tayade, D. S., Jawale, M. D. and Unchegaonkar, P. K. (1987). Effect of refrigeration on hatching of eggs of multivoltine races *Bombyx mori* L. *Sericologia, 27*(2), 297-299.

Venkatesh, H., Raghuraman, R. and Katti, S.R. (2004) Rearing performance in relation to different schedules of cold storing of silkworm *Bombyx mori* L. egg. *Indian Journal of Sericulture, 43*(2), 142-145

Verma, M. and Chuahan, T. P. S. (1996) Effect of longterm refrigeration of eggs on hatching and rearing performances of some polyvoltines silkworm races of *Bombyx mori* L. in Northern India. *Sericologia, 36*(2), 249-253.

How to cite this article: Y. Surjalata Devi, Anil Kumar Verma, Chandrakanth Nalavadi, Anowar Hossain and Biplab Pramanick (2024). Optimizing Preservation Duration of Mulberry Silkworm Crossbreed Eggs in Cold Storage. *Biological Forum – An International Journal, 16*(8): 349-356.