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Effect of cold storage of female pupae of silkworm *Bombyx mori* L. on survival and reproductive behaviours

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ABSTRACT

Present study was an attempt to investigate the effect on survival and reproductive behaviours of two multivoltine breeds of silkworm, *Bombyx mori* (L), Nistari and M.Con.4 under cold storage at 10±2˚C for 2, 4, 6, 8 and 10 days on 8th day of spinning in order to synchronize the emergence of female and male moths of required combination for mating. The female pupae had an adverse effect on its survival with the increased days of cold storage. Female moths emerged from cold treated pupae of both multivoltine breeds showed 100% pairing efficiency with fresh males of NB4D2, a pure bivoltine breed and (SK6 x SK7), (BHR2 x BHR3)-the newly evolved bivoltine foundation cross breeds. On the other hand, pair to laying percentage decreased progressively with the increased duration of cold treatment. In treated batches pair to laying percentage decreased maximum after 10 days of cold treatment in case of Nistari x NB4D2 (33.42%), whereas it was 65.49 and 64.39 % in case of [Nistari x (SK6 x SK7)] and [Nistari x (BHR2 x BHR3)] respectively. However, it was 92.45, 95.14 and 94.23% respectively in control. Similarly, in M.Con.4 x NB4D2, [M.Con.4 x (SK6 x SK7)] and [M.Con.4 x (BHR2 x BHR3)] after 10 days of cold treatment pair to laying percentage was 75.79, 82.23 and 79.58% against control which was 96.00, 95.74 and 96.71% respectively. Similar trend of significant ($P<0.05$) results were observed in recovery and hatching percentage of layings in cold treated batches of all the combinations when it was compared among the treatments and combinations.

Key words: Silkworm, Multivoltine, Bivoltine, Hybrid, Cold storage, Reproductive behaviour.

Introduction

The success of a commercial grainage depends upon several factors of which impact of abiotic factors - temperature, humidity and light play a major role on the growth and productivity of silkworm (Adkinson, 1965; Benchamin and Jolly, 1986). Virgin female silkworm requires optimum condition of environment for oviposition, failing of which it will lay only few eggs or none (Singh, 1998). Cold is a relative term which will be considered to encompass temperature too low to support normal development of the insects and its effect on them is many folds. Cold injury becomes more severe as the temperature is lowered or as the time of exposure is increased. The silkworm growth and development is heterogenic and varies greatly with the breeds, quality and quantity of food intake (Krishnaswami *et al.*,

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1973) and climatic conditions (Vijay, 1985). This information is very useful in planning for improvement in rearing and grainages under tropical condition (Mathur *et al.*, 1989). Literature available on the effect of cold on pupal / cocoon stage of insects revealed that the cold tolerance capacity varies in accordance with the age. Atwal (1960) found that the pupa of *Anagasta kiihniella* (Zell) were most cold tolerant and acclimated best when newly formed and decline thereafter. Contrary to this, the last stage of development of face fly *Musca autumnal* was mere resistant to the cold temperature than younger age pupae (Turner *et al.*, 1966). Forter *et al.*, (1984) and Patil and Govindan (1985) reported that egg production reduced at longer period of refrigeration of pupae of uzi fly and concluded that with the increase of pupal age and duration of refrigeration, the fly emergence tended to come down.

The production of silkworm seed involves a long chain of interdependent and highly specialized tasks. Since egg production of the silkworm is managed by graineurs, various processes such as procurement and preservation of seed cocoons, sexing, emergence of moth, pairing, egg laying, preservation and hatching of eggs are all important from the point of maximizing viable egg production (Ayuzawa *et al.*, 1972 and Jolly, 1983).

However, non-synchronization of emergence of male and female moths of required combination is the burning problem in all the commercial grainages for production of hybrid layings. This often need a great deal of planning in order to synchronize the emergence of female and male moths as there is variation in the larval periods, dates of spinning and period required from mounting of matured larvae to emergence of moths in required combination and also for climatic change in different seasons. Taking into account the above points and also to minimize the loss of production of layings due to non- synchronization of emergence of male and female moths of required combination, the present study was aimed to find out the effect of cold treatment on female pupae of two multivoltine breeds Nistari and M.Con.4 on their survival and reproductive behaviours when crossed with fresh bivoltine males for production of Multi x Bi hybrid layings.

Material and Methods

The breeds selected for the experiment were the productive popular breeds in the Eastern and North-Eastern parts of India. Cocoons of multivoltine (MV) breeds, Nistari, M.Con.4 and bivoltine (BV) breeds, NB4D2, (SK6 x SK7) and (BHR2 x BHR3) were produced under controlled condition in three seasons - Aswina (July –August), Aghrayani (September – October) and Falgooni(December – January). After sexing the female and male pupae of MV and BV breeds respectively were again put into the empty shells of respective breeds. Female pupae of MV breeds were kept in cold store at $10\pm2^{\circ}C$ on 8th day of spinning in perforated paper bags except the control batches. The MV females were treated at cold storage for 2, 4, 6, 8, and 10 days. (Control and treated MV females were mated with freshly emerged BV males. Afterwards treated pupae were kept separately for emergence). Collection of moth, pairing, depairing, egg laying, incubation of eggs etc. were conducted as per standard procedures (Jayaswal *et al.*, 2008). During conducting of grainage survival of female pupae, pairing efficiency, pair to laying percentage and recovery of layings in gram (g) per kg of MV seed cocoons and hatching percentage of layings were also studied for each treatment. Ranges of microclimatic condition maintained during rearing and grainage room is given in Table 5.

Results

Data contained in Tables 1-4 are the pooled data of three seasons and give an account of the effect of cold storage of female pupae on survival percentage, pair to laying percentage, overall recovery in g/ kg MV seed cocoon and percentage of hatching in different combinations of Nistari and M.Con.4 with BV males of pure breed, NB4D2 and foundation cresses, (SK6 × SK7) and (BHR2 × BHR3).

Survival Percentage: Data in Table 1 give an account of survival percentage of female moths after cold treatment in comparison to the control and also in between the two multivoltine (MV) breeds. It is revealed that under controlled condition female pupae of M.Con.4. showed higher survival percentage than Nistari. The survival percentage of female pupae of both the breeds of MV decreased significantly ($P<0.01$) in comparison to the control with the increase of duration of cold treatment. The effect was more pronounced in Nistari than M.Con.4.

Pairing efficiency: There had no effect on pairing efficiency of female moths emerged from cold

Table 1. Effect of cold storage of multivoltine female pupae on survival %

No. of days cold stored	Nistari	M.Con.4	Mean
0	95.49	98.90	97.197
2	90.17	98.48	94.327
4	85.42	95.80	90.608
6	77.84	91.58	84.710
8	75.33	88.67	82.725
10	72.50	86.67	81.000
Mean	83.51	93.35	88.43
Critical Difference (CD) at	Treatment	Breed	T x B
5%	0.99	0.99	2.42
1%	1.31	1.31	3.22
CV%	1.69		

treated pupae in both the MV breeds in comparison to the control i.e. there was 100% pairing efficiency in both controlled and treated pupae.

Pair to laying percentage: Pair to laying percentage significantly (P<0.01) decreased gradually among the T x T, C x C and also among the C x T with the increase of duration of cold treatment (Table 2). The cold effect on pair to laying percentage was more distinct in Nistati than M.Con.4. Again when this result is compared among the combinations it is pragmatic that M.Con.4. combined with all the BV breeds gave significantly (P<0.01) positive results than the combinations with Nistari. Concerning efficiency of BV male moths it is observed that the potentiality of (SK6 x SK7) is significantly (P<0.01) higher followed by (BHR2 x BHR3) and NB4D2.

Recovery of laying in g/kg MV seed cocoons: In this parameter recovery was significantly decreased (P<0.01) with the increase of duration of cold treatments in all Multi x Bi combinations compared to the control (Table 3). In case of Nistari x NB4D2 recovery of laying in g/kg of cocoon was significantly (P<0.01) lesser in both controlled and treated batches in comparison to the three way crosses, Nistari x (SK6 x SK7) and Nistari x (BHR2 x BHR3). Same trend of result was observed in case of all combinations of M.Con.4 with NB4D2, (SK6 x SK7) and (BHR2 x BHR3). Maximum recovery in g/kg. MV seed cocoon was observed in M.Con.4 x (SK6 x SK7). In treated batches in all the combinations egg recovery decreased significantly (P<0.01) from the 2nd day of the cold treatment and significantly (P<0.01) became lowest in all the treated lots after 10 days of cold treatments. All the results of egg recovery were statistically significant (P<0.01) among the treatments (T), combinations (C) and T X C except in control batches Nistari x Bi and M.Con4 x Bi

Hatching percentage: Hatching percentage in laying gradually decreased significantly (P<0.01) in treated pupae with increased duration of cold treatment (Table 4). Hatching percentage for Nistari x (SK6 x SK7), Nistari x (BHR2 x BHR3) and in all the combinations of M.Con.4 x BV simulate the trend of Nistari x NB4D2. All the treated groups of female pupae exhibited significantly lower frequencies of hatching and the incidence of unfertilized eggs continued to increase significantly (P<0.01) with increasing days of cold treatment.

Table 2. Effect of cold storage of multivoltine female pupae on pair to laying%

No. of days cold stored	Nistari x NB4D2	Nistari x (SK6 x SK7)	Nistari x (BHR2 x BHR3)	M.Con.4 x NB4D2	M.Con.4 x (SK6 x SK7)	M.Con.4 x (BHR2 x BHR3)	Mean
0	92.45	95.14	94.23	96.00	95.74	96.71	95.04
2	88.86	93.33	88.00	95.70	91.79	91.46	91.52
4	71.23	87.01	86.28	92.35	90.48	87.97	85.89
6	62.79	82.59	78.65	85.79	88.97	84.45	80.54
8	57.92	77.37	72.46	82.90	85.91	81.98	76.42
10	33.42	65.49	64.39	75.79	82.23	79.58	66.82
Mean	67.78	83.49	80.67	88.09	89.19	87.03	82.71
Critical Difference (CD) at	Treatment	Combination	T x C				
5%	2.00	2.00	4.91				
1%	2.66	2.66	6.52				
CV%	3.66						

Table 3. Effect of cold storage of multivoltine female pupae on recovery in g/kg MV cocoon

No. of days cold stored	Nistari × NB4D2	Nistari × (SK6 x SK7)	Nistari × (BHR2 x BHR3)	M.Con.4 × NB4D2	M.Con.4 × (SK6 x SK7)	M.Con.4 x (BHR2 × BHR3)	Mean
0	69.228	71.345	70.545	80.125	79.067	76.449	74.443
2	62.854	65.937	62.252	77.278	73.704	73.258	69.214
4	47.718	58.284	57.769	71.547	66.766	69.209	61.882
6	38.346	50.396	47.980	65.201	62.325	62.532	54.463
8	34.904	46.551	43.601	57.176	55.206	56.427	48.978
10	19.725	38.666	38.018	50.648	51.679	49.992	41.455
Mean	45.463	55.180	53.361	66.996	64.791	64.645	58.406
Critical Difference (CD) at	Treatment	Combinati-on	T x C				
5%	1.70	1.70	4.16				
1%	2.25	2.25	5.52				
CV%	4.38						

Table 4. Effect of cold storage of multivoltine female pupae on hatching%

No. of days cold stored	Nistari × NB4D2	Nistari × (SK6 × SK7)	Nistari × (BHR2 × BHR3)	M.Con.4 × NB4D2	M.Con.4 × (SK6 × SK7)	M.Con. 4 × (BHR2 × BHR3)	Mean
0	94.58	95.25	93.92	96.25	96.00	93.83	94.97
2	91.83	92.25	92.25	94.33	93.42	88.58	92.11
4	86.17	89.25	87.58	91.67	90.08	86.42	88.53
6	83.58	88.33	85.50	88.83	87.58	82.5	86.06
8	79.42	85.25	83.58	84.75	85.70	82.58	83.29
10	73.92	74.83	75.17	81.25	76.75	78.67	76.76
Mean	84.92	87.53	86.17	89.51	88.17	85.43	86.95
Critical Difference (CD) at	Treatment	Combination	T × C				
5%	1.31	1.31	NS				
1%	1.74	1.74	-				
CV%	2.27						

Table 5. Microclimatic condition during rearing and grainage period

Temperature (°C)		Relative humidity (%)	
Max.	Min.	Max.	Min.
24 - 29	19.5 – 27.5	93	64

Discussion

From the results it was observed that cold tolerance capability of M.Con.4. was more than Nistari. Variation in cold tolerance in pupae was also reported by Atwal, 1960 and Kovalev, 1970, Patil and Govindan (1985). Adverse effect on survival percentage in MV pupae irrespective of breeds refers to absence of inherent genetic adaptive mechanisms in the breeds for protecting themselves from low temperature. All the data in this regard were significant ($P<0.01$) when it was compared among the treatments (T x T) as well as between the breeds (B x B) and also with Treatment (T) x Breeds (B).

The results in respect of pairing efficiency depicted that mortality of pupae which may be due to physiological disorder and there was no harmful effect of cold storage on healthy pupae. Kovalev, 1970 reported that in *Bombyx mori* preservation of the seed cocoons at low temperature did not affect in normal growth and emergence of moths. This corroborates with the findings of 100% pairing efficiency of the female moths after cold treatment up to 10 days.

Variation in pair to laying percentage under longer duration of cold storage among all combinations of Multi x Bi is attributed to better effective rate of mating with male components of bivoltine hybrids than pure breed. Decrease of pair to laying percentage with increase of duration of cold storage is due to physical disorder of the female moths emerging from treated batches (Forter *et al.*, 1984).

Significant (P<0.01) decrease of egg recovery after 2 days of cold treatment in all combinations with increase of duration of cold treatment is in conformity with Kovalev, 1970 and Forter *et al.* 1984. Reduction of recovery is attributed to the fact that bulk of the energy is utilized by the pupae to withstand the cold environment instead of utilizing it for development of oocytes resulting less/poor development of oocytes. This is in agreement with Ayuzawa *et al.*, 1972 who advocated for preservation of female pupae for 2 days under 10°C. Jolly, 1983 also suggested for preservation of female cocoons / pupae for maximum of 3 days at 5-10°C on the 7th or 8th day of spinning stating that female moth emergence is affected significantly after exposing the pupae in cold environment. Significant variation in the results of [Nistari x (BHR2 x BHR3)], [N x (SK6 x SK7)] and Nistari x NB4D2 after cold treatment of female pupae is attributed to the result of mate preference / combining ability for this economically important characters. This is also in accordance with the view of Benchamin *et al.* (1990) who stated that the mating capacity of males of *Bombyx mori* pure breeds as compared with hybrids is less and the fecundity in MV female parent is not influenced by the male but other characters including rate of mating and fertility etc. which was significantly different among different crosses.

From the point of hatching percentage it is revealed that viability of ova is affected in case of cold treated MV female pupae as gonad formation starts in pupal stage in silkworm. Loss of viability is due to damage of gonad and thereby affects the reproductive potential of female moths. This finding also corroborates with the findings of Das and Kannantha (1996) who studied the effect of refrigeration of female pupae on fecundity, hatching percentage and fertility in silkworm *Bombyx mori* L. and observed significant difference (p<0.05) in hatching percentage between races and variation of temperature and days of cold storage.

Conclusion

Comparing the overall performance of cold treatment, it can be concluded that under unavoidable circumstances female pupae of Nistari and M.Con.4. can be preserved in cold storage for 4 and 6 days respectively on 8th day of spinning at 10±2°C without any harmful effect on quality parameters of Multi x Bi hybrid layings and also to minimize the production cost of layings by effective utilization cocoons and also to fulfill the demand of the hybrid layings in the field.

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