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Effect of Rearing Temperature and Humidity on Fecundity and Fertility of Silkworm, *Bombyx mori* L. (Lepidoptera: Bombycidae)

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Abstract.- The effect of temperature and humidity fluctuations on fecundity and fertility of the silkworm lines was investigated during autumn and spring by exposing 4^{th} and 5^{th} instar larvae of eleven inbred silkworm lines (M-101, M-103, M-104, M-107, Pak-1, Pak-2, Pak-3, Pak-4, PFI-1, PFI-2 and S-1) to 25±1°C, 30±1°C and 35±1°C in combination with 55, 65 and 75% relative humidity (RH) for 3hr daily. Temperature and relative humidity during larval rearing affected the fecundity and fertility of inbred silkworm lines adversely. The maximum average number of eggs were laid at 25±1°C with 75±5% RH (413.1) followed by 25±1°C with 75% RH (400.4) and 25±1°C with 65% RH (390.9). Significant difference in egg fertility was observed between all the treatments. Maximum average fertilized eggs were recorded at 25±1°C with 75±5% RH (88.77) followed by 25±1°C with 75% RH (86.88) and 25±1°C with 65% RH (85.09) while lowest fertility was shown by 35±1°C with 55% RH (67.52). The deleterious effect of temperature and humidity was observed on fecundity and fertility of inbred silkworm lines. Pak-4 was the best performer with maximum eggs (471.5) at 25±1°C with 75±5% RH followed by PFI-1 (465.2) and M-101(464.1) in the same environment. The best temperature and humidity for rearing silkworm lines for seed cocoon production (to obtain higher number of eggs per moth with increased fertility) is 25±1°C with 75±5% RH. Pak-2, Pak-4, Pak-3 and PFI-I were the better lines which may be utilized in seed cocoon production and hybridization. Investigations elucidated that temperature and humidity variations during larval rearing resulted in low fecundity and high incidence of unfertilized eggs. The study suggests that fecundity and egg fertility can be enhanced by avoiding temperature and humidity fluctuations during larval rearing.

Key words: Silkworm, fecundity, fertility, silk seed

INTRODUCTION

In sericulture, it is established fact that several factors contribute in the growth and development of silkworm for the production of quality eggs. Quality silkworm seed refers to richness of layings, egg viability, hatching uniformity and more importantly good rearing performance of the progeny (Ullal and Narashimhanna, 1981) and it depends on management practices *i.e.*, rearing temperature, humidity, nutrition, and genotype of the breed. The better rearing conditions, environment and nutrition during larval period may leads to higher fecundity by silkworm moths (Miller, 2005; Malik and Reddy, 2007).

Fecundity and fertility are the two main factors of seed cocoon production. Several factors affect the fecundity and fertility of silkworm races including aberrations in sex organ, faulty handling of moth during mating and egg laying, defective preservation of cocoons and environmental stress during larval rearing and cocoon storage (Biram *et al*., 2009). Temperature and humidity are key environmental factors that influence the physiology of insects. The range of adaptations to changing environments and maintenance of homeostasis is a complex and dynamic display of species inherent potential to keep internal changes within tolerable limits under wide fluctuations in their surroundings. The silkworm is sensitive to environmental fluctuations and unable to survive naturally due to continuous domestication since the dawn of sericulture. This has resulted in varied response to the environmental conditions (Temperature, humidity and photoperiod etc.) as compared to wild insect species. It means a significant interaction of environmental conditions and developmental stages governs the physiology of silkworm which affect the growth, development, productivity and quality of silk.

The attempts have been made to understand the effect of temperature and humidity (Kremky and

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Michalska, 1984; Gowda, 1988; Sing *et al*., 2009) on egg yield and egg fertility of silkworm, *Bombyx mori* L. during cocoon preservation (Govindan and Narayanswamy, 1986), mating (Jadhave and Gajare, 1978; Sarkar *et al*., 2009), oviposition (Narasimhanna, 1988) and egg storage (Upadhyay *et al*., 2006). Superiority of silkworm breed is determined by its ability to produce a good number of eggs which show uniform hatchability (Singh and Saratchandra, 2004). Several operations related to silk seed production are carried out within the optimum range of temperature and humidity to avoid reduction in egg yield and fertility rate (Kamble, 1997).

The silkworm egg production in Pakistan is lagging behind the potential of silkworm races and quite low as compared to other countries engaged in sericulture. The commercial seed production activities of sericulture need optimization to enhance returns to farmers, rural employment and raw silk productivity. The revival of sericulture in potential areas of silkworm rearing during the last decade resulted in increased egg demands i.e. 3000 silk seed packets are annually required in Punjab province for spring rearing (Sericulture Research Wing, 2008). The challenging task of developing silkworm breeds which can produce eggs under prevailing conditions of temperature and humidity. Perusal of literature reveals that no work has been carried out on the effect of temperature and relative humidity fluctuations on the fecundity and fertility of silkworm lines. The present study, therefore, was conducted to find out the effect of variations in temperature and humidity during rearing on silk worm egg production and egg-fertility of the silkworm, *B. mori*. The study would be helpful in establishing the causes of low egg yields and low egg fertility in Pakistan.

MATERIALS AND METHODS

The larvae of eleven silkworm lines (M-101, M-103, M-104, M-107, Pak-1, Pak-2, Pak-3, Pak-4, PFI-1, PFI-2 and S-1) were reared at Sericulture Research Laboratory, Lahore during autumn and spring seasons in 2007-2008. The eggs were incubated at optimum conditions of temperature, relative humidity and light/darkness ratio (12hr: 12hr). The eggs were spread on sheets in single layer to ensure uniform conditions for all the eggs and at pin head stage complete darkness was provided (black boxed) to ensure uniform hatching. The rearing rooms and all the appliances were washed, cleaned and disinfected by using standard methods following (Krishnaswami, 1978). The young larvae (1^{st} to 3^{rd} instar) were reared at 27-28°C with 85 to 90% relative humidity. In each replication 300 larvae were retained during 1^{st} to 3^{rd} instar. The larvae were served with four to five feedings at an interval of 5 hours starting at 0800 PST.

At the end of each instar bed cleaning nets were used to pick up the larvae and replace their bed. The larvae were reared under standard rearing conditions (Krishnaswami, 1983). At the beginning of 4^{th} instar, 50 larvae were counted from each replication and retained for further studies in each replication. The 4^{th} and 5^{th} instar larvae were subjected to the following treatments:

Table I.- Temperature and humidity treatments during 4^{th} and 5^{th} instar larvae of *B. mori.*

Treatments	Temp. (°C) for 3hrs	Humidity (%)
T_0	25±1a	70-80b
T_1	25±1	55
T_2	25±1	65
T_3	25±1	75
T_4	30±1	55
T_5	30±1	65
T_6	30±1	75
T_7	35±1	55
T_8	35±1	65
T_9	35±1	75

a, standard rearing temperature; b, standard relative humidity.

On 5^{th} day of the 5^{th} instar, ripe larvae were collected manually and transferred to mountages for cocooning. The cocoons were made within 72hr of mounting and seed cocoons were harvested on eighth day of spinning. Cocoons were preserved at 25±1°C and 75±5% RH. After accomplishment of emergence, male and female moths in equal number were kept on wooden trays and were allowed for copulation for 4hr after which they were decopulated gently. Female moths were then placed on sheets and covered with funnel for egg laying. After completion of egg laying, the female moths were packed and then crushed for examining the hereditary diseases. The total number of eggs and number of fertilized eggs per female were counted. The response of the silkworm lines was assessed for fecundity and fertility (Rao *et al*., 2006). The experiment was laid out in Completely Randomized Design (3-Factor Factorial) with three replications. The analysis of the data was carried out to assess the effect of treatments and the means were subjected to Duncan's Multiple Range Test (DMRT) to find out the significance among treatments, silkworm lines and their interactions following Steel and Torrie (1981).

RESULTS AND DISCUSSION

The data on fecundity and fertility of eleven inbred silkworm lines reared during spring and autumn seasons were statistically analyzed and means were compared for significance. The significant differences ($P<0.05$) among the treatments, silkworm lines and interaction between treatments x silkworm lines were observed. The data on fecundity of silkworm lines at different temperature and humidity are given in (Table II). The data showed great variations in egg laying of silkworm lines in different treatments. The maximum average number of eggs were laid in control ($T_{0:}$ 25±1°C & 70- 80 % RH) and minimum egg laying was recorded in T_7 (35±1°C and 55 % RH). The data showed that treatments affected the performance of all silk worm lines. The silkworm lines under stress humidity yielded low number of eggs even at standard rearing temperature (25±1°C). The variations in number of eggs occurred due to fluctuations of temperature and humidity and interaction with silkworm lines. The moths which were emerged from the larvae which were reared at standard condition showed better results for fecundity as compared to other combinations of temperature and relative humidity. Similarly, the performance of silkworm lines at different temperatures was observed i.e. lower numbers of eggs were produced at higher temperature and lower humidity level.

The variable performance of silkworm lines

Table II.- **Fecundity of eleven inbred silkworm lines at different environmental conditions.**

Lines	T_0	T_1	T_2	T_3	T_4	T_5	T_6	T_7	T_8	T_9	Mean
M-101	464.8 AB	422.7 IJ	428.8 GHI	442.0 DE	376.2 STU	378.5 R-U	385.2 P-S	332.2]	344.2 Z]	352.2 YZ	**392.7 D**
M-103	367.3 LM	333.0 W-Z	343.2 Q-V	355.8 NOP	312.5 _	318.0]	324.3 Z]	285.2 fg	292.5 def	302.5 abc	**323.4 H**
M-104	372.5 UV	347.7 Z]	357.8 XY	363.2 WX	324.8 _a	328.8 _	336.3]	393.7 NOP	366.8 VW	311.5 b	**350.3 G**
M-107	438.7 EF	402.0 MN	416.0 JK	430.8 F-I	374.0 TUV	377.0 R-U	387.8 PQ	339.2]	344.8 Z]	352.2 YZ	**386.3 E**
PAK-1	358.3 XY	326.2 _	338.2]	344.8 Z]	309.8 b	316.5 ab	322.0 a	384.8 d	295.7 c	300.8 c	**319.7 I**
Pak-2	462.7 B	426.5 HI	432.8 E-H	442.2 DE	336.7]	336.8]	332.2]	324.2 _a	325.2 _a	325.7 _	**374.6 F**
Pak-3	437.5 EFG	415.0 JK	427.3 HI	434.3 E-H	405.8 LM	412.2 KL	417.3 JK	379.0 Q-U	385.7 PQR	385.0 P-S	**409.9 C**
Pak-4	471.5 A	435.5 E-H	448.0 CD	453.0 C	435.7 E-H	431.5 F-I	422.8 IJ	391.8 OP	397.5 MNO	398.2 MNO	**428.5 A**
PFI-I	465.2 AB	428.3 GHI	438.3 EF	450.2 CD	404.7 LM	410.8 KL	417.5 JK	374.2 TUV	382.2 Q-T	392.7 OP	**416.4 B**
PFI-II	351.0 O-R	324.7 Z]	335.0 V-Y	345.7 Q-T	305.0 _ab	310.0 _	316.2]	276.5 h	282.2 gh	291.8 ef	**313.8 J**
S-1	354.7 NOP	328.0 XYZ	334.7 V-Y	342.7 R-V	312.5 _	317.0]	323.3]	296.5 b-e	306.2 _a	316.3]	**323.2 H**
Mean	**413.1 A**	**380.9D**	**390.9 C**	**400.4 B**	**354.3 G**	**357.9 F**	**362.4 E**	**334.3 I**	**338.4 H**	**339.0 H**	

Means sharing same letters/symbols are not different significantly.

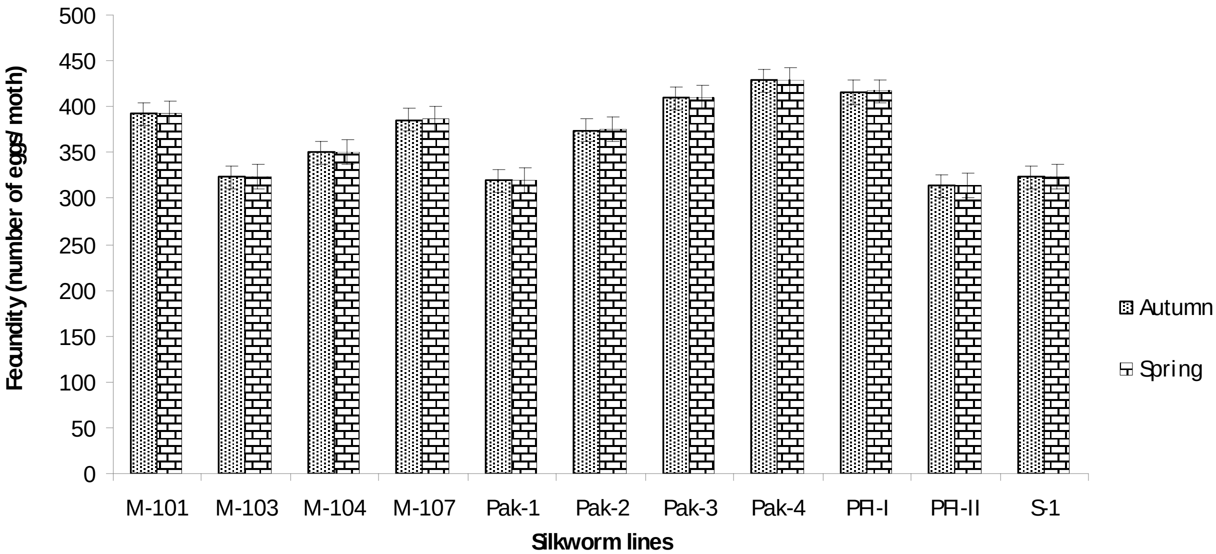

Fig. 1. Mean performance of fecundity of silkwork lines in autumn and spring.

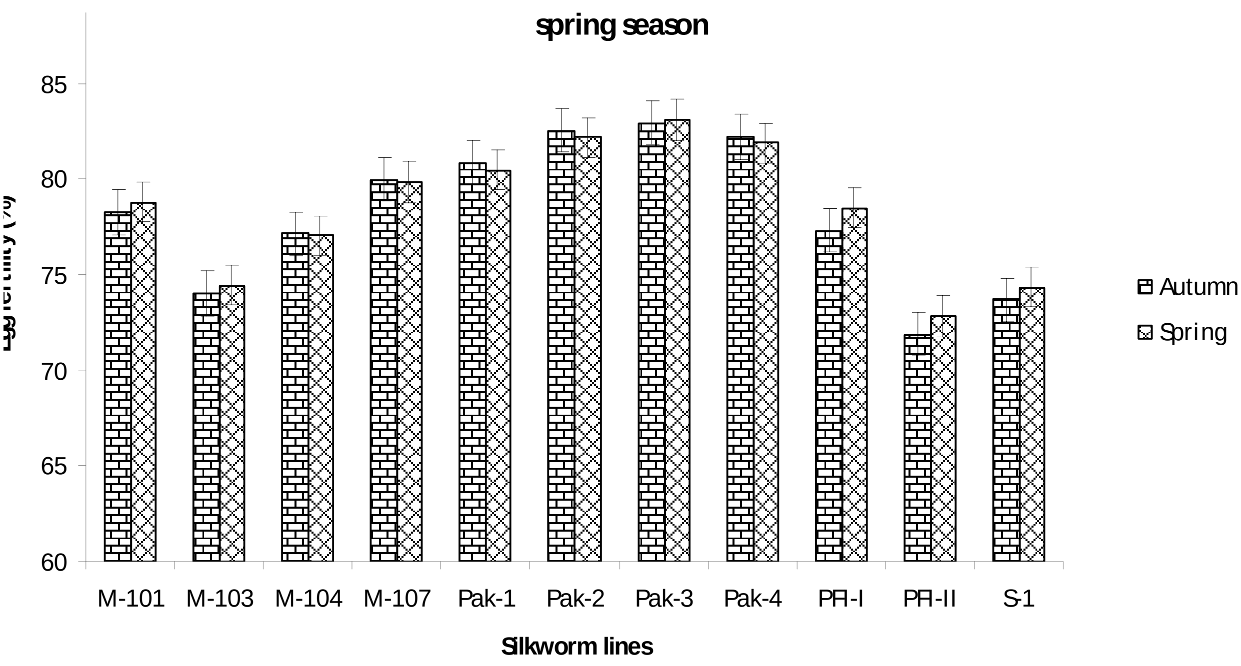

Fig. 2. Mean performance of fecundity of eleven silkwork lines in autumn and spring season.

was observed at various treatments. The mean fecundity of all the silkworm lines was statistically significant with each other. Pak-4 was the best silkworm line with maximum eggs (471.5) in T_0 and minimum (391.8) in T_7. The gradual decrease in the egg number was observed with the increase in temperature. The response of silkworm lines to various treatments showed that expression of genetic potential for egg laying trait is strongly governed by environmental conditions during larval rearing. The data indicated that the performance of silkworm lines was affected significantly at 25, 30 and 35˚C with all three combinations of humidity (55, 65 and 75%). However, rearing of silkworm larvae at 25°C produced higher number of eggs as compared to other temperatures. It showed that temperature and relative humidity governed the expression of genetic potential of the silkworm lines. It is found that variations in relative humidity below 75% at 25°C resulted in poor fecundity and evident from the results that increasing of temperature and low humidity (T_1, T_4 and T_7) have more pronounced effect on egg laying of female moths than the vice versa. The data in Figure I depicted that the performance of silkworm lines during two rearing seasons remained non significant. The comparison of means revealed that seasons, treatments x seasons, and silkworm lines x seasons were not significantly different. The effect of season (autumn and spring) on the performance of inbred silkworm lines was not much pronounced as the larvae were provided with similar set of environmental conditions. The slight variations in the means during autumn and spring season may be due to variations in nutrients in mulberry leaves fed to the larvae (Ito, 1978). Differences among silkworm strains in egg laying capabilities and other biological aspects have been reported by Ahsan *et al*. (2000). Genetic diversity among silkworm breeds may cause low egg recovery under different environments. Similar findings have been reported by Ahsan and Rahman, (2008) that varietal differences affect the silkworm egg yield. The fecundity is genetically controlled character (Robertson, 1957; Sidhu *et al*., 1969) and expression of trait has genetic limitations of an insect species like silkworm and it shows direct correlation with many physiological and ecological factors including temperature, humidity and light (Yamaoka *et al.*, 1971).

The results on the effect of fluctuations in temperature and humidity on the fertility of silkworm moths are shown in Table III. The highest fertility was shown by Pak-3 followed by Pak-2 and Pak-4. There were no significant differences between the means of these silkworm lines. The lowest egg-fertility was recorded in PFI-II. The data presented in Table I shows that there is gradual decrease in fertility with decrease in relative humidity at a given temperature. Significant difference in egg-fertility was observed between all the treatments. Maximum average fertilized eggs

Table III.- Egg-fertility of eleven inbred silkworm lines at different environmental conditions.

Lines	T_0	T_1	T_2	T_3	T_4	T_5	T_6	T_7	T_8	T_9	Mean
M-101	89.33 b-f	82.50 l-q	85.00 h-n	85.83 f-l	79.16 q-v	75.83 v-z	76.66 t-z	69.83 b-f	69.50 _c-g	71.50 _a-e	**78.52 C**
M-103	85.33 g-m	74.16 xyz	79.66 p-u	81.16 o-r	70.50 a-f	71.66 _a-d	75.50 w-z	63.17 h	70.83 a-f	70.33 a-f	**74.23 E**
M-104	81.33 n-r	75.50 v-z	82.00 m-t	84.83 h-o	75.33 w-z	82.00 m-r	76.16 uz	67.66 fg	72.16 _a-d	73.83 xyz	**77.08 D**
M-107	90.66 n-r	86.83 v-z	87.00 m-r	88.16 h-o	75.83 w-z	78.66 m-r	83.00 u-z	66.00 fg	71.00 _a-f	71.83 _a-f	**79.90 B**
Pak-1	89.50 b-f	88.83 c-g	87.00 c-j	89.66 b-e	77.33 s-x	77.50 s-x	85.50 g-m	68.66 d-g	69.16 _c-g	73.50 yz	**80.67 B**
Pak-2	94.83 a	88.17 c-h	90.50 bcd	90.17 b-e	79.17 q-v	80.50 p-s	86.83 d-j	67.67 fg	71.83 _a-d	73.83 x-z	**82.35 A**
Pak-3	92.66 ab	87.00 c-j	92.66 ab	92.66 ab	80.33 p-t	84.33 i-o	83.00 k-p	70.33 a-f	72.66 _abc	74.50 x-z	**83.02 A**
Pak-4	93.00 ab	86.50 e-k	87.66 c-i	93.00 ab	80.17 p-t	81.33 n-r	83.33 j-p	70.17 _a-f	72.66 _abc	72.50 _abc	**82.03 A**
PFI-I	87.33 bc	82.50 d-j	84.33 c-j	85.50 c-h	73.00 v-z	77.16 r-w	77.33 k-p	67.83 gh	71.16 _a-f	72.66 _a-d	**77.88 CD**
PFI-II	86.66 g-m	73.00 yz	78.17 s-x	80.67 n-r	70.17 a-f	66.83 fg	69.33 _c-g	65.00 fg	66.50 fg	67.17 fg	**72.35 F**
S-1	85.83 g-m	74.66 xyz	82.00 m-r	84.00 h-o	71.50 _a-d	71.50 _a-d	73.16 yz	66.33 fg	66.33 fg	64.66 gh	**74.00 E**
Mean	**88.77 A**	**81.78 D**	**85.09 C**	**86.88 B**	**75.68 G**	**77.03 F**	**79.08 E**	**67.52 J**	**70.35 I**	**71.49 H**	

Means sharing same letters/symbols are not different significantly.

were observed in control ($T_{0:}$ 25±1°C & 70-80% RH) followed by T_3 and T_2 while lowest fertility was recorded by T_7. The fluctuation in the temperature up to 30°C is within the tolerable limits of the silkworm lines provided that the relative humidity may remain between 70-80%. It was found that the number of unfertilized eggs increased almost all the silkworm lines with the increase in temperature during 4^{th} and 5^{th} instar. It was also observed that the effect of high temperature on egg-fertility was more pronounced when the relative humidity was low (T_1, T_4 and T_7). There was a significant difference between the silkworm lines and treatments. Treatments deleteriously affected the performance of lines in comparison to controls (Table III). The effect of autumn and spring season on the performance of inbred silkworm lines has been given in Figure 2. There was no significant difference in the fertility of silkworm lines during autumn and spring.

The egg laying capability of *Bombyx mori* L. has been noticed to be influenced by the genotype of silkworm line and rearing temperature. Rearing under stress environments results consumption of energy in the metabolic activity (Gowda, 1988). Unfavorable environment during rearing results in poor performance of parental lines. Exposure of seed cocoons to high temperature of 35˚C in combination with low relative humidity results in decreased egg recovery and increased incidence of unfertilized eggs (Ayuzawa *et al.,* 1972). The occurrence of unfertilized eggs was more common in summer as compared to other seasons (Biram *et al.,* 2009). Larvae and cocoon exposures to 35˚C or above results in poor performance of silkworm moths in relation to egg number and egg fertility and high temperature during rearing, cocooning, mating and oviposition induced unfertilized egg layings (Gowda, 1988). Rate of development and physiology of larvae may be influenced by high temperature which results in alteration in metabolism causing reduced egg yield, increased mortality and enhanced disease incidence due to the production of apyrene sperm which results in the production of unfertilized eggs (Kovalev, 1970). The apyrene production reduces at 35˚C by 50% (Osanai *et al*., 1989) resulting in increased egg infertility.

The number of investigations suggests that exposure of male silkworms at higher temperature from the time of spinning to pre-pupal period brings male sterility (Sugai and Takahashi, 1981; Das *et al*., 1996) and results in increasing of unfertilized eggs (Biram and Gowda, 1987; Ming, 1994). The results indicated that variations in temperature and humidity during the rearing of larvae have significant effect on the fecundity and fertility of silkworm moths. The number of eggs laid by a moth decreased significantly when the larvae were exposed to increased temperature and low humidity (T_1, T_4 and T_7). Fluctuations in temperature and humidity during rearing of larvae disturbed rhythm and efficiency of the larvae to harvest the energy during 5^{th} instar for the synthesis of cocoon before pupation and complete developmental process during the non-feeding stages. Thus, much of energy resources are utilized to restore internal homeostasis when external environment exerts stress. The fecundity and fertility are two major components of silkworm seed production. The study showed that fluctuations in rearing temperature and humidity have adverse effects on the egg production and egg fertility of the silkworm moths. The best temperature and relative humidity combination was 25±1°C with 75±5% and the promising silkworm lines identified were M-101, M-107, Pak-2, Pak-3, Pak-4 and PFI-I. These silkworm lines may be utilized for silkworm seed production and hybridization programmes. The study also suggests that other factors of seed cocoon (hatchability, pupation and larval mortality) production should be evaluated along with commercial traits by utilizing Evaluation Index Method (EIM) for selection of silkworm lines for further studies.

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