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Full Length Research Paper

Studies on the influence of temperature and humidity on biological traits of silkworm (*Bombyx mori* L.; Bombycidae)

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Impact of variations in temperature and humidity on pupation, hatchability and larval mortality of eleven inbred silkworm lines M-101, M-103, M-104, M-107, Pak-1, Pak-2, Pak-3, Pak-4, PFI-I, PFI-II and S-1 was investigated during autumn and spring, 2007-2008 at Sericulture Research Laboratory, Lahore. The experiment was laid out in factorial design with replications and the data was pooled over season. The larvae of 4th and 5th instar were exposed to three temperature regimes (25, 30 and 35 ± 1°C). Significant variations in hatchability, pupation and mortality were noticed due to the effect of temperature and relative humidity on 4th and 5th instar larvae of inbred silkworm lines. The maximum mean values of hatchability (93.15), pupation (93.12) and the lowest mean larval mortality (2.60) was observed at 25°C and 70-80% RH. Lower RH of (55 and 65%) even at 25°C lowered the hatchability and pupation of the silkworm lines and contributed significantly in higher larval mortality. The lowest mean value of hatchability (68.96) and pupation (76.55) was recorded at 35°C and 55% RH while highest larval mortality (11.92) was noticed at 35°C with 55% RH. The results indicate that the mean performance of inbred silkworm lines under various conditions of temperature and humidity was significantly different from each other at various temperature and humidity exposures during 4th and 5th instar. At 25°C with 75% RH, the performance of silkworm lines remained consistent but variations in temperature or humidity for three hours significantly affected all three parameters (hatchability, pupation and larval mortality). The results illustrate that hatchability percentage of M-101(84.98), Pak-2 (84.52), Pak-3 (84.32) and Pak-4 (84.05) and pupation rate of Pak-4 (86.60), Pak-2 (86.08), PFI-I (85.33) and M-101(84.88) was significantly better as compared to other silkworm lines. The mean values of larval mortality observed in Pak-2 (5.56), Pak-3 (5.76), PFI-I (6.03) and M-107 (6.20) showed significantly lower mortality. The lower relative humidity level (less than 65%) is not conducive for seed cocoon production even at the optimum temperature of 25°C. The study clearly underlines the importance of optimization of environmental conditions during larval rearing in relation to seed cocoon production. The investigations strongly recommend that temperature and relative humidity in the range of 25-26°C and 70-80% respectively are mandatory for excellent results of egg hatchability, pupation and survival rate (low larval mortality). The results also emphasize that Pak-2, Pak-3, Pak-4, PFI-I, M-101 and M-107 showed better potential for seed production and commercial exploitation.

Key words: Sericulture Pakistan, silkworm rearing.

INTRODUCTION

The utilization of silkworm (*Bombyx mori* L.) for the production of natural silk in the form of cocoons has been exploited worldwide since its discovery in 2700 BC. China, India and Korea are among the world's leading silk producing countries. In Pakistan, sericulture has been practiced by farming communities wherever the mulberry is grown in various parts of the country including Azad Jammu and Kashmir. The main activity of natural silk

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production is practiced around the irrigated forest plantations of Changa Manga, Daffar, Sargodha, Khushab (Joharabad), Kamalia, Chichawatni and Multan in Punjab province (Sericulture Research Wing, 2008). The cocoon crop success is determined by several factors including weather conditions during rearing, nutrition and ability of the silkworm races to deliver under stress environment. Silkworm is highly sensitive to changes in the environment (temperature, humidity and photoperiod) which govern the life cycle of the silkworm and ultimately the cocoon crop. Voltinism of the successive generations of the silkworm strains depends upon the environmental conditions during various stages. Several researchers have tried to establish the rearing requirements of the silkworm for successful cocoon crop on commercial basis as well as for egg production to ensure continuity of the process. Rearing performance affects growth and development of larvae and cocoon production (Thapa and Ghimire, 2005). The silk cocoon production is determined by various factors including environment and genotype of the silkworm (Chatterjee et al., 1993). Many of the silkworm characters are not only controlled by genes but also influenced by environmental factors such as temperature and relative humidity. High temperature affects nearly all biological processes including the rates of biochemical and physiological reactions (Hazel, 1995; Willmer et al., 2004) ultimately affecting the quality and quantity of cocoon crops. Several researchers reported (Tazima and Ohuma, 1995; Hussain et al., 2011) that silkworm larvae are sensitive to high temperature (above 25 ± 1°C) during 4th and 5th instars. Fluctuations in environmental conditions and poor management practices during larval rearing coupled with nutrient deficient mulberry leaves emphasized the need to use silkworm eggs with higher viability and ability to produce excellent cocoon crop under stress conditions. Thus, there was need to evaluate the existing silkworm strains for their performance under different climatic conditions with respect to cocoon production for seed purpose (ussain et al., 2010). The present study was planned to find out the effect of variations in temperature and relative humidity on silkworm egg hatchability, pupation rate and larval mortality.

MATERIALS AND METHODS

The experiment was carried out to evaluate eleven inbred silkworm lines (Pak-4, PFI-I, M-103, M-104, Pak-2, PFI-II, S-1, M-107, Pak-1, M-101 and Pak-3) against various treatments of temperature and humidity during autumn and spring 2007-2008 at Sericulture Research Laboratory, Lahore. The larvae were reared in three replications in Completely Randomized Factorial Design. In each replication, 300 larvae were retained and were reared at standard conditions of temperature and humidity (Rao et al., 2006). At the beginning of 4th instar, 50 larvae were maintained in each replication and were subjected to various treatments (Table 1) of temperature and humidity during 4th and 5th instar for three hrs daily in addition to controlled condition rearing.

The temperature and relative humidity were controlled inside the rearing chamber (BOD incubator) according to treatments. Rearing room temperature and humidity were managed by using air conditioner and humidifier, respectively. The feeding, bed cleaning and sanitation was maintained according to Krishnaswami, (1978). After the completion of spinning process, cocoons were kept for moth emergence at standard conditions of cocoon storage (Greiss et al., 2002). The female moths were individually inspected for their disease freeness. The eggs were incubated to observe the hatchability of eggs. The data were recorded following Rao et al. (2006) and Ramesh et al. (2009). The hatchability and pupation was calculated as:

Hatchability

$$\text{Hatchability (\%)} = \frac{\text{No. of hatched eggs per laying}}{\text{No. of fertilized eggs per layings}} \times 100$$

Pupation rate (%)

The percentage of cocoons obtained from the larvae retained after 3rd instar was calculated by following formulae:

$$\text{Population Rate (\%)} = \frac{\text{No. of good cocoons } + (\text{No. of double cocoons} \times 2)}{\text{larvae retained after 3rd moult (300)}} \times 100$$

Mortality (%)

The percentage of larvae died after the 3rd instar was calculated and larvae were inspected to identify the infected ones as per the signs and symptoms of disease.

Statistical analysis

The data was analyzed in completely randomized design (Factorial) by using MSTATC statistical package. Duncan's Multiple Range Test (DMRT) was applied (P < 0.05) to compare treatment means (Steel and Torrie, 1981).

RESULTS AND DISCUSSION

The results indicate that various treatments of temperature and humidity affected the egg hatchability of silkworm lines significantly. The significant differences among the treatments ($F_{9, 440}$ = 444.788, P < 0.000), silkworm lines ($F_{10, 440}$ = 16.115, P < 0.000) and interaction between treatments × silkworm lines ($F_{90, 440}$ = 2.580, P < 0.000) were observed. The maximum egg hatching was observed at 25 ± 1°C with 70-80 % RH (93.15) followed by 25 ± 1°C with 75% RH (92.20), 25 ± 1°C with 75% RH (86.64) and lowest hatchability was

Table 1. Exposure of silkworm larvae to various temperature and RH conditions during 4th and 5th larval instar in addition to controlled conditions of temperature and humidity.

Treatment	Temperature (°C) for 3h	Humidity (%)
T_o (Controlled condition)	**Standard rearing temperature (25±1)**	**Standard relative humidity (70- 80)**
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

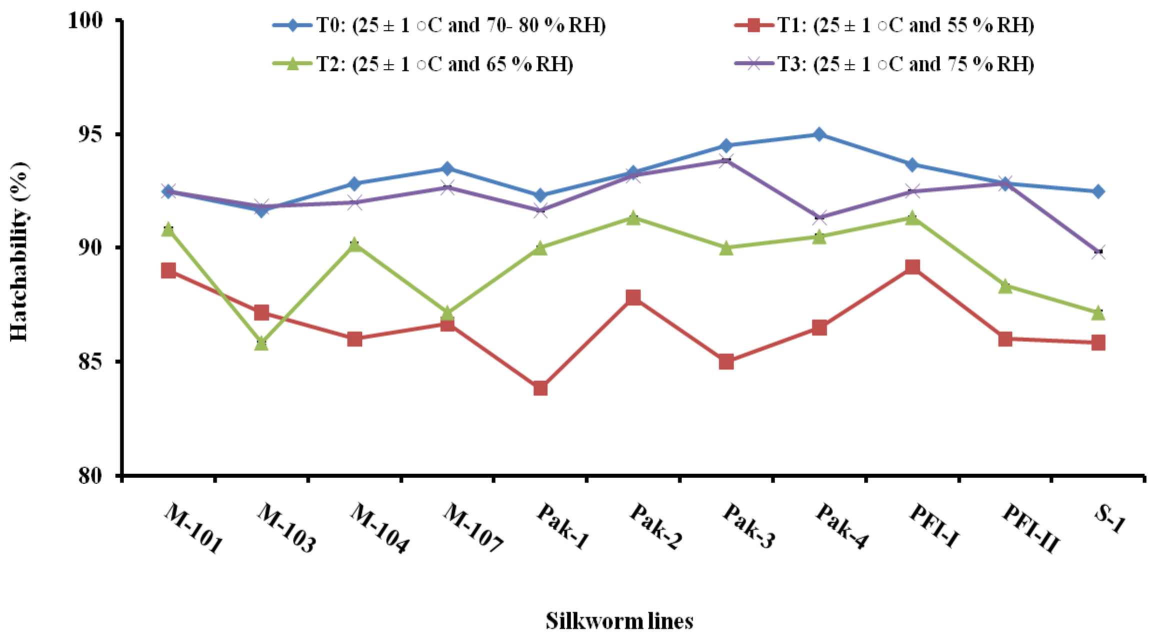

Figure 1. Comparative performance of hatchability (Mean ± SE) of eleven silkworm lines at 25 ± 1°C in combination with 55, 65 and 75% RH and controlled conditions of temperature and RH (25 ± 1 °C and 70- 80%, respectively).

recorded in 35 ± 1°C with 55% RH (68.96). Mean hatchability in all the treatments was significantly different except at controlled conditions (25 ± 1°C with 70-80 % RH) and 25 ± 1°C with 75% RH (T_3) was not significantly different. Maximum average egg hatching percentage was observed in M-101(84.98) followed by Pak-2 (84.52) and Pak-3 (84.32) while the lowest hatchability was observed in S-1(80.55). The eggs obtained from moths which were the outcome of the larvae reared at controlled conditions of temperature and humidity (25 ± 1°C and 70-80% RH) and 25 ± 1°C and 75% RH) showed better results for hatchability as compared to other combinations of temperature and RH (Figure 1). The analysis of data also indicated that effect of seasons during larval rearing on hatchability ($F_{1, 440}$ = 1.011, P > 0.05), interaction between treatments × season ($F_{9, 440}$ = 5.166, P > 0.05), line × season ($F_{10, 440}$ = 0.827, P > 0.05) and line × treat-ments × season ($F_{90, 440}$ = 0.735, P > 0.05) were not significantly different.

The results depict that rearing of 4th and 5th instar larvae under temperature and humidity stress conditions affected hatchability of inbred silkworm lines adversely. Tempe-rature and relative humidity variations (30°C ± 1 and 35°C ± 1 in combination with 55, 65, 75% RH) during larval rearing affected the development process of silkworm lines which resulted in egg laying with reduced

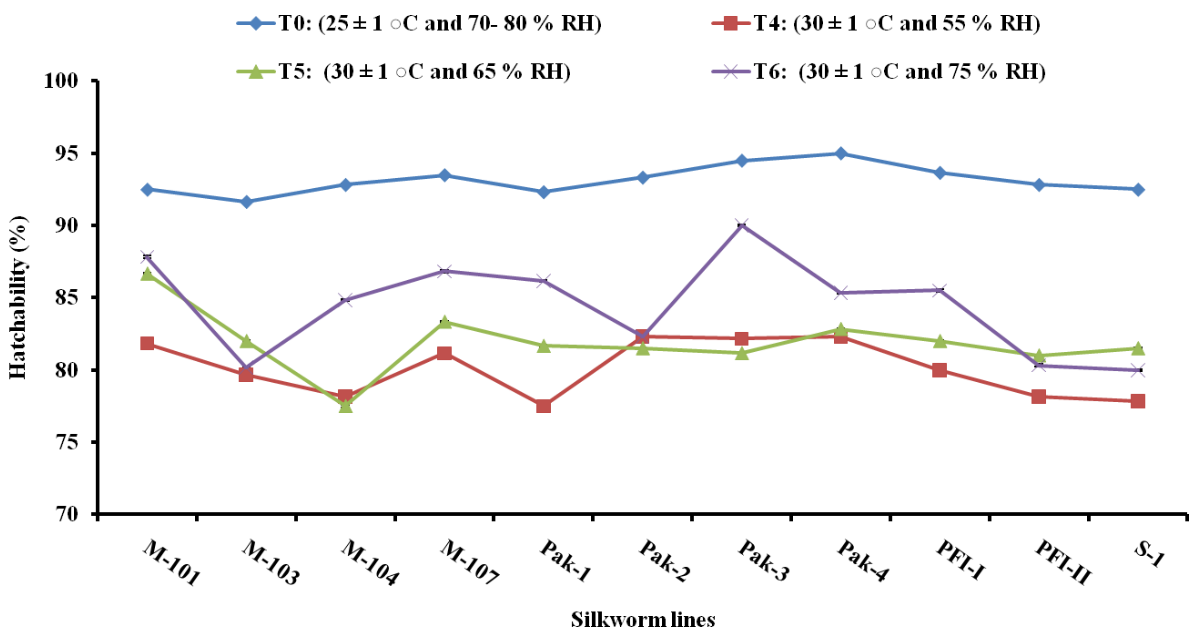

Figure 2. Comparative performance of hatchability (Mean ± SE) of eleven silkworm lines at 30 ± 1 °C in combination with 55, 65 and 75% RH and controlled conditions of temperature and RH (25 ± 1 °C and 70- 80%, respectively).

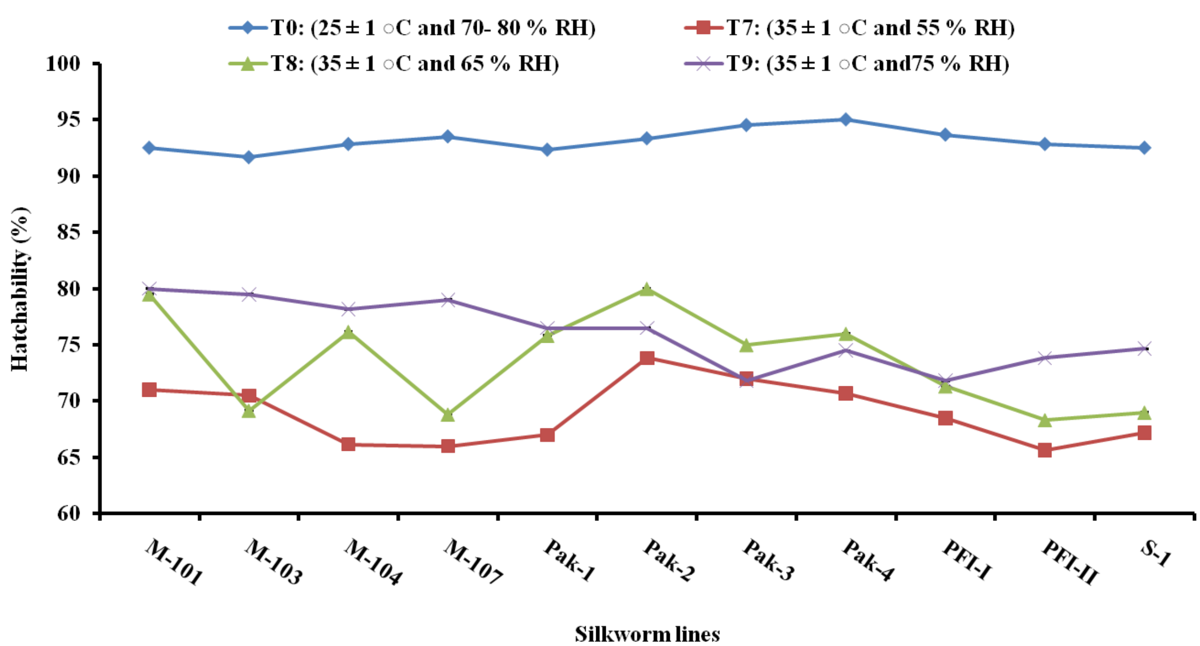

Figure 3. Comparative performance of hatchability (Mean ± SE) eleven silkworm lines at 35 ± 1 °C in combination with 55, 65 and 75% RH and controlled conditions of temperature and RH (25 ± 1 °C and 70- 80%, respectively).

hatchability (Figures 2 and 3). These observations support earlier findings which explained that race characteristics for various biological and economic traits are under the influence of rearing conditions and place of rearing due to variations in temperature and humidity (Begum et al., 2008). The results are also in line with the results which reported that biotic and physical factors exert pressure on silkworm larvae during rearing resulting in low productivity (Kobayashi et al., 1986).

The mean values of pupation showed significant differences between different treatments of temperature and relative humidity ($F_{9, 440}$ = 343.100, $P < 0.000$). The highest pupation rate was observed at 25 ± 1°C with 70-80 % RH (93.12) followed by 25 ± 1°C with 75 % RH

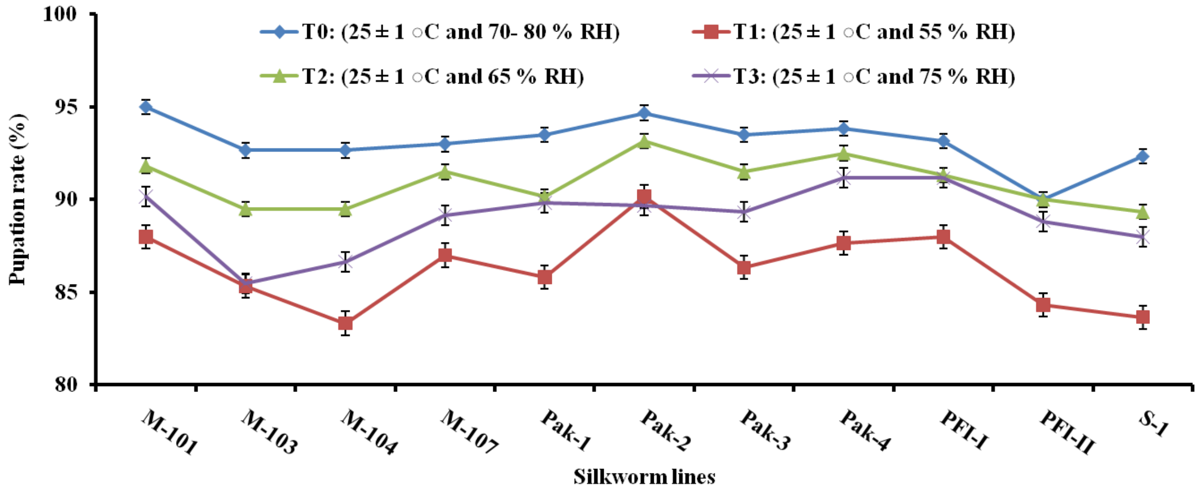

Figure 4. Comparative performance of pupation rate (Mean ± SE) eleven silkworm lines at 25 ± 1 °C in combination with 55, 65 and 75% RH and controlled conditions of temperature and RH (25 ± 1 °C and 70- 80%, respectively).

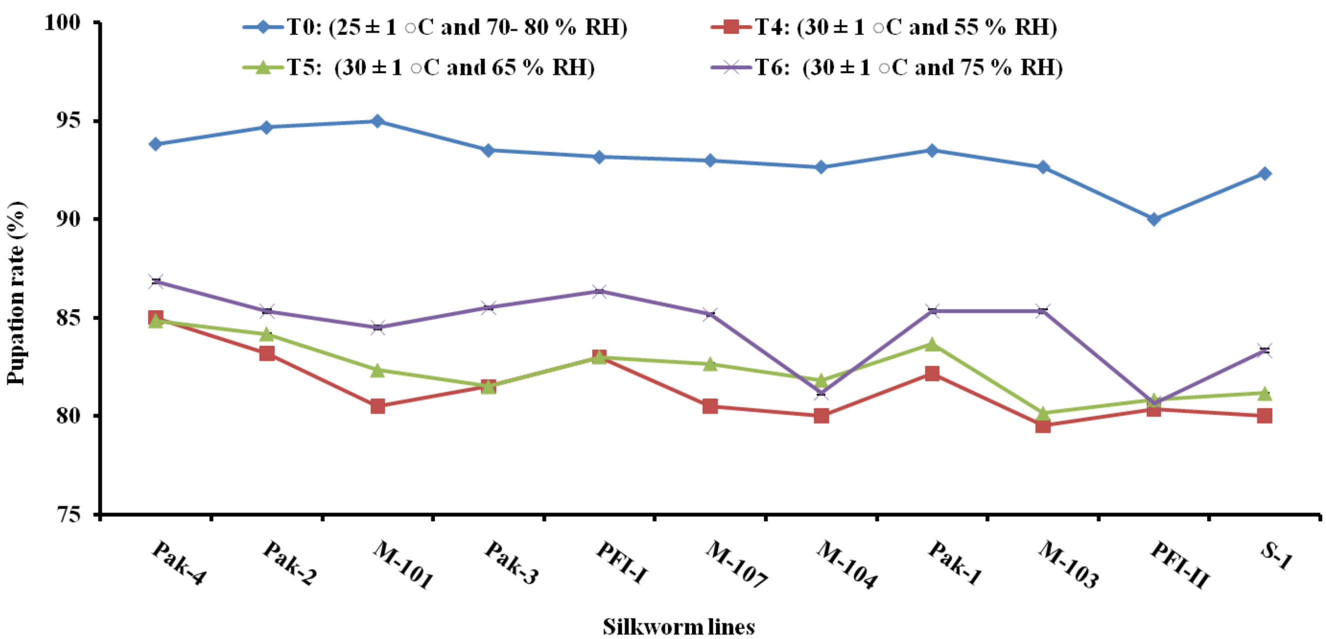

Figure 5. Comparative performance of pupation rate (Mean ± SE) of eleven silkworm lines at 30 ± 1°C in combination with 55, 65 and 75% RH and controlled conditions of temperature and RH (25 ± 1 °C and 70- 80%, respectively).

(90.94), 25 ± 1 °C with 65 % RH (89.05) and the lowest pupation was recorded in 35°C ± 1 with 55% RH (76.55). Maximum pupation rate was noticed in the larvae of M-101 (95.00) followed by Pak-2 (94.67), Pak-4 (93.83), Pak-3 (93.50) and Pak-1(93.50) at controlled conditions of temperature and humidity. Overall performance of silkworm lines under various treatments of temperature and humidity was significantly different ($F_{90, 440} = 1.283$, $P < 0.000$). The mean performance of pupation is presented in Figures 4, 5 and 6 which depicted that the performance of silkworm lines under different treatments of temperature and humidity was significantly different. The data clearly indicate that adverse temperature and relative humidity influenced the growth and development of silkworm larvae which resulted in decreased pupation rate (Figures 5 and 6). The data also depict that with the increase in temperature and decrease in RH, the pupation rate decreased significantly (Figures 5 and 6).

The data show that larval mortality was influenced by exposing larvae to temperature and humidity variations (30 ± 1°C and 30 ± 1°C with 55, 65 and 75% RH). The larval mortality was much higher in the treatments with

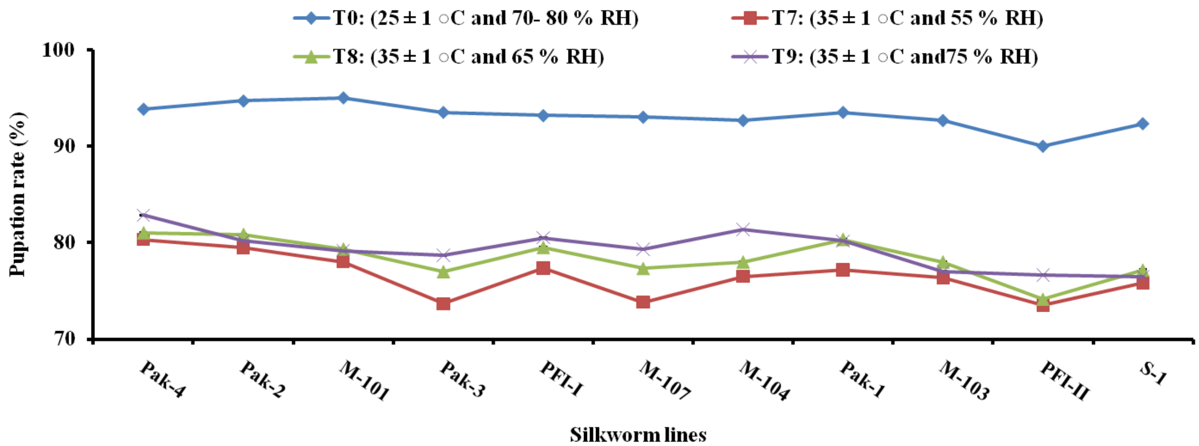

Figure 6. Comparative performance of pupation rate (Mean ± SE) of eleven silkworm lines at 35 ± 1 °C in combination with 55, 65 and 75% RH and controlled conditions of temperature and RH (25 ± 1 °C and 70- 80%, respectively).

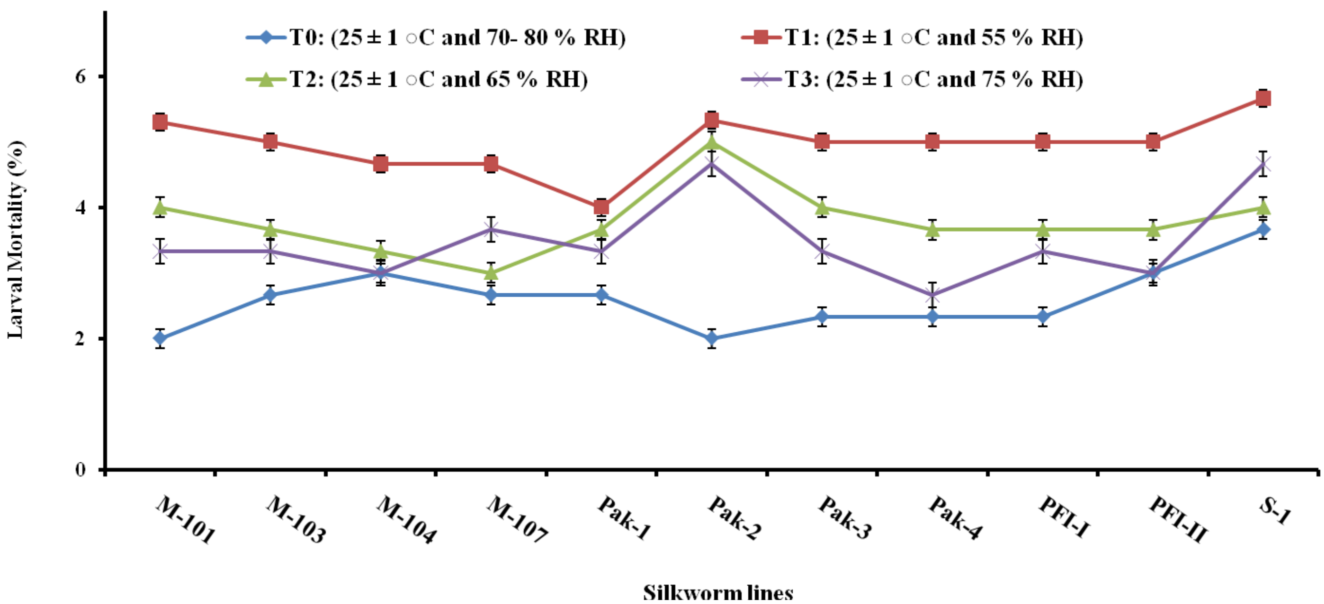

Figure 7. Comparative performance of larval mortality (Mean ± SE) of eleven silkworm lines at 25 ± 1 °C in combination with 55, 65 and 75% RH and controlled conditions of temperature and RH (25 ± 1 °C and 70- 80%, respectively).

high temperature and low humidity (Figures 7, 8 and 9). The maximum mean larval mortality was recorded in T_7 (9.23), followed by T_4 (8.96), T_8 (7.85) and T_1 (7.85) which clearly indicated that at lower RH at all three temperatures (25, 30 and 35°C), larval mortality was higher. The maximum larval mortality was shown by S-1 (8.52) followed by PFI-II (7.65), M-103(7.35) and Pak-1(7.10) while the lowest larval mortality was observed in Pak-2 (5.57). The lower larval mortality rate was shown by Pak-4, Pak-2, Pak-3, PFI-I and M-107 (Figures 7, 8 and 9). The results reveal that these silkworm lines had lower larval mortality showing significant difference with other silkworm lines ($F_{10, 440}$ = 12.004, P < 0.000). The effect of different treatments on larval mortality indicated that low humidity has immense effect on the larval mortality (Figures 7, 8 and 9). The analysis of data showed that influence of season during larval rearing on larval mortality was not significantly different ($F_{1, 440}$ = 3.001, P > 0.05), interaction between treatments × season ($F_{9, 440}$ = 0.157, P > 0.05), line × season ($F_{10, 440}$ = 0.240, P > 0.05) and line × treatments × season ($F_{90, 440}$ = 0.345, P > 0.05) were statistically not significant.

The results of the study emphasized that the performance of silkworm lines was greatly influenced by the temperature and humidity regimes during larval rearing. In the present study, Pak-3, Pak-4, PFI-I and M-101 showed better performance for hatchability, pupation rate and showed lower larval mortality as compared to other

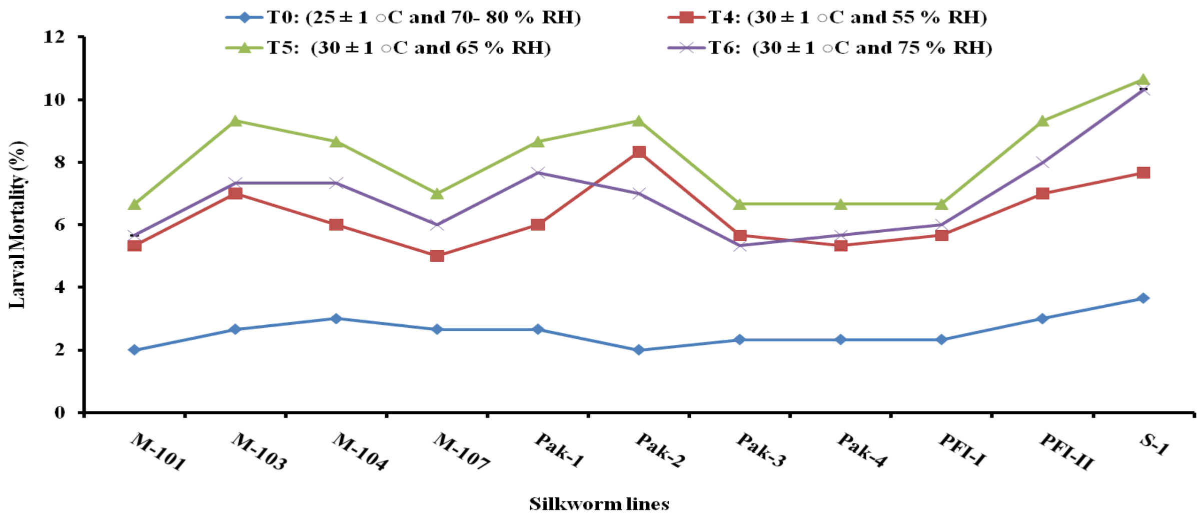

Figure 8. Comparative performance of mean larval mortality of eleven silkworm lines at 30 ± 1 °C in combination with 55, 65 and 75% RH and controlled conditions of temperature and RH (25 ± 1 °C and 70- 80%, respectively).

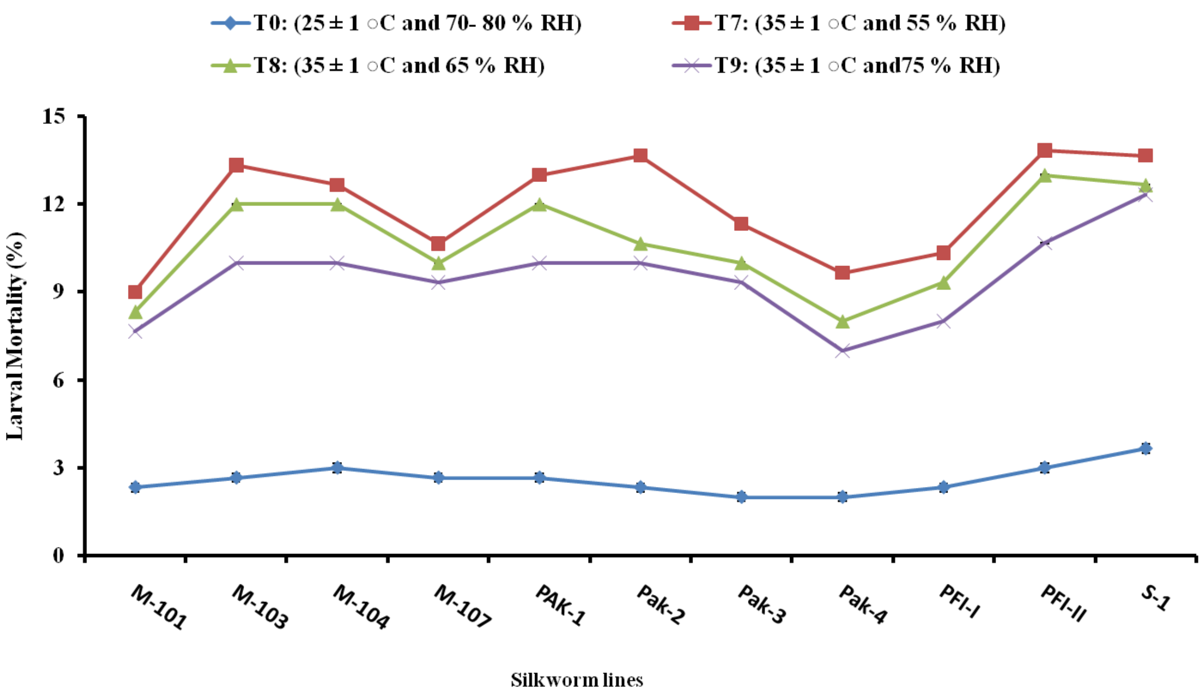

Figure 9. Comparative performance of mean larval mortality of eleven silkworm lines at 35 ± 1 °C in combination with 55, 65 and 75%
RH and controlled conditions of temperature and RH (25 ± 1 °C and 70- 80%, respectively)

silkworm lines which may be due to their ability to resist stress environment during rearing. Similar findings were reported by other researchers validating our results. Genetic stability of the silkworm strain determines the adaptability of silkworm strains to the variations in the environmental conditions (Li et al., 2001; Gangwar et al., 2009). Datta et al. (2001) and Kumar et al. (2002) deleterious effect of high temperature is more pronounced in productive hybrids as compared to robust hybrids. Pandey et al. (2006) reported that rise in temperature from 24 -36 °C produces considerable decline in larval duration which may affect the lateral stages of the life cycle of *Bombyx mori* L. The findings of this study are also in confirmation with the works of Benchamin et al. (1983) and Tazima (1988) who found similar results in separate studies conducted on the effect of temperature and humidity on the growth and development of silkworm. This may be ascribed to differences in the genetic potential and racial specificity as pointed out by Nagaraju, (2002). Pandey and Tripathi (2008) observed the effect of relative humidity between the range of 55-80% RH on survival rate and larval mortality of the silk worm. Li et al. (2001) reported that variations in environmental factors during larval rearing cause higher mortality and lowers cocoon yield significantly. Hugar and Kaliwal (1998) observed that the 5^{th} instar larvae were most active instar and hub of larval activity during which larvae build up large quantities of food reserves which are harvested for cocoon spinning, metamorphosis and reproduction.

Conclusion

In practice, the seed cocoons are generally collected from farmers, which usually rear silkworm larvae under natural conditions with little modification in the micro environment of the rearing rooms. This makes the larvae to strive for their survival against the stress environment depleting much of their energy resources in maintaining homeostasis ultimately influencing quantitative and qualitative traits adversely. It can be concluded that exposure of later age (4^{th} and 5^{th} instar) silkworm larvae to variations in temperature and humidity (above 25 ± 1°C and below 70% RH respectively elicited low hatchability and pupation rate. The study also illustrated that higher larval mortality resulted when larvae were exposed to higher temperature (30 and 35°C) and lower RH (55 and 65%). This implies that cocoon crops for seed purpose require optimum conditions of temperature and humidity during larval growth and development.

REFERENCES

Begum NAR, Basavaraja HK, Joge PG, Palit AK (2008). Evaluation and Identification of Promising Bivoltine Breeds in the Silkworm, *Bombyx mori* L. Int. J. Ind. Entomol. 16(1):15-20.

Benchamin KV, Jolly MS, Benjamin DAI (1983). Study on the reciprocal crosses of multivoltine × bivoltine with special reference to the use of bivoltine hybrid as a parent. National Seminar on Silk Research and Development, Bangalore March 10-13.

Chatterjee SN, Rao CGP, Chattergee GK, Aswath SK, Patnaik AK (1993).Correlation between yield and biochemical parameters in the mulberry silkworm, *Bombyx mori* L. Theor. Appl. Genet. 87:385-391.

Datta RK, Kumar NS, HK Basavaraja, Kumar CMK, Reddy NM (2001). "CSR18 x CSR19"-a robust bivoltine hybrid suitable for all season rearing in the tropics". Indian Silk. 39: 5-7.

Gangwar SK, Jaiswal K, Dwivedi P, Gupta, V (2009). Synthesis of promising bivoltine breed UP of the silkworm (*Bombyx mori* L.) for Uttar Pradesh. J. Agric. Biol. Sci. 4: 25-27.

Greiss H, Petkov N, Yungen M (2002). Optimized rearing conditions for silkworm, *Bombyx mori* L. egg production under Egyptian conditions. Philip. J. Sci. 131(2): 137-141.

Hazel JR (1995). Thermal adaptation in biological membranes: Is homeoviscous adaptation the explanation? Annu. Rev. Physiol. 57: 19-42.

Hugar II, Kaliwal BB (1998). Effect of Benzyl-6-aminopurine and indole –3-acetic acid on the biochemical changes in the fat body and haemolymph of the bivoltine silkworm, *Bombyx mori* L. Bullet. Sericult. Res. 9: 63-67.

Hussain M, Khan SA, Aslam M (2010). Evaluation of genetic potential of inbred pure lines of silkworm for breeding and cocoon production in Pakistan. Afr. J. Food Sci. 4(5): 300-302

Hussain M, Khan SA, Naeem M, Mohsin AU (2011). Effect of relative humidity on factors of seed cocoon production in some inbred silk worm (*Bombyx mori*) lines. Int. J. Agric. Biol., 13: 57-60.

Kobayashi J, Edinuma H E , Kobayashi N (1986). The effect of diapause egg production in the tropical race of the silkworm, *Bombyx mori* L. J. Seric Sci. Jpn., 55: 345-348.

Krishnaswami S (1978). New technology of silkworm rearing. CSR and TI, Bulletin No. 2. Central Silk Board, Bangalore, India. pp. 1-23.

Kumar NS, Basavaraja HK, Kumar CMK, Reddy NM, Datta RK (2002). "On the breeding of "CSR18 x CSR19"-a robust bivoltine hybrid of silkworm, *Bombyx mori* L. for the tropics". Int. J. Ind. Entomol. 5: 155-162.

Li M, Yao W, Hou Q, Lin CQ, Chen KP (2001). Studies of some special characters in the silkworm (*Bombyx mori* L.) germplasm in China. Sericologia, 41: 527-535.

Nagaraju J (2002). Application of genetic principles for improving silk production. Curr. Sci. 83: 409-414.

Pandey P, Tripathi SP (2008). Effect of humidity in the survival and weight of *Bombyx mori* L. Larvae. Malays. Appl. Biol, 37:37-39.

Pandey P, Tripathi SP, Shrivastav VMS (2006). Effect of ecological factors on larval duration of silkworm (*Bombyx mori* L.). J. Ecophysiol. Occup. Health, 6(3-4): 3-5.

Ramesh C, Seshagiri SV, Rao CGP (2009). Evaluation and identification of superior polyvoltine crossbreeds of mulberry silk worm, *Bombyx mori* L. J. Entmol., 6: 179-188

Rao CGP, Seshagiri SV, Ramesh C, Basha K, Ibrahim H, Nagaraju J, Chandrashekaraiah M (2006). Evaluation of genetic potential of the polyvoltine silkworm, (*Bombyx mori* L.) germplasm and identification of parents for breeding programme. J. Zhejiang Univ. Sci. 7(3): 215-220.

Sericulture Research Wing (2008). Annual Research Report. Punjab Forestry, Fisheries and Wildlife Department, Punjab. pp. 21-25.

Steel RGD, Torrie JH (1981). Principles and Procedures of Statistics. McGraw Hill Book Co. Inc., New York.

Tazima Y (1988). A view on the improvement of Mysore breeds. Proceedings of International Congress on Tropical Sericulture.pp. 18-23.

Tazima Y, Ohuma A (1995). Preliminary experiments on the breeding procedure for synthesizing a high temperature resistant commercial strain of the silkworm, *Bombyx mori* L. Silk Sci. Res. Inst. Jpn. 43: 1-16.

Thapa RB, Ghimire NP (2005). Performance of mulberry silkworm (*Bombyx mori* L.) under leaf and shoot feeding methods. J. Inst. Agric. Anim. Sci. 26:83-86

Willmer CW, Stone G, Johnston I (2004). Environmental Physiology of Animals. Blackwell Science, Oxford.