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Effect of Relative Humidity on Factors of Seed Cocoon Production in some Inbred Silk Worm (*Bombyx mori*) Lines

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ABSTRACT

The evaluation of silk worm lines for seed cocoon production was studied in humidity stress conditions during spring 2009. The selected silk worm lines viz. Pak-1, Pak-2, Pak-4, PFI-1, M-103, M-107 were reared at 25±1˚C in combination with different relative humidity (RH) levels i.e., 80±5% (control), 50%, 60%, 70% and 90% RH. The maximum fecundity (number of eggs laid by a single moth) was observed at 80% RH (436) followed by 70% RH (320), 60% RH (305) and 90% RH (218). The highest hatchability was observed at RH of 80% (88.66) followed by 90% (71.05), 50% (70.28) and 60% (60.10). The best pupation occurred at 80% RH (88.44) followed by at RH of 70% (81.44), 60% (73.16) and 50% (68.94). The highest larval mortality was noticed at 90% RH (32.66) followed 50% (27.77), 60% (19.72) and 70% (13.77) RH, whereas the lowest mortality was found in controls set (10.94). Pak-1 showed better performance at all humidity levels for fecundity (300), hatchability (73.328), pupation (79.66) and mortality (16.33). Pak-1, Pak-2 and Pak-4 were not significantly different for fecundity, pupation and mortality when larvae were reared at 75±5% RH, while significant differences were observed between all the other silkworm lines at this RH. The investigations showed that either low (50%) or high (90%) RH levels have adverse effect on the fecundity of female moth, egg hatchability, pupation and larval mortality. Three silkworm lines (Pak-1, Pak-2 & Pak-4) were better performer for all the four traits with no significant differences for hatchability, pupation and larval mortality among them. These three silkworm lines may be utilized for commercial seed production and hybridization. The study elucidated that rearing of larvae under humidity stress conditions resulted in poor performance of the silk worm lines. Silkworm seed cocoon production under unfavorable humidity conditions should be avoided to maximize the exploitation of genetic potential of silkworm lines.

Key Words: Inbred silk worm lines; Humidity stress; Seed cocoon

INTRODUCTION

The silk worm (*Bombyx mori* L.) is domesticated insect, which feeds exclusively on mulberry leaves to produce raw silk in the form of cocoon. The silkworm has been extensively utilized as model organism in biological studies as well as for economic gains. Commercial rearing of silkworms has been in practice for over 5000 years in different parts of the world (Nagaraju & Goldsmith, 2002) and an estimated 4310 silkworm germplasm strains are being reared worldwide (Goldsmith *et al*., 2005).

The life cycle of silk worm is greatly influenced by environmental stress and nutrition particularly during larval period. Relative humidity (RH) affects all stages of the insect. Deviations in humidity levels below and above certain critical limits affect larval growth and development. Low RH levels affect survival of early instars and have great impact on pupation and fecundity (Bursell, 1970; Rockstein, 1974). The cations binding proteins decrease due to dehydration stress (Malik & Malik, 2009). Low RH affects hatching percentage and survival of early instars (Pandey & Tripathi, 2008). The revival of sericulture in Pakistan requires the production of good quality silk seed with resistance to diseases and ability to cope with environmental stress. Day to day change in weather during the larval rearing poses great threat to the cocoon crop. The larval growth of silkworm is under direct influence of temperature and humidity. Silk worm larvae spun best quality cocoons at 22°C and 65% RH (Ramachandra *et al*., 2001; Srivastava *et al*., 2007; Suresh Kumar *et al*., 2008). RH levels, above and below the optimum, result in poor performance of the silkworm larvae (Furdui *et al*., 2010). Temperature and RH variations may cause incomplete fecundity and prolongation in oviposition and extreme conditions result in poor development of embryo and increase in sterility (Jha, 1997).

The six inbred silkworm lines used in this study were the outcome of the inbreeding of various races of indigenous and exotic origin. The impact of RH on commercial traits of cocoons as well as biological aspects has been investigated

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extensively in various countries. In Pakistan little work has been done on silkworm rearing under stress environment with reference to silkworm seed cocoon production. Seed cocoons are collected from farmers for obtaining egg layings by sericulture department. These seed cocoons may be obtained from larvae, which were reared under humidity stress environment resulting not only in larval mortality but also in poor performance of silkworm moths.

In this study, the effect of different levels of RH was investigated on silkworm larvae of inbred lines to know the effect on mortality, hatchability, pupation and fecundity with reference to seed cocoon production. The present study evaluated the silkworm inbred lines against different RH levels for their utilization in insect breeding and hybrid silk worm lines development with improved silk production, adaptability to stress environments and their capability to resist or tolerate diseases.

MATERIALS AND METHODS

The experiment was carried out in Sericulture Research Laboratory, Lahore during spring 2009. The larvae were reared in three replications in Completely Randomized Factorial Design. The eggs of Pak-1, Pak-2, Pak-4, PFI-1, M-103 and M-107 silkworm lines were obtained from Sericulture Research Laboratory, Lahore, Pakistan. The young larvae (1^{st} to 3^{rd} instars) were reared at 27±1°C with 85% ± 5% RH, while 4^{th} and 5^{th} instar larvae were maintained at 25±1°C with different treatments of RH (Rao *et al*., 2006; Nezhad *et al*., 2010; Hussain *et al*., 2010). At the beginning of 4^{th} instar, 200 larvae were counted from each line and subjected to 80±5% RH (control), 50, 60, 70 and 90% RH levels.

Rearing was carried out under hygienic conditions of feeding, cleaning and sanitation following Krishnaswami *et al*. (1973). Rearing beds were prepared according to the treatments and replications. Clean bedding was provided at the inception of each instar. The larvae were reared under standard conditions of temperature and humidity until the end of 3^{rd} instar. At the beginning of the 4^{th} instar the larvae were reared in five groups with three replications. At the end of 5^{th} instar, larvae were collected manually and were provided with branches for mounting. Cocoons were harvested after 72 h of their completion and stored for moth emergence.

After emergence female moths were assorted in wooden trays followed by pouring of equal number of male moths. The selected combinations of male and female moths were allowed to copulate for 4 h. The males after copulation were rejected and the females were allowed to lay eggs on sheets and were covered with funnel. After the completion of oviposition total number of eggs per female was counted. The eggs were incubated at temperature of 25±1°C and RH of 75±5% with 16 h of light and 8 h of darkness. During the pinhead stage, the eggs were black boxed to get uniform development of embryo and simultaneous hatching of larvae on a single day. Required temperature was managed by using air conditioner. Required RH was maintained by humidifier. Maximum and minimum room temperatures were recorded using maximum-minimum thermometer.

The data were recorded following Rao *et al.* (2006) and Ramesh (2009) with little modifications, which were as follows:

Fecundity: The number of eggs laid by a single female moth and taken as an average of the egg laying of three females in the replications for each treatment for every silk worm line.

Pupation: The number of cocoons with live pupae recovered out of the number of larvae retained after 3^{rd} moult expressed in percentage. The pupation rate was calculated by dividing total number of good cocoons present in all the replications of a treatment for a given line by total number of larvae retained after 3^{rd} moult in all the replications of a treatment for given line.

$$\text{Pupation (\%)} = \frac{\text{No of good cocoons} + (\text{No. of double cocoons} \times 2)}{\text{Larvae retained after } 3^{rd} \text{ moult (200)}} \times 100$$

Hatchability: It was calculated on the 3^{rd} day after hatching by the following formula:

$$\frac{\text{Normal eggs in the layings - unhatched eggs in the layings}}{\text{Number of normal eggs in the layings}} \times 100$$

Mortality: The data on mortality was taken after the third instar larvae. The number of larvae died in all three replications of the treatments for in silk worm lines were counted during 4^{th} and 5^{th} instar. The percentage of the larvae died was calculated. The larvae were observed daily to identify the infected ones as per the signs and symptoms of disease.

Statistical analysis: The data recorded for the above parameters were analyzed in completely randomized design factorial using MSTATC package. Means were compared by applying Duncan's Multiple Range Test (DMRT) and the significance of means was checked at $P< 0.05$ (Steel & Torrie, 1981).

RESULTS AND DISCUSSION

The results showed that the average number of eggs laid by a single silkworm moth was maximum when larvae were reared in 80% RH (436) followed by RH levels of 70% (320), 60% (304) and 50% (241) and minimum in 90% (218). Pak-1 laid maximum eggs (461) when reared at standard RH (80±5%), while minimum eggs were recorded in 90% RH (183). The larvae reared at standard humidity conditions showed better egg laying as compared to all other treatments. The exposure of silkworm larvae to different levels of relative humidity adversely affected fecundity of the silk worm moths (Table I). The maximum average eggs were laid by Pak-1 followed by Pak-2, Pak-4 and PFI-1. The fecundity is significantly different between all the

Table I: Effect of different humidity levels on fecundity and egg hatchability of inbred silkworm lines during larval rearing

	Fecundity (Number of eggs per moth)							**Hatchability (%)**						
	Pak-1	**Pak-2**	**Pak-4**	**PFI-1**	**M-103**	**M-107**	***Mean**	**Pak-1**	**Pak-2**	**Pak-4**	**PFI-1**	**M-103**	**M-107**	***Mean**
80±5% RH	461.7a	463.3a	457a	434b	407c	394.3d	436.21 A	88.67a	90.67a	91.33a	90a	86a	85.33a	88.66 A
50% RH	269m	254.7n	248.7n	236.3o	231.7o	210.3p	241.78 D	75.67b	68def	71.67bcdef	66.67efg	71bcdef	68.67cdef	70.28 C
60% RH	332.3f	322gh	315.7hij	308.3jkl	300kl	249.3n	304.6 C	61.3ghi	60.67hi	59i	59.67i	59.67i	60.33i	60.10 E
70% RH	361e	329fg	318.3hi	309.3ijk	306kl	299l	320.43 B	72.67bcde	71.67cdef	69cdef	61.33ghi	59.67i	57.33i	65.27 D
90% RH	300kl	231.7o	211.7p	197.3q	187.3r	183r	218.5 E	68.33def	74bcd	74.33bcd	68.67cdef	66fgh	75bc	71.05 B
*Mean	344.8 A	320.14 B	310.28 C	297.04 D	286.4 E	267.18 F	*Mean	73.32A	73.00A	73.06 A	69.26 B	68.468 B	69.33 B	

Table II: Effect of different humidity levels on pupation and larval mortality of inbred silkworm lines

	Pupation							**Larval Mortality**						
	Pak-1	**Pak-2**	**Pak-4**	**PFI-1**	**M-103**	**M-107**	***Mean**	**Pak-1**	**Pak-2**	**Pak-4**	**PFI-1**	**M-103**	**M-107**	***Mean**
80±5% RH	93.67a	93a	93a	85.67bc	83cd	82.33cde	88.44 A	6.33l	8 kl	12.67 ijk	9 kl	13.33 ijk	16.33 hij	10.94 E
50% RH	78def	75.33fg	71gh	64.67ij	62.67ijk	62jk	68.94 D	23ef	30.33 cd	32.33 bc	22.67 ef	22.67 ef	35.67 b	27.77 B
60% RH	76.67efg	72fgh	76fg	74.67fg	68.33hi	71.33gh	73.16 C	16.67 hij	22 efg	23 ef	15.67 hij	15.33 hij	25.67 de	19.72 C
70% RH	86bc	90.33ab	83cd	77.67def	76.33fg	75.33fg	81.44 B	10 kl	17 ghi	12.33 ijk	13.33 ijk	11.33 jkl	18.67fgh	13.77 D
90% RH	64ijk	67hij	66.67hij	65ij	62jk	58.33k	63.83 E	25.67de	34.33 bc	29.67 cd	32.67 bc	30 cd	43.67 a	32.66 A
*Mean	79.66 A	79.53 A	77.93 A	73.53 B	70.46 C	69.86 C	*Mean	16.33 D	22.33 B	24.33 B	18.66 C	18.53 CD	28.002 A	

Means sharing similar letters are not significantly different (P< 0.05)
*Overall effect of treatment and performance of silkworm lines

inbred silk worm lines (Table I). Ayuzawa *et al.* (1972) concluded that low humidity exposures to various stages of silkworm resulted in low egg yield and increased egg infertility. The egg laying capability of *Bombyx mori* L. is under direct influence of genotype of silkworm race and rearing conditions (Gowda, 1988).

Egg hatchability found to be influenced at various humidity levels (Table II). The results indicated that maximum egg hatching was shown by eggs collected from 80±5% RH (88.66) followed by 50% RH (70.28) and 70% RH (71.05). Rearing under stress environments results consumption of energy in the metabolic activity (Gowda, 1988). Low RH (less than 70%) causes occurrence of weak egg laying with high proportions of unfertilized eggs, resulting in low hatchability, while high RH (above 80%) causes larval mortality by favoring disease infestation (Ifantidis, 1982; Patil & Vishweshwara, 1986). Results of this study for fecundity and hatchability of silkworm lines (Table II) are in conformity with Sen *et al*. (1999)**.** Silkworm adaptability to the fluctuations in the environment is dependent on genetic stability of the silkworm strain (Li *et al*., 2001; Gangwar *et al*., 2009). The mean values of pupation showed significant differences between different treatments of relative humidity. The highest pupation rate was observed in 80±5% RH (88.44) followed by 70% RH (81.44), 60% RH (73.16) 50% RH (68.94) and 90% RH (63.83). Pak-1, Pak-2 and Pak-4 were not significant (P< 0.05) for pupation, while M-103 and M-107 were not significantly different (Table II).

The larval mortality was much higher in 90% RH (32.66) followed by 50% RH (27.77) and 60% RH (19.72). The results revealed that PFI-1 and M-107 had lower mortality with no significant (P>0.05) difference between them but remarkably different from other lines (Table II). The contribution of abiotic components of silkworm environment is immense. 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 strive for their survival against the stress environment depleting much of their energy resources in maintaining homeostasis.

Several factors play important role during the rearing of silk worm for successful cocoon crop production (Benchamin & Jolly, 1986). Performance of silkworm (*Bombyx mori* L.) race for different parameters greatly depends upon genetic potential as well as environmental conditions during the rearing period (Mathur *et al*., 1997). Some researchers (Yokoyama, 1962; Mutswmura, 1975) reported adverse effect of decline in RH during larval rearing on the physiology the silk worms which was resulted in poor cocoon production. At low humidity larvae lose water from their body in considerable amount which result in inefficient metabolism and less conservation of energy for pupal and adult stage and this may affect egg laying ability of the female moths (Anonymous, 1987). Hugar and Kaliwal (1998) concluded that the 5th larval instar was the most active period during which larvae accumulate large quantities of food reserves to conserve nutritional resources for cocoon spinning, metamorphosis and reproduction.

Some workers (Vishwanath *et al.,* 1987; Sharma *et al.,* 1988; Sarkar *et al.,* 1995; Zaman *et al.,* 1996) are of the opinion that seasonal variations in temperature and relative humidity and feeding of different mulberry leaves of different varieties influence the performance of silkworm larvae. The survival rate of the silkworm larvae were found to be significantly (P < 0.01) influenced by the variation in RH in the range 55 to 80% (Pandey & Tripathi, 2008).

Sericulture industry has great potential in Pakistan but unable to flourish due to many reasons such as no coordination of sericulture wing with agriculture department, lack of research for commercial utilization of the silkworm and mulberry, rearing infrastructure in the areas near mulberry plantations, untrained farming community, severity of weather with fluctuation in the environment, dearth of research staff with specialization in insect breeding, insect pathology and no ecological studies on the existing stock for development of hybrid silk worm races with improved economical characteristics. The stress environment during the rearing of silkworm larvae resulted in stress on the development of larvae and lateral stages were influenced with low production and less survival rates. The crop failure at final stages of larval instars results in deformed cocoons, low pupations and poor fecundity.

CONCLUSION

The variations in rearing conditions of temperature and RH resulted in poor performance of non-feeding stages (pupa & adult) of moth. Rearing of silkworm larvae at lower levels of RH resulted in lower fecundity, hatchability and pupation and higher larval mortality. The best RH recommended for seed cocoon production on the basis of results shown by study is 75±5% at 24-26^{o}C temperature. All the silkworm lines produce poor quality eggs when larvae were reared under low (50%) and high (90%) RHs. Thorough studies on silkworm lines for temperature, humidity and their combinations with other factors should be carried out in relation to silkworm seed cocoon production to popularize sericulture industry in Pakistan.

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