EFFECT OF TEMPERATURE ON REPRODUCTIVE AND EGG LAYING BEHAVIOR OF SILK MOTH *BOMBYX MORI* L.

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ABSTRACT

In present investigation a study was conducted to determine the effect of changes in temperature on reproductive behavior, fecundity, site selection for egg laying and longevity of adult *Bombyx mori* L. crossbreed of PM X CSR2.The present investigation showed that adult of *B. mori* were died when kept at 40$\pm$ 5 ^{o} C and unable to lay eggs, at 30$\pm$ 1 ^{o} C they lay least number of eggs i.e. 2494 and died within 72 hours. At 10 $\pm$ 1 ^{o} C they exhibit diapauses mechanism and delayed in egg laying showed higher number of egg laying i.e. 2619 and found live on the day15th of termination of experiment. No change in temperature cause normal reproduction behavior and laid highest number of eggs i.e.2922 and died after seven days. Silk worm prefer egg lying at the bottom of the beaker than upper side of beaker.

Keywords: Temperature, Egg, Silk Moth, Bombyx mori.

INTRODUCTION

The silk worm *Bombyx mori* L is economically important insects which feed on specifically mulberry leaves and produce silk in the form of cocoon. Silk worm were domesticated for the silk production for about 50000 years (Nagaraju and Goldsmith, 2002). Silk is a high value but low volume products accounting for only 0.2 % of world's total textile production. Geographically, Asia is the main producer of silk in the world and produces over 95 % of the total global output. China is the leading supplier of silk to the world with an annual production of 153942 MT (2006) out of which the mulberry raw silk product is 115092 MT. India is the second largest producer of silk with 18475 MT (2006-07) and also the largest consumer of silk in the world (Anonymous, 2011). Over the last three decades, India silk production has steadily grown outpacing countries like Japan and erstwhile USSR which were once the leading silk producers. India is now the second largest producer of raw silk in the world after China, with a production of about 19,690 tons in 2009-10 (Malhotra, 2011). The silk worm has been extensively used as a model organism (Mubashar *et al.,* 2011).

Life cycle of silk worm is greatly influenced by physical factors of environment. Temperature is one of the most important physical environmental factors. Ramchandra *et al.,* (2001) reported silk worm larvae spun best cocoon at 22^{0} C and 65 % RH. Some researcher showed that good quality cocoon are produced within 22 - 27^{0} C and level above these marks cocoon quality worse (Penzaman and Jagdeeshkumar, 2010). PM x CSR2 hybrid is suggested by commercial rearing by the mulberry silkworm race authorization committee for south zone including Andhra Pradesh, Karnataka and Tamil Nadu (Anonymous 2011). PM x CSR2 hybrid is meant for irrigated areas in all the seasons and is superior over the existing hybrid in yield, cocoon wt., shell wt., SR%, filament length, raw silk %, neatness and renditta (Anonymous 2011). In present investigation a study was conducted to determine the effect of sudden change in temperature on reproductive behavior, fecundity, selection of site for egg laying and longevity of *Bombyx mori* L. Cross breed of PM X CSR2.

MATERIALS AND METHODS

Silkworm pupae of *Bombyx mori* L. crossbreed of PM X CSR_2 were collected from field and brought to the laboratory. The pupae were then kept in insect raring chamber at room temperature and allow emerging into adults. After emergence of adults, a pair of healthy copulating male and female was isolated and kept into 500 ml beaker, internally completely lined by filter paper and mouth covered by mosquito net. One set with five replicate was then placed at bottom in refrigerator at $10\pm1\ ^{O}$C, one set with five replicates was transferred in thermostatic oven at $30\pm1\ ^{O}$C and another set of five replicates was kept in another thermostatic oven with $40\pm5\ ^{O}$C. The control set with five replicates maintained at room temperature (27 ± 3^{O}C). The experiment was terminated after 15 days. The observations were recorded on reproductive behavior site for egg laying, fecundity and longevity. Data collected was analyzed using statistical software Graph pad prism3.

RESULTS AND DISCUSSION

Effects of changes in temperature on egg laying are shown in table 1. Drastic effect of changes in temperature was observed at $40^{O}\pm5^{O}$C where all five pairs were died in first seventeen hours without egg laying. At $30^{O}\pm1^{O}$C it was observed all five pairs are able to lay eggs up to 48hours and then copulate again and able to survive for 72hours. At this temperature site preferred for egg laying was bottom of the beaker and laid least number of eggs that is 2494.At $10^{O}\pm1^{O}$C there was no egg laying up to the first 72 hours. After 72 hours all females start's egg lying for next 24 hours and then copulate with male again, no further egg laying was takes place. They were able to survive for 15 day and when experiment was terminated they were found live. They laid second highest number of eggs that is 2619. While the most preferred site for egg lying was bottom side of beaker. At room temperature all five pairs was observed laying eggs within 24 hours and lasts for 48 hours. After 48 hours all pairs copulate again, no further egg lying was observed. All pairs were found alive for seven days and then died. They laid highest number of eggs that is 2922. Preferred site for egg laying was bottom of the beaker.

Table 1: Number of eggs lay by of *Bombyx mori* at different Temperature.

Sr.No.	Replicate no.	$10^0\pm1^0$C		$30^0\pm1^0$C		$40^0\pm5^0$C		Room temp27 ± 3^0C	
1	1	Us= 0	T=235	Us= 31	T=529	Us= 0	T=0	Us=20	569
		B=235		B=498		B=0		B=549	
2	2	Us= 281	T=610	Us= 7	T=569	Us= 0	T=0	Us= 649	649
		B=329		B=562		B=0		B=0	
3	3	Us= 24	T=595	Us=116	T=398	Us= 0	T=0	Us=608	608
		B=571		B=282		B=0		B=0	
4	4	Us= 9	T=558	Us 585	T=590	Us= 0	T=0	Us=0	472
		B=549		B=5		B=0		B=472	
5	5	Us= 41	T=621	Us=274	T=408	Us= 0	T=0	Us= 25	624
		B=580		B=134		B=0		B=599	
6	Sum	2619		2494		0		2922	
	Mean	523.8		498.8		0		584.4	
7	SD	163.189		90.22		0		69.22	

Us = Upper side; B= Bottom

T= Total number of egg laid by single pair = Us + B

Results of One-Way Analysis of Variance (ANOVA)

The P value is <0.0001, considered extremely significant.

Variation among column mean is significantly greater than by chance.

Table 2: Tukey-Kramer Multiple Comparisons Test.
If the value of q is greater than 4.046 then the value is less than 0.05.

Comparison	Mean difference	q	P value	95% confidence interval	
				From	To
10$\pm$^{O}C Vs 30$\pm$1^{O}C	25.000	0.5621	Ns P>0.05	-154.95	204.95
10$\pm$^{O}C Vs 40$\pm$5^{O}C	523.80	11.777	*** P<0.001	343.85	703.75
10$\pm$^{O}C Vs Room (27$\pm$3^{O}C)	-60.600	1.363	Ns P>0.05	-240.55	119.35
30+1^{O}C Vs Room (27$\pm$3^{O}C)	498.80	11.215	*** P<0.001	318.85	678.75
30$\pm$1^{O}C Vs 40$\pm$5^{O}C	-85.600	1.925	Ns P>0.05	-265.55	94.353
40$\pm$5^{O}C Vs Room (27$\pm$3^{O}C)	-584	13.139	*** P<0.001	-764.35	-404.45

ANOVA assumes that the data are sampled from population with identical SDs. This assumption is tested using Bartlett.
Bartlett's test cannot be performed because at least one column's standard deviation is zero

Table 3: Assumption test: are the data sampled from Gaussian distribution.
ANOVA assumes that the data are sampled from population that follows Gaussian distributions. This is tested using the method Kolmogorov and Smirnov:

Group	KS	P value	Passed normality test
10$\pm$^{O}C	0.3830	0.0157	NO
30$\pm$1^{O}C	0.2429	>0.10	YES
40$\pm$5^{O}C		>0.10	NO
Room (27$\pm$3^{O}C)	0.2334	>0.10	YES

At least one column failed the normality test with P<0.05.
Consider using a nonparametric test or transforming the data (i.e. converting to logarithms or reciprocals)

Table 4. Intermediate ANOVA Table

Variation	Freedom	Squares	Square
Treatment (between columns)	3	1095395	365132
Residuals (within columns)	16	158255	9890.9
Total	19	1253650	

F= 36.916 = (MStreatment/MSresidual)

Result of ANOVA showed in Table 2, 3, 4. The P value is <0.001, considered significant variation among column mean is significantly greater than by chance. Tukey-Kramer multiple comparison test showed there is significant variation when column compared with each other i.e. 10$\pm$1^{o}C Vs40$\pm$5^{O}C, 30$\pm$1 ^{O}C Vs room temperature (27$\pm$3^{O}C) and 40$\pm$5^{O}C Vs room temperature (27$\pm$3^{O}C). When data was tested using Kolmogorov and Smirnov method 10$\pm$1^{O}C and 40$\pm$5^{O}Cwhile 30$\pm$1^{O}C and room temperature (27$\pm$3^{O}C) The F value is 36.916.

Fecundity, derived from the word fecund, generally refers to the ability to reproduce.

In demography, fecundity is the potential reproductive capacity of an individual or population. In biology fecundity is under both genetic and environmental control, and is the major measure of fitness (Anonymous, 2011.)The present investigation shows that fecundity of silk worm *Bombyx mori* L. was affected by temperature.

In present investigation all pairs of silk worm died at 40$\pm$5 ^{O} C. Oman and Karumathil (1995) reported temperature above 43^{O} C found to be lethal to all developmental stages of the multi voltine race, strains *C. Nicini* of *Bombyx mori*. SYLVIA G. R. (1951) reported that common cloth moth *Tineola bisselliella* complete mortality occurred at relatively low temperatures, 41^{O}C for four hours being lethal to all stages of the insect. Insect pests of stored foods are killed within seconds when exposed to temperatures above 60^{O}C. Lower treatment temperatures can also be effective however, the exposure time required to kill all insect stages increases with decreases in temperature, taking a few hours at 50^{O}C and days at 45^{O}C.At temperatures that are not instantly lethal, insects die through heat stress and dehydration (Anonymous, 2011)
Silk worm *Bombyx mori* survived and showed delayed egg lying of about two days lateral 10 $\pm$ 1^{O}C this may be because of diapauses mechanism. This mechanism is observed first time in adult of *Bombyx mori.* Studies conducted by Robert A. Bell showed successive age groups, incubated at 25°C then transferred to 5°C, showed that eggs of gypsy moth must beat least 12-13 days old to survive prolonged chilling and to hatch successfully after re-incubation at higher temperature (Robert Bell, 1996).

Specific conditions of humidity between 70 to 80 percent and temperature range 27^{O}Cis required for cultivation of silkworm (Anonymous, 2012.) however temperature 30$\pm$1^{O} C was little higher than recommended and may affect reproductive behavior and fecundity cause less number of egg laying in silkworm. Death at this temperature caused much early than silkworm kept at room temperature due to heat stress and dehydration. As there was no sudden change in temperature of silkworm kept at room temperature hence there was highest level of egg laying.

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