International Journal of Research in Engineering, Science and Management
Volume-2, Issue-1, January-2019
www.ijresm.com | ISSN (Online): 2581-5792

Effects of Mating Temperature on Female Reproductive Output and Longevity in Silkworm, *Bombyx Mori*

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***Abstract*: Temperature has profound effects on reproductive success of the domesticated silkworm, Bombyx mori. This study was aimed to investigate the effect of constant mating temperatures (15, 20, 25, 30 and 36°C) on reproductive success and female adult longevity of a bivoltine (BHR3) and multivoltine (Cambodge) silk moths. Though, BHR3 and Cambodge are habitant of thermally different regions, mating temperature had similar effects on their reproductive traits and female adult longevity. Analysis of variance (ANOVA) revealed significant differences ($p < 0.05$) in reproductive traits and female adult longevity of experimented silkworm breeds. Lifetime fecundity and hatching percentage of eggs increased as temperature increased from 15 to 25°C, and then declined from 25 to 36°C. Fertilization percentage of eggs and female adult longevity were decreased with the increase of mating temperature. However, fertilization percentage was not significantly affected by lower mating temperatures (15 and 20°C). The present investigation confirms that 25°C as an ideal temperature for mating of silk moths and further demonstrates the reproductive consequences of mating silk moths at relatively higher and lower temperatures.**

***Keywords*: Bombyx mori, lifetime fecundity, female adult longevity, fertilization percentage, hatching percentage**

1. Introduction

Sericulture is known as agro-based cottage industry. It plays a crucial role in removing poverty from rural area by providing employment and income generation capability with minimum investment (Hiware, 2001). The mulberry silkworm, Bombyx mori is an insect of economic importance and a major source of silk production in sericulture. It is a holometabolous insect that has four distinct life stages including egg, larva, pupa and moth. Egg yield is directly related to the quality of seed cocoons, therefore egg yield is of primary interest for silkworm farmers while procuring silkworm laying (Rao et al., 1989). Egg yield is influenced by many abiotic factors such as photoperiod, temperature, humidity, duration and frequency of mating (Saha et al., 2013). Among them, temperature has a major effect on the egg yield and other reproductive traits of silkworm as it is a poikilothermic insect. Therefore, the various activities pertaining to egg production are performed within optimum limits of temperature to avoid reduction in egg yield and fertility rate (Kamble, 1997). Above or below the optimum limits of temperature during silkworm rearing, cocoon preservation and egg production leads to increase in number of unfertilized eggs (Yokoyama, 1963). Temperature stress during larval and cocoon stages reduces fertilization and hatching percentage of eggs (Biram Saheb et al., 2005). Even mating of moths exposed to high temperature of 35°C results in decline in egg yield and fertility rate. Similarly, lower temperatures also adversely affect the frequency of fertilized eggs (Ayuzawa et al., 1972). Studies of Engelmann (1970) indicates that egg production in insects do not necessarily give a true picture of the actual reproduction potential unless factors like atmospheric condition during mating period and oviposition, and nature of laying substratum are considered either individually or on a cumulative effect on the egg production. Even egg laying capacity of various silkworm breeds vary under identical atmospheric, mating and egg laying conditions (Hassanien and EL-Sharaawy, 1962; Saha et al., 2013). During unfavourable seasons, the total number of eggs laid by a female silk moth (Fecundity), fertilization and hatching percentage of eggs are reduced when compared to favourable seasons (Spring and autumn) (Khan et al., 2003; Saha et al., 2013). Though, these findings show that the temperature changes during mating has negative effect on reproductive traits of silkworm but they failed to give an exact picture of the effect of different constant temperatures during mating on reproductive traits. Keeping this point in view the current study was conducted to investigate the effect of different mating temperatures on reproductive traits of two silkworm breeds by assessing lifetime fecundity, fertilization and hatching percentage, and female adult longevity that can be utilized by the silkworm seed grainier for maximum egg recovery.

2. Materials and methods

A. Silkworm breeds and rearing

Two silkworm breeds namely BHR3 and Cambodge were considered for this study. BHR3 is a bivoltine (Two generations per year) silkworm breed that is habitant of temperate regions and is sensitive to environmental stress. Cambodge is a multivoltine (5-6 generations per year) silkworm breed that is habitant of tropical regions and it can withstand environmental stress. Rearing of these silkworm breeds was conducted by following standard procedure (Krishna swami, 1978). A standard well-practiced mulberry variety S1635 was used for the experiment purpose. The silkworm larvae were grown by feeding mulberry leaves until the final day of fifth instar. On the final day, ripened larvae were manually picked up and spread over the mountages for spinning cocoons, where they transformed to pupa and ultimately emerge as an adult.

B. Experimental design

Adult stage in silkworm is a non-feeding stage therefore the adult silkworms were maintained without any food source thorough out the experiment. Same day emerged male and female moths were used in the experiment to negate the age factor. Each virgin female was weighed, and those with relatively equal weight were introduced with male mates for mating at 15°C, 20°C, 25°C, 30°C and 36°C temperatures in a special chamber, SERICATRON (CHUO, Japan), which has a provision to control temperature and humidity, simultaneously. Male and female moths were randomly selected and allowed to copulate for 210 minutes (Saha et al., 2013) under different temperatures. Decoupling was done by holding female body and pulling back male, physically. Care was taken to have at least fifteen inseminated females for each treatment. Inseminated females were allowed to lay eggs on egg cards in semi dark environment. Every day, female moth was transferred to new egg cards to monitor female adult longevity (from adult emergence to death).

Eggs laid by bivoltine females undergo diapause, therefore in order to break the diapause these eggs were treated with hot hydrochloric acid (Specific gravity =1.075; temperature =46°C) for 5 minutes as suggested by Rajanna et al. (2008). The traces of acid were removed by washing eggs in running water and air dried. The eggs were then incubated at 25°C. Multivoltine eggs were directly incubated at 25°C as they are of non-diapausing type. Eggs were incubated at 25°C until they turned to blue colour which indicates the time for wrapping the egg cards in a black box. The process is called black boxing (It ensures uniform hatching of eggs), which was continued till the stage of pinhead formation. The eggs were then exposed to light to trigger hatching signal continuously for 72 hours to ensure complete hatching of eggs. The total number of eggs laid by a female throughout her life (Lifetime fecundity), total number of fertilized and hatched eggs was counted. Female adult longevity was recorded on daily basis by examining the moth twice in a day with an interval of twelve hours.

C. Statistical analysis

Data sets were analyzed by ANOVA if they initially met the assumptions of normal distribution (Kolmogorov-Smirnov test) and equal variance (Levene test), or met after being square root or arcsine-square root transformed with mating temperature as a fixed factor. The data sets for two experimented silkworm breeds were analyzed separately. Least Significant Difference (LSD) post hoc test was performed if significant differences ($p \leq 0.05$) were observed in different mating temperatures. But the lifetime fecundity in multi voltine and adult longevity in both bi- and multi-voltine were not normally distributed even after being square root transformed therefore Kruskal-Wallis H test was done. Once the significant difference of Kruskal-Wallis H test was detected, Mann-Whitney U test at Bonferroni corrected P value was subsequently applied. All the values are represented as mean ± SD. All statistical analyses were performed using SPSS 11.5.

3. Results

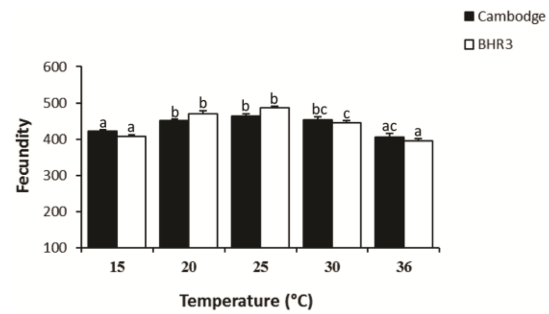

Fig. 1. Effect of mating temperatures on fecundity of silkworm breeds. Bars topped with same letter are not significantly different

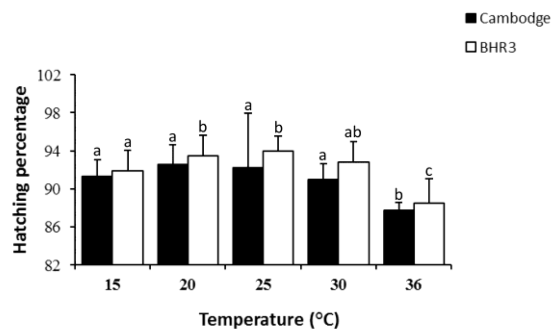

Fig. 2. Effect of mating temperatures on hatching percentage of eggs of silkworm breeds. Bars topped with same letter are not significantly different

Mating temperature significantly affected female reproductive output and adult longevity in both bivoltine and multivoltine silkworm breeds. However, the effect was relatively similar in both the silkworm breeds. Generally, higher mating temperatures had severe effects on reproductive traits and longevity than lower mating temperatures. The maximum lifetime fecundity was observed at 25°C and was significantly reduced at relatively lower and higher mating temperatures for both silkworm breeds (ANOVA, $F(4,70) = 37.308$, $p = 0.0001$ for bivoltine and Kruskal Wallis H test: $H = 28.57$, $df = 4$, $p = 0.0001$ for multivoltine). The effect of mating temperatures on lifetime fecundity in two silkworm breeds is showed in Fig. 1. Hatching percentage was highest at 25°C in both bivoltine and multivoltine silkworm breeds. Hatching percentage of the eggs differed significantly between mating temperatures in bivoltine (ANOVA, $F(4,70) = 12.828$, $p = 0.0001$) and multivoltine breed (ANOVA, $F(4,70) = 5.839$, p

=0.0001). Multiple comparisons revealed that the hatching percentage was significantly low at 15°C and 36°C in bivoltine and at 36°C in multivoltine. The effect of mating temperatures on hatching percentage in two silkworm breeds is showed in Fig. 2.

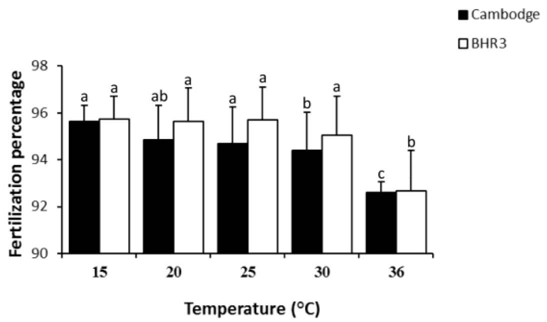

Fig. 3. Effect of mating temperatures on fertilization percentage eggs of silkworm breeds. Bars topped with same letter are not significantly different

With the increase in mating temperature, fertilization percentage was significantly decreased (ANOVA, F(4,70) =9.963, p =0.0001 for bivoltine and F(4,70) =7.741, p =0.0001 for multivoltine). Number of fertilized eggs laid by females mated at 15°C, 20°C and 25°C were nearly constant indicating that there was no influence of lower mating temperature on fertilization percentage. The effect of mating temperatures on fertilization percentage in two silkworm breeds is showed in Fig. 3. Female adult longevity was also significantly decreased with the increase in mating temperature in the experimented breeds (Kruskal Wallis H test: H =16.908, df =4, p =0.002 for bivoltine and H =21.774, df =4, p =0.0001 for multivoltine). Females mated at 36 °C had shorter life span relatively to that under lower temperatures. The effect of mating temperatures on female adult longevity in two silkworm breeds is depicted in Fig. 4.

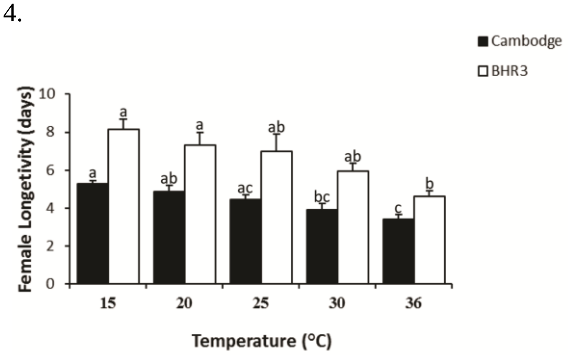

Fig. 4. Effect of mating temperatures on female adult longevity of silkworm breeds. Bars topped with same letter are not significantly different

4. Discussion

The results of current study revealed that the reproductive traits viz., lifetime fecundity, hatching and fertilization percentage of eggs, and female adult longevity were apparently affected by mating temperatures. The results showed maximum fecundity and hatching percentage of eggs at 25°C and were comparatively low at relatively higher and lower mating temperatures. Fertilization percentage of eggs and female adult longevity were also drastically reduced at higher mating temperatures relative to 25°C. Kamble (1997) and Saha et al. (2013) suggested 25-28°C as ideal temperature for silkworm rearing and egg production, any fluctuation from this temperature reduces reproductive traits. The reduction in the reproductive traits is probably due to insufficient transport of viable sperms and/or seminal fluids inside the female genital tract as a result of thermal damage caused to male accessory glands and female reproductive tract of silk moths (Fugo and Arisawa, 1992; Ouyang and Wu, 1993; Katsuki and Miyatake, 2009). Several reports have also showed reduction in the production of oviposition-stimulating substance, hormones and protein contents due to the high temperature, which negatively affected female reproductive output (Ouyang and Wu, 1993; Biram Saheb et al., 2005). The results of current study reflect the outcome of earlier investigations that higher or lower temperatures relative to 25°C reduce the reproductive traits and female adult longevity. Jha (1997) showed that variations in temperature during mating causes in-complete oviposition and poor development of embryo resulting in decrease in fecundity and hatching percentage of eggs, respectively. Bhasker et al. (1992) recorded the gradual decline in the fecundity of the moths due to high temperature. Mathur and Lall (1994) showed that maximum fecundity in silkworm was obtained at 25.36 ± 0.17°C (optimum) temperature. Mahmoud and Yehia (2009) found significant reduction in the fecundity of female silk moths mated with cold stored male moths compared to the normal mated moths. Saha et al. (2013) investigated the effects of seasonal variation on fecundity and hatching percentage of eggs and reported maximum fecundity and hatching percentage during favourable seasons (20-30°C) against unfavourable seasons (30-38°C) and suggested that temperature could be considered as an influential factor in determining the growth and fecundity of silkworm through the mediation of juvenile hormone. Dash et al. (1993) reported the effects of seasonal variation on fecundity and egg hatching percentage in Antheraea mylitta (Indian tasar silk moth), which are consistent with the results of B. mori presented in this study. In contrast, Chen et al. (2010) reported that the lifetime fecundity of Pardasa astrigera female was positively related to copulation temperature.

Sugai and Hanoaka (1972) showed that continuous exposure to high temperature induces sterility in male moths of silkworm. Mona (2013) exposed male silk moths at different temperatures (7 to 34°C) and crossed them with the normal females which resulted in significant reduction of the fertilization percentage at above and below 25°C with lowest fertilization percentage at 34°C. Wanule and Balkhande (2013) reported reduction in fecundity and female adult longevity of B. mori by mating silk moths at low (10 ± 1°C) and high (30 ± 1°C) temperatures compared to normal (27 ± 3°C) temperature and continuously allowed the female moths to lay eggs for 15 days in respective temperatures. Reduction of female adult longevity at higher mating temperatures can be explained by the non-feeding behaviour of adult silk moths, which depends entirely on metabolic resources assembled by their larvae for reproduction and other activities. Osanai (1978) demonstrated that the entire metabolism and the rate of living of silk moth appeared to be accelerated by temperature of 30°C and above, resulting in shortening their life span. Therefore, in the present study the females mated at higher temperatures had shorter life span than the females mated at lower temperatures. Similar results were also reported in tropical butterfly, Bicyclus anynana (Janowitz and Fischer, 2011). Further investigation is needed to establish the relationship between mating temperatures and the amount of sperm and/or seminal fluid transferred during mating in B. mori. Saheb et al. (2009) pointed out the occurrence of higher number of unfertilized eggs during the hot summer months compared to that in other seasons. In this context, current investigation confirms the role of higher mating temperatures in increasing the number of unfertilized eggs during hot summers. Therefore, the recommended mating temperature should be strictly followed during unfavorable seasons for maximum egg recovery with desired characters.

5. Conclusion

The present investigation confirms that 25°C as a ideal temperature for mating of silk moths and further demonstrates the reproductive consequences of mating silk moths at relatively higher and lower temperatures.

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