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Research Article

EFFECT OF THERMAL STRESS ON THE BIOLOGICAL TRAITS OF PINK BOLLWORM (*PECTINOPHORA GOSSYPIELLA* SAUNDERS) (LEPIDOPTERA: GELECHIIDAE)

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ABSTRACT

The pink bollworm (*Pectinophora gossypiella*) is a significant pest of cotton worldwide. Its occurrence and distribution across various cotton-growing regions are influenced by multiple factors, with temperature being a critical determinant. This study aimed to analyze the life history traits of the pink bollworm under different temperature regimes. Biological and demographic parameters of *P. gossypiella* were systematically examined at five specific temperatures: 20°C, 25°C, 30°C, 35°C, and 40°C, under a controlled photoperiod of 16 h of light and 8 h of darkness (LD 16:8 h). The results revealed that the total developmental period of the pink bollworm decreased with increasing temperatures, with the highest larval survival rates observed at 30°C and 40°C, reaching 78.93% and 63.89%, respectively. Moreover, pupal weight, mating success, and fecundity were significantly higher at 30°C and 35°C. In contrast, lower egg hatching rates, larval survival, and adult emergence were recorded at the extremes of 20°C and 40°C. These findings offer valuable insights into the population dynamics of *P. gossypiella* under field conditions and highlight the need for climate-resilient management strategies to mitigate its impact on cotton production.

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INTRODUCTION

Insect pests pose a significant challenge to cotton production, reducing yields not only in Pakistan but also in other major cotton-producing countries (Shuli et al., 2018; Ali et al., 2019a; Naeem-Ullah et al., 2020; Shahzad et al., 2022). The diversity of insect pests attacking cotton is extensive, with approximately 1,327 pest species reported globally. Among the most critical pests are the whitefly, pink bollworm, spotted bollworm, tobacco caterpillar, spiny bollworm, dusky cotton bug, American bollworm, thrips, green leafhoppers, and aphids (Rajendran et al., 2018; Anees and Shad, 2020; Kaur et al., 2021; Kaur and Kumar, 2024). Of these, the pink bollworm (*Pectinophora gossypiella* Saunders) is regarded as a pandemic pest of cotton, causing substantial damage to cotton fields worldwide (Garg et al., 2022; Nagrare et al., 2023; Nagaraju et al., 2024).

The pink bollworm undergoes complete metamorphosis, progressing through four major life stages. The developmental cycle begins with eggs laid by adult females (Sarwar, 2017; Ihsan et al., 2021; Mushtaq et al., 2021). Female pink bollworms take 3-4 days to mate and develop eggs internally. Within ten days, the females lay eggs either singly or in groups of 4-5 on flowers, petiole axils, the underside of leaves, or young bolls (Ghoneim, 2018; Borkar et al., 2022). The eggs are approximately 0.25 mm wide and 0.5 mm long, featuring longitudinal lines. They appear iridescent, pearly white, oval, and slightly flattened.

The eggs require an incubation temperature of 27±1 °C, with developmental rates increasing as the temperature rises. However, temperatures exceeding 35 °C result in significant mortality of pink bollworm eggs (Masry and El-Wakeil, 2020; Peddu et al., 2020; Kaur and Kumar, 2024).

Pink bollworm eggs typically take 3-4 days to hatch. Upon hatching, larvae begin to emerge. The newly emerged larvae are pale in color and approximately 1-2 mm in size, while mature larvae grow to a length of 12-15 mm. As they develop, the color of the larvae changes from white in the first instar to pink, which becomes noticeable during the third or fourth instar (Sahu et al., 2023; Salem et al., 2024).

The next stage in the life cycle of pink bollworm is the pupal stage, which occurs after the larval stage. Newly formed pupae are light brown, gradually darkening to dark brown as pupation progresses. The pupae measure around 7 mm in length (Rajendran et al., 2018; Rosentrater, 2022). During this stage, the pupae remain motionless for 7-8 days, typically within the top layer of soil beneath cotton plants. Mature pupae are reddish-brown in color and range from 8-10 mm in length (Walker, 2013; Islam and Khan, 2021; Kaur and Kumar, 2024).

The duration of the life cycle of pink bollworm, from egg to adult, is greatly influenced by temperature. Under hot summer conditions, the complete cycle can take approximately one month. Adult moths are small, greyish in appearance, with black bands on their forewings and silvery-grey hindwings (Nawaz et al., 2019; Balikai et al., 2020; Sindhu et al., 2023; Rakhesh et al., 2024).

To survive unfavorable conditions, pink bollworm larvae undergo diapause (Khakwani et al., 2022). Adult moths are about 5 mm long, with males exhibiting a reddish-brown head and pale scales. The male-to-female emergence ratio from pupae is approximately 1:1. Females are ready to mate and lay eggs 2-3 days after emerging as adults. The lifespan of both male and female adults is about one month. Female moths release pheromones to attract males, aiding in successful mating (Umer et al., 2019; Mushtaq et al., 2021).

Among various environmental factors, temperature has been identified as a significant parameter influencing the survival, reproduction, development, mortality, and infection dynamics of the pink bollworm (Hussain et al., 2023; Akhtar et al., 2024). Temperature profoundly affects the pre-oviposition period of the pink bollworm, with lower temperatures significantly delaying gonadal maturation compared to higher temperatures. At elevated temperatures, gonadal development accelerates, leading to a shorter pre-oviposition period (Nagrare et al., 2023; Nagaraju et al., 2024).

High temperatures, specifically in the range of 32.2-36.2°C, have been shown to cause elevated mortality rates among pink bollworm larvae in laboratory conditions. Mortality further increases as temperatures rise beyond this range. Furthermore, high temperatures impose stress on the behavior of adult pink bollworms, reducing their fertility (Umer et al., 2019; Mushtaq et al., 2021; Nagaraju et al., 2024). Conversely, at lower temperatures, larval mortality decreases; however, there is a marked increase in the proportion of diapausing larvae, with diapause intensity increasing as temperatures decline further (Balikai et al., 2020; Sindhu et al., 2023; Rakhesh et al., 2024).

Female longevity is particularly sensitive to temperature variations, while humidity exerts a relatively minor effect. However, extremely high or low humidity levels can still impose stress on female longevity. Temperatures exceeding 35 °C abruptly reduce the lifespan of females (Rajendran et al., 2018; Rosentrater, 2022; Sahu et al., 2023; Salem et al., 2024).

Despite these findings, there remains a paucity of information regarding the thermal effects on the life history traits of *P. gossypiella*, particularly its transgenerational impacts. Consequently, it is imperative to investigate how temperature influences the life parameters of *P. gossypiella* in the context of ongoing climate change. In this study, we systematically examined the developmental and reproductive traits of *P. gossypiella* across six different temperatures to assess its thermal fitness.

MATERIAL AND METHODS

Collection of plant material

After the cotton plants were infested with pink bollworm (PBW), the bolls were collected and brought to the laboratory. The collected bolls were then placed in large plastic cages. Daily observations were conducted to monitor the emergence of pink bollworms.

Rearing

Rearing of pink bollworms was carried out in the laboratory under controlled conditions that closely mimicked their natural environmental requirements in the field. Large plastic containers were used for rearing, following the protocol described by Borkar et al. (2022). The laboratory was maintained with a photoperiod of 16:8 h (light:dark cycle), 65% relative humidity, and conditions conducive to studying the carryover effect of temperature on the F1 generation.

Collection of insects, eggs, larvae, and pupae

Emerging adults were captured from the rearing boxes. Male and female moths were separated and released into glass chimneys in a 1:1 ratio (6 males and 6 females). These adults were provided with a 5-8% honey solution as a diet. The chimneys were covered with gauze or tissue paper to allow egg laying. Females laid their eggs on the gauze/tissue paper, which was then collected for further processing.

The eggs were incubated for 2-4 days until hatching. After hatching, larvae were collected and transferred to small plastic cups containing an artificial diet. Under standard laboratory conditions, larvae required 10-12 days to pupate. The pupae then developed into adults within 5-8 days.

Experiment layout

The study investigated the effects of temperature on the biology of pink bollworm. Various biological parameters of the pink bollworm were examined under controlled, constant temperature conditions. Five constant temperature levels (20°C, 25°C, 30°C, 35°C, and 40°C) were selected to evaluate thermal stress on the species.

Reared adults were exposed to each temperature level, and adult longevity was recorded under these conditions. Following this, female fecundity was assessed across the same temperature ranges. The incubation period and hatching percentage of eggs were determined using an incubator set to each temperature level separately. The experiment was conducted in a completely randomized design with three replicates per treatment.

Statistical analysis

A life table was developed by analyzing the data recorded for individual pink bollworms at different temperatures. The observed effects of thermal stress on F1 generation parameters, such as fertility, adult longevity, pre-oviposition and oviposition periods, pupal weight, and the developmental duration of eggs, larvae, and pupae, were analyzed using a one-way analysis of variance (ANOVA) performed with Statistix® 8.1 software.

RESULTS

The developmental stages of *P. gossypiella*, from egg to pupa, were significantly influenced by varying thermal conditions (Table 1). Egg developmental time was significantly longer at 20°C and shorter at 40°C, with no notable differences observed between 20°C and 25°C or between 30°C and 35°C ($F = 17.65$, $p < 0.001$).

Larval developmental time decreased consistently with increasing temperatures across all instars, with F-values ranging from 18.64 to 21.54 ($p < 0.001$). Both larval and pupal developmental times were significantly prolonged at 20°C and shortest at 40°C ($F = 96.54$, $p < 0.001$). The larval survival rate was highest at 30°C but declined at both 20°C and 40°C.

Pupal weight also varied significantly with temperature ($F = 39.51$, $p < 0.001$). The highest average weights were recorded at 35°C (27.57 ± 2.15 mg) and 30°C (25.61 ± 1.95 mg), while the lowest weight was observed at 40 °C (19.65 ± 1.84 mg) (Table 1).

The percentage of adult emergence in *P. gossypiella* was significantly influenced by temperature ($F = 88.46$, $p < 0.001$). The highest emergence rates were observed at 30°C, 35°C, and 25°C, with values of 77.99 ± 3.57%, 75.79 ± 3.55%, and 71.79 ± 3.34%, respectively. In contrast, a significantly lower emergence rate of 52.62 ± 2.59% was recorded at 20°C.

Egg hatching rates also varied with temperature, peaking at 30 °C (73.69 ± 2.41%) followed by 25°C (68.85 ± 2.59%) and 35°C (63.99 ± 2.44%). The lowest hatching rate, 48.43 ± 2.38%, was observed at 40°C. The differences in egg hatching rates across the temperature regimes were statistically significant ($F = 19.64$, $p < 0.001$).

Adult longevity for both males and females was significantly affected by temperature ($F = 12.54$ for males and $F = 11.64$ for females, $p < 0.001$). Male longevity ranged from 6.82 ± 1.39 days to 14.59 ± 1.79

days, while female longevity ranged from 9.54 ± 1.52 days to 14.76 ± 2.25 days. Longevity decreased with increasing temperature, though females consistently lived longer than males across all temperature treatments.

Mating success rates of *P. gossypiella* were significantly influenced by temperature (F = 32.65, $p < 0.001$). The highest success rates were recorded at 35°C (85.77 ± 3.79%) and 30°C (81.56 ± 3.56%), while the lowest rate, 65.81 ± 2.89%, was observed at 20°C.

Fecundity also varied significantly with temperature (F = 56.72, $p < 0.001$). The highest fecundity was observed at 35°C (78.44 ± 3.53 eggs/female) and 30°C (69.92 ± 3.41 eggs/female), while the lowest fecundity, 54.37 ± 2.59 eggs/female, was recorded at 20°C. However, no significant differences were observed in sex ratios across the temperature treatments (p = 0.604) (Table 2).

DISCUSSION

Pink bollworms, notorious chewing pests, pose a significant threat to cotton crops by severely damaging cotton fibers and reducing their market value. Based on comprehensive surveys conducted in the cotton-growing districts of Punjab, this report underscores the infestation status of the pink bollworm (PBW). Alarmingly, PBW was detected at every sampling site across the cotton-growing regions of Punjab Province. The widespread distribution and rapid population increase of PBW have raised serious concerns for the cotton industry in Punjab.

P. gossypiella appears to have evolved from its previous status to become a pandemic pest, adapting its behavior to a broader range of environmental conditions in the cotton-growing regions of Punjab (Kaur and Kumar, 2024). The monsoon season, in particular, creates favorable conditions for the buildup of PBW populations (Garg et al., 2022; Khakwani et al., 2022).

Interestingly, as temperatures decrease, the emergence rate of PBW appears to increase. This hypothesis is supported by temperature-based experiments (Tables 1 and 2). The findings suggest that PBW is spreading more rapidly across the diverse climatic conditions of cotton-growing regions of Pakistan. This accelerated spread is attributed to its unique life cycle, as well as its evolving resistance to various pesticides and biological control agents (Bardin et al., 2015; Wang et al., 2019; Javed et al., 2020; Ahmed et al., 2022; Kumar and Grewal, 2023).

P. gossypiella is notoriously difficult to control. Given the current situation in Punjab, it is not surprising that PBW may lead to pandemic-like infestations in Pakistan's Bt cotton fields if timely actions are not taken (Lykouressis et al., 2005; Hutchison et al., 2015; Sarwar, 2017; Kumar and Grewal, 2023). The lack of prompt intervention and the poor performance of the agricultural department have resulted in an absence of substantial research reports or policy guidelines addressing the recent surge in PBW attacks on Bt cotton in Pakistan (Kumar and Grewal, 2023). As a result, it is predicted that *P. gossypiella* will continue to cause widespread infestations in cotton-growing areas across the country.

The resurgence of *P. gossypiella* in Bt cotton fields may be attributed to the failure to adopt the refuge strategy, which was recommended as part of integrated pest management (IPM) to mitigate the pest's resistance to Bt cotton (Sabry et al., 2014; Agrawal et al., 2020; Rao, 2022; Hussain et al., 2023). In an IPM program, it is crucial to plant at least 20% non-Bt cotton alongside Bt cotton to prevent the development of pest resistance. This strategy helps maintain genetic diversity within the pest population, reducing the likelihood of pests evolving resistance to Bt toxins (Naranjo, 2011; Hutchison et al., 2015).

Climate change has altered temperature variability and extremes, which may impact the natural development and successful colonization of the pest (Moreno and Møller, 2011). Therefore, understanding the life history traits of *P. gossypiella* is essential for developing management strategies resilient to climate change. In this study, *P. gossypiella* exhibits traits that enable it to thrive as a successful colonizer across a range of temperatures. The mean duration of egg, larval, and pupal development was significantly longer at 20°C and shorter at 40°C. However, findings from other studies were somewhat inconsistent with our results, indicating that egg development lasted approximately 10 days at 20°C and 4 days at 35°C (Ali et al., 2019b; Peddu et al., 2020; Ihsan et al., 2021). Consequently, *P. gossypiella* can complete more generations within a single season or year under elevated thermal conditions. This is primarily linked to metabolic activity, as numerous studies have shown that rising temperatures accelerate metabolic processes at every developmental stage, facilitating the generation of additional cohorts. Thus, temperature is a critical factor influencing the lifespan of arthropods (Mohan et al., 2014).

Table 1: Mean duration (± SE) of each developmental stage of *P. gossypiella* at varying temperatures.

Temperature	Developmental time (days)						Larval Survival (%)	Avg. Pupal Weight (mg)
	Eggs	I instar	II instar	III instar	IV instar	Pupa		
20°C	4.99 ± 0.46a	5.79 ± 0.52a	5.98 ± 0.58a	6.92 ± 0.64a	8.01 ± 0.91a	12.09 ± 1.43a	67.98 ± 2.88d	19.65 ± 1.84d
25°C	4.89 ± 0.42a	5.17 ± 0.46 a	5.81 ± 0.51a	6.89 ± 0.56a	7.55 ± 0.88b	11.24 ± 1.29b	74.39 ± 3.25b	22.54 ± 1.89c
30°C	3.94 ± 0.44b	4.65 ± 0.33b	4.78 ± 0.44b	5.73 ± 0.51b	6.15 ± 0.62c	9.76 ± 1.18c	78.93 ± 3.84a	25.61 ± 1.95b
35°C	3.76 ± 0.36b	3.63 ± 0.31c	3.61 ± 0.42c	4.76 ± 0.45c	5.07 ± 0.49d	8.21 ± 1.11d	71.76 ± 3.25c	27.57 ± 2.15a
40°C	3.42 ± 0.28c	3.18 ± 0.24c	3.18 ± 0.38c	4.51 ± 0.41d	4.31 ± 0.42e	7.12 ± 1.08e	63.89 ± 2.73e	20.54 ± 1.88d
F value	17.65	19.31	18.87	21.54	18.64	19.63	96.54	39.51
p value	*P < 0.0001*	*P < 0.0001*	*P < 0.0001*	*P < 0.0001*	*P < 0.0001*	*P < 0.0001*	*P < 0.0001*	*P < 0.0001*

Means (± SE) within the same row that are followed by different letters indicate a statistically significant difference at the 0.01 level, as determined by one-way ANOVA and Tukey's HSD multiple comparisons.

Table 2: Effect of thermal stress on the life history traits of *P. gossypiella*.

Temperature	Adult Emergence (%)	Egg Hatching (%)	Adult Longevity (days)		Mating Success (%)	Fecundity/female	Sex ratio (F:M)
			Male	Female			
20°C	52.62 ± 2.59e	59.64 ± 2.78d	9.89 ± 1.54^{C}	12.56 ± 1.87^{B}	65.81 ± 2.89e	54.37 ± 2.59^{E}	1.11 ± 0.03a
25°C	71.79 ± 3.34c	68.85 ± 2.59b	11.63 ± 1.66^{B}	14.76 ± 2.25^{A}	76.59 ± 3.21d	61.45 ± 3.28^{D}	1.13 ± 0.02a
30°C	77.99 ± 3.57a	73.69 ± 2.41a	14.59 ± 1.79^{A}	11.56 ± 1.54^{B}	81.56 ± 3.56b	69.92 ± 3.41^{C}	1.14 ± 0.04a
35°C	75.79 ± 3.55b	63.99 ± 2.44c	10.51 ± 1.62^{BC}	9.65 ± 1.49^{C}	85.77 ± 3.79a	78.44 ± 3.53^{A}	1.12 ± 0.02a
40°C	64.92 ± 2.71d	48.43 ± 2.38e	6.82± ± 1.39^{D}	9.54 ± 1.52^{C}	78.64 ± 3.71c	64.54 ± 2.86^{B}	1.10 ± 0.01a
F value	88.46	19.64	12.54	11.64	32.65	56.72	7.05
p value	*P < 0.0001*	*P < 0.0001*	*P < 0.0001*	*P < 0.0001*	*P < 0.0001*	*P < 0.0001*	*P < 0.6041*

Values (± SE) within the same row that are followed by different letters indicate a statistically significant difference at the 0.01 level, as determined by one-way ANOVA and Tukey's HSD multiple comparisons.

CONCLUSION

In conclusion, *Pectinophora gossypiella* is identified as a major pest of cotton, demonstrating a remarkable ability to thrive under various environmental conditions. Our study highlights the critical role of temperature in shaping the developmental and biological traits of *P. gossypiella* across different thermal regimes. The optimal temperature range for egg and larval development, as well as overall reproductive success, was found to be between 30 and 35°C. Deviations from this range led to reduced egg hatchability and increased larval mortality. Moreover, the study revealed the long-term effects of thermal stress on the F1 generation, with some recovery observed as they progressed to later developmental stages. These findings are crucial for predicting population dynamics in field conditions and for formulating effective management strategies to ensure sustainable cotton production amidst climate-related challenges.

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AUTHORS' CONTRIBUTIONS

MI and SA designed the study, formulated the experiments, and executed them; MI, AJ and MBC collected and organized the data, analyzed the results, and wrote the manuscript; TH, MK and ZM assisted in writing the manuscript and proofreading the paper.

CONFLICTS OF INTEREST

The authors declare no conflict of interest.

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