



# Influence of Constant Temperatures on Growth and Development of *Pectinophora gossypiella* (Saunders) in Bt cotton

P. Harshavardhan, Amandeep Kaur\*, Vijay Kumar and Ravinder Singh

Department of Entomology, Punjab Agricultural University, Ludhiana-141 004, India

\*E-mail: [amankaur17@pau.edu](mailto:amankaur17@pau.edu)

**Abstract:** The study on 'Influence of temperature on growth and development of *P. gossypiella* in Bt cotton' was studied at various constant temperatures of 15, 20, 25, 30, 35 and 40°C in Integrated Pest Management Laboratory, Department of Entomology, PAU, Ludhiana during *khari* 2022-23. The results revealed that duration of all the larval instars were significantly lower at constant temperature of 35°C. The pupal duration, adult longevity and total developmental period of male and female were recorded significantly lower at 35°C. The fecundity and egg, larval and pupal survival was recorded highest at 30°C whereas at 40°C, no egg hatching and all the larvae experienced mortality. The larval and pupal weights were recorded highest at constant temperature of 35°C. The correlation was worked out where the developmental parameters have shown significant negative correlation with constant temperatures. All the larval instars and male pupal weights show significant positive correlation with constant temperatures and female pupal weight, egg, larval and pupal survival show non-significant positive correlation with constant temperatures. Constant temperature dependant set with Stinner nonlinear and inverse second order polynomial model for the development of *P. gossypiella*.

**Keywords:** Adult longevity, Constant temperatures, Fecundity, *Pectinophora gossypiella*, Total developmental period, Survival

Cotton (*Gossypium* spp), often referred to as the 'King of Fibers,' is a highly valuable fiber crop cultivated in both tropical and warm temperate regions worldwide. China is the leading producer of cotton globally, with India as the second-largest producer, yielding an estimated 343.74 lakh bales during 2022-23 (Anonymous 2023). In Punjab, cotton ranks as the second most significant *khari* crop after rice, covering approximately 3.25 lakh hectares in the 2021-2022 season which was an increase of about 75 thousand hectares from the previous cropping season, yielding 21.86 lakh quintals of cotton (Anonymous 2022). The introduction of Bt cotton hybrids to India in 2002 resulted in a significant reduction in bollworm infestations (Manjunath 2004). After a decade, since the introduction of Bt cotton, the pink bollworm (PBW), *Pectinophora gossypiella* (Saunders) (Lepidoptera: Gelechiidae), has emerged as a significant threat in the cotton-growing regions of Central and Southern India. There have been reports of the insect feeding and surviving on both single-gene Bt cotton (Bollgard I) and dual-gene Bt cotton (Bollgard II) in Gujarat (Dhuria et al 2011). This pest has become a major concern for cotton production in the Southern and Central regions of India, where it has developed resistance to Cry1Ac and Cry2Ab expressing cotton. Additionally, it has shown resistance to insecticides and has started infesting late-season cotton (Naik et al 2018). The survival of this insect is notably influenced by temperature and relative humidity under climatic conditions. Therefore, understanding how pink bollworm responds to variations in temperature and relative humidity is crucial. This

knowledge is essential for modifying pest management strategies in response to climate change (Shrestha 2019). The status of this pest species can be significantly affected by changes in temperature. Therefore, studying the impact of temperature on insect development is vital for estimating seasonal and phenological development, understanding pest population dynamics, conducting risk analysis, forecasting pest outbreaks, and developing effective management strategies to minimize pest populations in the field.

## MATERIAL AND METHODS

**Raising of test plant:** The pure seed of RCH 773 BG-II cotton cultivar was procured from the local market. Two to three seeds of this cultivar were sown at 10 days interval in earthen pots filled with soil and farmyard manure to get a continuous supply of required stages of bolls for conduct of various experiments.

### Initiation and maintenance of culture of *P. gossypiella*:

The larvae of the pink bollworm were collected from infested cotton fields in the cotton belt (Bathinda, Mansa, Abohar and Mutksar districts) of Punjab. The larvae were fed on flower/cotton bolls of non-Bt cotton cultivar, F 2228 in the laboratory at room temperature. To ensure their survival, food was provided on a daily basis. Male and female pupae were kept in plastic jar with 10×15 cm diameter. To facilitate the females to lay eggs in the oviposition cage, 10-15 days old boll was provided as substrate for the egg laying. The cage was covered with black muslin cloth at the top and side.

**Experimental procedure:** The mentioned set of minimum and maximum temperature treatments where T1- 15±1°C, T2- 20±1°C, T3- 25±1°C, T4- 30±1°C T5- 35±1°C and T6- 40±1°C along with five replications each was maintained for 14:10 hour in an incubator. The relative humidity was maintained 65±5 per cent throughout the experiment. Twenty neonate larvae were used in each treatment and three such replications were maintained in different plastic jars (10 cm diameter and 18 cm height) by using camel hair brush and the larvae was fed on the locules of Bt cotton cultivar, RCH 773. For recording the duration of each instar, cut the locules and examine it for casted skin. To record the fecundity, detached cotton bolls of 10-15 days old was wrapped with moist cotton wick and placed in eppendorf tubes filled with 10 per cent sucrose solution to keep the cotton bolls fresh and this was placed in the oviposition cage of 14.5 x 20.5cm diameter (Fand et al 2019). For mating purposes, five pairs of newly emerged adults were introduced into an oviposition cage (14.5x20.5 cm diameter). The eggs which were laid by the adult female moth were collected separately. The cotton shoots were replaced after every two days. The number of eggs deposited on the old twig was counted.

Data for temperature dependent development were analyzed by three different models depending on ordinary regression method. Campbell et al (1974) as linear model  $r(T) = a + b.T$  where  $r(T)$  is mean development rate (1/day) at temperature  $T$  (°C),  $a$  is slope,  $b$  is intercept and  $T$  is the temperature. Rate of development (1/day) was analyzed by linear and nonlinear model given by Stinner et al (1974) as nonlinear model  $(C/1+e^{(a+b.T)})$  where  $r(T)$  is mean development rate at temperature  $T$  (°C),  $C$  is the constant,  $e$  is the exponential to the equation and third model was Inverse second order polynomial model  $[1/D = a/(1 + bT + cT^2)]$  given by Damos and Savopoulou (2021) where  $D$  is the days of development and  $c$  is constant.

**Observations to be recorded:** The observations on various biological parameters including fecundity, incubation period,

larval period (1<sup>st</sup>, 2<sup>nd</sup>, 3<sup>rd</sup> and 4<sup>th</sup> instar), pupal period, larval weight, pupal weight, adult longevity, total developmental period, per cent survival of egg, larva and pupa. From the models, Regression coefficient ( $R^2$ ) and Residual Sum of Square (RSS) were worked out.

## RESULTS AND DISCUSSION

The study unveiled significant lowest incubation period in Treatment-5 (35±1°C) at 2.27±0.10 and highest in Treatment-1 (15±1°C) with a duration of 5.24±0.17 days (df=4, f=51.21,  $p < 0.001$ ) (Table 1). The significant lowest duration of first larval instar was recorded in T-5 (35±1°C) with 2.36±0.05 days and highest in T-1 (15±1°C) (df=4, f=32.33,  $p < 0.001$ ). The significant lowest and highest durations of second larval instar was recorded in T-5 (35±1°C) and T-1 (15±1°C) with 3.12±0.04 and 7.30±0.09 days, respectively. Durations of fourth larval instar was lowest in T-5 (35±1°C) lasting for 6.85±0.09 days, whereas the highest was recorded in T-1 (15±1°C) with 13.63±0.06 days. Similarly, the total larval duration was significantly lowest in T-5 (35±1°C), lasting for 16.87±0.28 days and highest was recorded in T-1 (15±1°C) with a duration of 36.52±0.15 days (Table 1).

As insects are ectothermic, their growth, development, and reproduction are primarily influenced by temperature as it plays crucial roles in their metabolism, metamorphosis, mobility, host availability etc. Higher temperatures lead to accelerated metabolic activities in insects, which, in turn, results in a shortened duration of their developmental stages (Shreshta 2019). The findings on larval duration are consistent with the results of Likitha et al (2022) and Shrinivas et al (2019), who observed a total larval period of 25.10±0.99 and 26.10±0.66 days, respectively when the pink bollworm was reared on artificial medium at a temperature of 25±2°C. Additionally, Peddu et al (2019) found that the development of larva increased from 20°C to 35°C and rapidly declined at the temperatures above 35°C. However, the findings are in contrast with earlier results of Rajput et al

**Table 1.** Effect of constant temperatures on incubation, larval period and total larval duration of *P. gossypiella* in Bt cotton

Sr.No.	Temperature (°C)	Incubation period (days)	Larval period (days)				
			1 <sup>st</sup> Instar	2 <sup>nd</sup> Instar	3 <sup>rd</sup> Instar	4 <sup>th</sup> Instar	Total larval duration
T1	15±1	5.24±0.17 <sup>a</sup>	5.64±0.02 <sup>a</sup>	7.30±0.09 <sup>a</sup>	9.43±0.05 <sup>a</sup>	13.63±0.06 <sup>a</sup>	36.52±0.15 <sup>a</sup>
T2	20±1	4.35±0.20 <sup>b</sup>	4.56±0.02 <sup>b</sup>	5.00±0.20 <sup>b</sup>	7.55±0.02 <sup>b</sup>	11.95±0.09 <sup>b</sup>	29.10±0.13 <sup>b</sup>
T3	25±1	3.68±0.16 <sup>c</sup>	3.74±0.04 <sup>c</sup>	4.66±0.02 <sup>b</sup>	5.71±0.03 <sup>c</sup>	10.01±0.08 <sup>c</sup>	24.24±0.10 <sup>c</sup>
T4	30±1	3.19±0.12 <sup>c</sup>	3.12±0.03 <sup>d</sup>	4.04±0.06 <sup>c</sup>	5.21±0.09 <sup>d</sup>	8.79±0.08 <sup>d</sup>	21.89±0.45 <sup>d</sup>
T5	35±1	2.27±0.10 <sup>d</sup>	2.36±0.05 <sup>e</sup>	3.12±0.04 <sup>d</sup>	4.16±0.05 <sup>e</sup>	6.85±0.09 <sup>e</sup>	16.87±0.28 <sup>e</sup>
T6	40±1	--	--	--	--	--	--
CD (p=0.05)		0.46	0.11	0.30	0.17	0.25	0.77

Mean of five replications; Data followed by superscript indicate significant differences as per Tukey's HSD test; Relative humidity = 65±5 per cent; (--) shows 100% mortality

(2018), who reported a maximum fourth instar larval duration of 11.36±0.30 days at 27±2°C.

The male and female pupal duration was recorded lowest in Treatment 5 (35±1°C) and highest in Treatment 1 (15±1°C). The adult longevity of male and female was recorded minimum in Treatment 5 (35±1°C) with 6.44±0.13 and 7.46±0.12 days, respectively and maximum in Treatment 1 (15±1°C) with 9.66±0.23 and 10.40±0.06 days, respectively (Table 2). These align with the observations made by Cacayorin et al (1992) who found that the average duration of pupal stage was 7.42 days. The total developmental period of male and female follows the same trend with the lowest in T-5 (35±1°C), lasting for 25.25±0.50 and 26.24±0.36 days, respectively and highest in Treatment 1 (15±1°C) with duration of 50.65±0.26 and 52.56±0.22 days, respectively (Fig. 1). The findings are supported by Zinzuvadiya et al (2017) who reported that the total developmental period of male was 38.40±4.48 days at 28.34±3.15°C and 49.71±0.50 days at 21°C. Sapna et al (2017) found that the complete life cycle of female from egg to adult emergence was 52.3±6.51 days at 25±1°C. Shrinivas et al (2019) from Raichur,

Karnataka, reported that females survived longer than males. An increase in body size and a decrease in development time with respect to increase in temperature are commonly observed outcomes of natural selection acting on individual organisms (Kingsolver et al 2004 and Harrison et al 2013).

Fecundity was significantly lowest in Treatment 1 (15±1°C) with 23.60±0.61 eggs per female and significantly higher in Treatment 4 (30±1°C) with 61.30±0.89 eggs per female (df=4, f=22.59, p<0.001) (Fig. 2) and sex ratio was

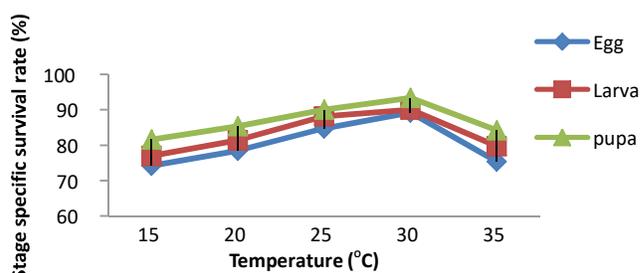


Fig. 1. Effect of constant temperatures on Survival of egg, larval and pupal stages of *P. gossypiella* in Bt cotton

Table 2. Effect of constant temperatures on pupal duration, adult duration and sex ratio of *P. gossypiella* in Bt cotton

Sr. No.	Temperature (°C)	Pupa duration (days) (Mean±SE)		Adult longevity (days) (Mean±SE)		Sex ratio (male:female)
		Male	Female	Male	Female	
T1	15±1	8.88±0.16 <sup>a</sup>	10.80±0.11 <sup>a</sup>	9.66±0.23 <sup>a</sup>	10.40±0.06 <sup>a</sup>	1.50:1
T2	20±1	7.50±0.07 <sup>b</sup>	9.56±0.02 <sup>b</sup>	8.47±0.09 <sup>b</sup>	9.99±0.13 <sup>ab</sup>	1.32:1
T3	25±1	7.28±0.09 <sup>b</sup>	8.72±0.04 <sup>c</sup>	7.51±0.11 <sup>c</sup>	9.68±0.12 <sup>b</sup>	1.17:1
T4	30±1	6.80±0.21 <sup>bc</sup>	8.38±0.26 <sup>c</sup>	7.15±0.06 <sup>c</sup>	9.04±0.06 <sup>c</sup>	1:1.08
T5	35±1	6.10±0.26 <sup>c</sup>	7.10±0.13 <sup>d</sup>	6.44±0.13 <sup>d</sup>	7.46±0.12 <sup>d</sup>	1:1.38
T6	40±1	--	--	--	--	--
CD (p=0.05)		0.52	0.42	0.41	1.31	

Mean of five replications; Data followed by superscript indicate significant differences as per Tukey's HSD test; Relative humidity = 65±5 per cent; (--) shows 100% mortality

Table 3. Effect of constant temperatures on larval weight of first, second, third and fourth instars of *P. gossypiella* in Bt cotton during 2022

Sr. No.	Temperature (°C)	Larval weight (mg) (Mean±SE)			
		1 <sup>st</sup> Instar	2 <sup>nd</sup> Instar	3 <sup>rd</sup> Instar	4 <sup>th</sup> Instar
T1	15±1	2.81±0.02 <sup>b</sup>	8.72±0.03 <sup>d</sup>	20.10±0.26 <sup>d</sup>	18.95±0.08 <sup>b</sup>
T2	20±1	2.94±0.04 <sup>b</sup>	9.20±0.09 <sup>cd</sup>	20.89±0.11 <sup>cd</sup>	19.88±0.26 <sup>ab</sup>
T3	25±1	3.16±0.03 <sup>a</sup>	9.68±0.13 <sup>bc</sup>	21.04±0.17 <sup>bc</sup>	20.85±0.41 <sup>ab</sup>
T4	30±1	3.30±0.03 <sup>a</sup>	10.07±0.19 <sup>ab</sup>	21.67±0.22 <sup>ab</sup>	21.19±0.27 <sup>a</sup>
T5	35±1	3.36±0.08 <sup>a</sup>	10.56±0.33 <sup>a</sup>	22.79±0.23 <sup>a</sup>	21.76±0.32 <sup>a</sup>
T6	40±1	--	--	--	--
CD (p=0.05)		0.14	0.55	0.95	0.72

Mean of five replications; Data followed by superscript indicate significant differences as per Tukey's HSD test; Relative humidity = 65±5 per cent; (--) shows 100% mortality

recorded highest in Treatment 5 (35±1°C), with sex ratio of 1:1.38 (Table 2). These findings are supported by Hussain et al (2023) who reported that the fecundity of 49.82±1.46 eggs/female at 35°C. Awaknawar (1976) reported a fecundity range of 9-34 eggs per female, with an average of 20.6 eggs per female. Philipp et al (2014) found that the higher the temperature, lower fecundity rates.

The egg, larval and pupal survival was recorded highest in Treatment 4 (30±1°C) with 89.27±0.85, 90.09±0.87 and 93.39±1.12 per cent respectively and lowest in Treatment 1 (15±1°C) (Fig. 3). In the face of extreme heat, numerous insect species struggle to survive, primarily due to their lack of sufficient heat tolerance during gradual heat stress in their natural habitats (Bodlah et al 2023). The alternate temperatures led to elevated overall survival rates in *H. armigera* compared to the survival rates observed under corresponding constant temperatures, (Mironidis et al 2008). This outcome is possibly due to the fact that fluctuating temperatures offer intervals for recovery or enable adaptation to extreme hot or cold conditions (Vargas et al 2000).

The larval weights of first, second, third and fourth larval

instars was maximum in Treatment 5 (35±1°C) with 3.36±0.08, 10.56±0.33, 22.79±0.23 and 21.76±0.32 mg, respectively and minimum in Treatment 1 (15±1°C) with 2.81±0.02, 8.72±0.03, 20.10±0.26 and 18.95±0.08 mg, respectively (Table 4). The male and female pupal weight was significantly higher in Treatment 5 (35±1°C) with 10.89±0.42 and 16.90±0.14 mg, respectively. Whereas, the significant lowest was recorded in Treatment 1 (15±1°C)

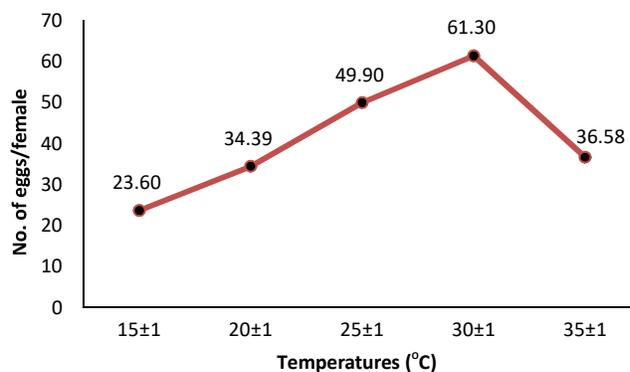


Fig. 3. Effect of constant temperatures on fecundity of *P. gossypiella* in Bt cotton during 2022

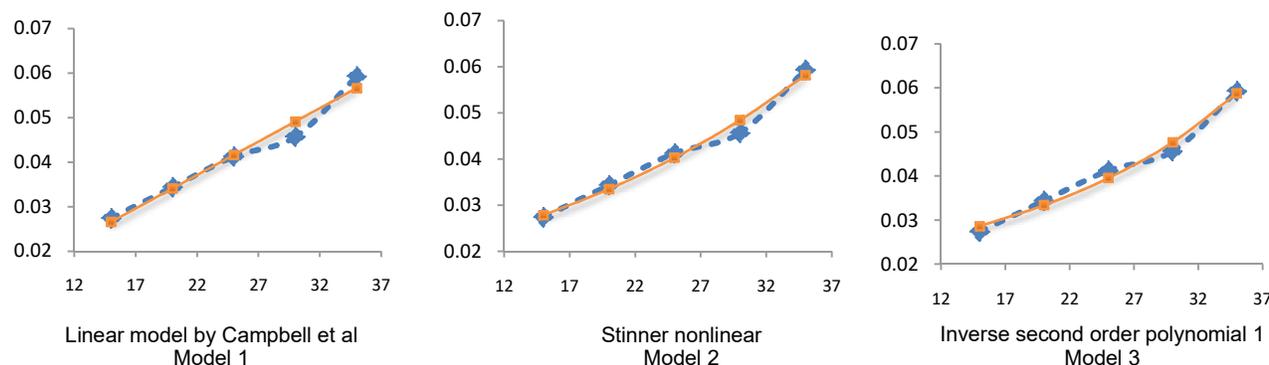


Fig. 4. Fitting different linear and nonlinear models based on development rate (1/day) for total immature larval stage of *P. gossypiella* versus temperature. Blue squares represent observed data

Table 4. Effect of constant temperatures on pupal weight of *P. gossypiella* in Bt cotton during 2022

Sr. No	Temperature (°C)	Pupal weight (mg) (Mean±SE)		Growth Index	
		Male	Female	Larva	Pupa
T1	15±1	9.16±0.18 <sup>b</sup>	15.61±0.14 <sup>bc</sup>	2.10	8.32
T2	20±1	9.34±0.39 <sup>b</sup>	15.81±0.13 <sup>bc</sup>	2.79	10.15
T3	25±1	10.11±0.23 <sup>ab</sup>	15.19±0.16 <sup>c</sup>	3.63	11.33
T4	30±1	10.29±0.18 <sup>ab</sup>	16.23±0.22 <sup>ab</sup>	4.11	12.43
T5	35±1	10.89±0.42 <sup>a</sup>	16.90±0.14 <sup>a</sup>	4.72	12.81
T6	40±1	--	--	--	--
CD (p=0.05)		0.89	0.48		

Mean of five replications; Data followed by superscript indicate significant differences as per Tukey's HSD test; Relative humidity = 65±5 per cent; (--) shows 100% mortality

**Table 5.** Regression analysis of three models describing the effect of constant temperature on development time

Model 1	Parameters	1 <sup>st</sup> Instar	2 <sup>nd</sup> Instar	3 <sup>rd</sup> Instar	4 <sup>th</sup> Instar	Total larval duration
Linear model by Campbell ( $r(T) = a + bT$ )	a	-0.015	0.016	0.005	0.015	0.004
	b	0.011	0.008	0.006	0.003	0.001
	R <sup>2</sup>	0.96	0.94	0.98	0.95	0.96
	RSS ( $\times 10^{-4}$ )	13.84	9.165	1.891	1.502	0.196
Model 2						
Stinner nonlinear model ( $C/1 + e^{(a+b.T)}$ )	a	3.205	3.285	3.424	3.896	4.786
	b	-0.051	-0.041	-0.042	-0.037	-0.037
	c	2.12	2.257	1.881	2.054	1.921
	R <sup>2</sup>	0.98	0.95	0.98	0.98	0.98
	RSS ( $\times 10^{-4}$ )	3.90	7.90	1.96	0.05	0.10
Model 3						
Stinner nonlinear model ( $1/D = a/(1 + bT + cT^2)$ )	a	8.376	14.378	11.605	17.977	18.126
	b	-0.200	-0.570	-0.550	-0.283	1.378
	c	0.001	0.007	0.006	-0.001	-0.0406
	R <sup>2</sup>	0.99	0.93	0.98	0.99	0.88
	RSS ( $\times 10^{-4}$ )	0.991	13.53	2.02	0.13	0.65

Comparison between linear and non-linear models shows close relation between development rate and temperature for 1<sup>st</sup>, 2<sup>nd</sup> instar and overall larval development

weighing  $9.16 \pm 0.18$  and  $15.61 \pm 0.14$  mg, respectively (Table 5). The larval and pupal growth index was higher in Treatment 5 ( $35 \pm 1^\circ\text{C}$ ) and lowest in Treatment 1 ( $15 \pm 1^\circ\text{C}$ ) (Table 5). The recent observations regarding the larval weight of the pink bollworm align with previous research conducted by Dharajothi et al (2016) and found larval weight of  $21.40 \pm 3.63$  mg at  $27 \pm 0.5^\circ\text{C}$ . These results are consistent with the observations made by Fand et al (2019) who reported larval weight of 21.96 mg when fed on bolls of Bt cotton.

Correlation between developmental parameters like incubation period, larval duration, pupal duration, adult longevity and total developmental period has shown significant negative correlation with constant temperatures. Different temperature based model studied by various scientist on different lepidopteran. The development rate of *Spodoptera litura* when predicted for linear and non-linear models showed that thermodynamic SSI non-linear and Lactin-2 models were most fitted and estimated  $34.5^\circ\text{C}$  and  $33.7^\circ\text{C}$ , respectively as best suited for its development (Prasad et al 2021). Similarly Damos and Savopoulou (2021) advised that for particular specie there is an inverse relation between development of specie and threshold temperature that fitted the model.

### CONCLUSION

It can be concluded that the shortest duration of *P.*

*gossypiella* was recorded at constant temperature of  $35 \pm 1^\circ\text{C}$  and increased with decrease in temperature. The larval and pupal weight of *P. gossypiella* were highest at constant temperature of  $35 \pm 1^\circ\text{C}$ . An increase in body size and a decrease in development time with respect to increase in temperature are commonly observed outcomes of natural selection acting on individual organisms. The fecundity and per cent survival of *P. gossypiella* were highest at constant temperature of  $30 \pm 1^\circ\text{C}$  and decreased with both rise and fall of temperature. All the developmental parameters showed highly significant negative correlation with constant temperature. Including forecasting model in relation to different temperature will help in timely prediction of any outbreak and sustainable management.

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