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DIAPAUSE DYNAMICS IN PINK BOLLWORM (*PECTINOPHORA GOSSYPIELLA*) TRIGGERED BY EPISODES OF PHOTOPERIOD AND TEMPERATURE REGIMES

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ABSTRACT

Pink bollworm, *Pectinophora gossypiella* is considered as the most injurious insect-pest of cotton causing significant yield losses in the world including Pakistan. The ability of pink bollworms to enter in diapauses before the onset of harsh condition increases the survival rate to greater extent and determine to attain the status of major insect-pest. The present investigations were conducted under laboratory conditions set as 26±2 °C, 65±5% and 15:9 (L/D) temperature, relative humidity, photoperiod respectively. Change in temperature to varied extent and episodes of photoperiod for specific intervals were the key variables to study their influence on diapausing ability in pink bollworms. The results elaborated that the temperature of 14 °C found to be optimum for diapause induction as maximum (68.21±1.83%) larvae of pink bollworms at 4th instar were in diapause state. While the temperature of 26 °C found to be lethal regarding diapause induction of 0.00, 3.11±1.03 and 21.96±0.13% when 4th instar larvae were held for one, four and seven days respectively. Influence of photoperiod revealed that the highest diapause (28.01±0.20%) was induced when episode of 07:17 (L/D) photo phase was maintained for a period of seven days. Peak adult emergence (92.13±3.74%) was recorded at 26 °C, 15:9 L/D of temperature and photoperiod respectively. However, maximum adult mortality (31.66±4.59%) was found at 14 °C when the pupae were held for 14 days. These findings lead to the conclusion that the temperature and photoperiod are the key determinants among the abiotic variable, which govern diapausing phenomena in pink bollworm.

Keywords: Cotton, Pink Bollworm, Diapause, Induction, Adult Emergence, Termination, Photoperiod, Relationship

INTRODUCTION

Cotton (*Gossypium hirsutum* L.) also known as white gold, is one of Pakistan's most valuable cash crop ranking the country's 4th largest cotton producing 33 million bales (Ali *et al.*, 2019; Khan, 2019; Khan *et al.*, 2020; Siyal *et al.*, 2021). It contributes around 0.6 percent to GDP and 3.1 percent of the value added in agriculture. This crop was grown on 2.8 million hectares in the country with an annual yield of 12.3 million bales (Akhtar *et al.*, 2016). Cotton is vulnerable to a variety of variables that contribute to its low yield, the most prominent of which are biotic factors, such as insect pests, diseases and weeds. Insects play a critical role in yield reduction, as the crop is highly susceptible to insect-pests causing 87% losses at the final harvest (Dhawan, 2016; Razaq *et al.*, 2019; Naeem-Ullah *et al.*, 2020). These insect-pests deteriorate the quality and quantity by cutting, staining and blackening of the fiber and poor seed quality (Ghosh, 2001; Karar *et al.*, 2020).

Among cotton insect-pests, Pink bollworm (*Pectinophora gossypiella*) is considered as the most injurious pest of cotton crop causing yield loses up to 20-90% annually (Patil, 2003; Fand *et al.*, 2020). Pink bollworms remain active throughout the year at varied developmental rates governed by a number of a-biotic factors (Gutierrez *et al.*, 2015). During June when the temperature is 40°C the activity found to be low (Henneberry, 2007; Milonas *et al.*, 2016). In the month of September, attack is more severe (Arshad *et al.*, 2015). The adults feed on nectar while the larvae are concealed feeders that feed inside the flowers, squares and cotton bolls (Ingole *et al.*, 2019). The larvae not only attack cotton bolls but the flowers and bolls also. The larvae of pink bollworm damage the flowers that resulted in developing a visual symptom called rosette formation (Peloquin *et al.*, 2001). Inside the flowers, larvae spin webbing, which causes inappropriate flower opening, resulting in "rosetted-bloom" or "rosetted-flower" (Tabashnik *et al.,* 2003). After harvesting, the larvae survive in the cotton sticks and unopened bolls of cotton stalks in farmers' fields (Mallah *et al.,* 2000).

One of the important phenomenon regarding biology of pink bollworm is the occurrence of diapause that determines the survival potentials (Gutierrez *et al.,* 2006; Sarwar, 2017). It is one of the basic means, by which the insects deal adverse environmental conditions to enhance the survival chances. Apparently, diapause includes diapause induction, diapause maintenance, diapause termination, post-diapause quiescence, and post-diapause development. The electrophoretic analysis of the diapausing stages of pink bollworms showed an accumulation of Pectinophora Diapausing Proteins (PDP) projected to be 490000 subunits. It was found that the concentration of PDP increases at the initiation of diapause, continue to increase with the progress of diapausing stage and decreases as the larvae undergo the process of termination from 17.4% to 38.6% irrespective of the total proteins (Salama and Miller, 1992). The external stimuli or the clues that triggers the initiation of diapause included photoperiod, temperature, relative humidity and nutritional imbalance along with a number of unexplored variables (Bale and Hayward, 2010). Genetic variation in the host plant has identified is one the most important factors influencing diapause along with other physiological and biochemical changes as reported the resistance in pink bollworms association with cadherin genes, controlled by recessive alleles and their frequency (Wang *et al.,* 2022).

A number of scientists have reported insecticide resistance and open field resistance in pink bollworms from various regions of the world (Tabashnik *et al.,* 2005). In Indian strains of pink bollworms, open field resistance has also been reported (Naik *et al.,* 2020). With the advent of increasing resistance to insecticides, resistance to *Bt* cotton a huge yield losses were recorded due to the infestation of pink bollworm and cotton industry suffered a great downfall (Karar *et al.,* 2020). Under such challenging circumstances, there is a dire need to reorient the studies for understanding the biological processes underlying such mechanism in pink bollworms and resurgence as major pest in the recent decade. Keeping in view all these challenges, the present investigations on diapause was designed to explore more concluding findings on this phenomenon ultimately to devise concrete IPM for pink bollworm.

MATERIALS AND METHODS

Population Homogenization: The research trial was performed in Integrated Pest Management (IPM) laboratory (Pink Bollworm Rearing Unit), Department of Entomology, University of Agriculture Faisalabad. Pink bollworm, *Pectinophora gossypiella* infested materials (flowers, squares, bolls) were collected from farmer's fields (31º17′410″ N and 72º52′570″ E), University Research area Young Wala (31º26′110″ N and 73°03′450″ E) and Ayub Agricultural Research Institute Faisalabad (31°24′17″ N and 73°03′02″).

The infested samples were packed in the plastic bags after proper labelling and brought to the laboratory where the larvae of pink bollworms were separated. The larvae were reared on artificial diet (Wu *et al.,* 2006)) till pupation. The pupae of uniform age were collected and kept for adult emergence. Male and females of pink bollworms were identified at larval stage isolated for proper adult pairing (Dharajothi *et al.,* 2010). The adults emerged on the same date were released in adult chamber and provided with 10% honey solution soaked cotton swabs (adult diet) (Hariprasad, 1999; Wang *et al.,* 2005; Gary *et al.,* 2009; Ahir *et al.,* 2020; Nadeem *et al.,* 2023).

Experimental Plan: Newly emerged larvae (1st instar) were collected from the laboratory culture, reared on standard laboratory diet (Wu *et al.,* 2008) and observed till reached 4th instar stage. As soon as the larvae reached at 4th instar stage (stop feeding), the larvae were exposed to different regimes of temperature and photoperiods episodes. Freshly pick cotton bolls (10~20 days old) were excised with the help of scalpel knife (Peddu *et al.,* 2020; Naik *et al.,* 2021). The stem of these cotton bolls were embedded in cotton soaked in distilled water and wrapped with Para film layer to keep them succulent and provided to the larvae for rearing on natural diet (Attique *et al.,* 2004; Sapna *et al.,* 2017). In the study, the experiment comprised of ten replications and in each replication, five larvae were released in each of the treatments to be applied. Prior to the larval release in the cups, the larvae were released on a layer of paper towel to monitor their activity. The larvae showing maximum activity were picked with the help of a camel hair brush and placed gently in plastic cups containing dissected green cotton bolls. These larvae were kept isolated and encaged in separate plastic cups to avoid cannibalism. Different limits of temperature and various episodes of photoperiod were set at treatments (table#1). The experiment was conducted following completely randomized design (CRD) and the laboratory conditions were maintained as (26±2ºC, 65±5% and 9:15 L/D), (Dharajothi *et al.,* 2016; Reyaz *et al.,* 2018; Shrinivas *et al.,* 2019) at different time intervals

The larvae were observed throughout the developmental period (1st to 4th instar) until pupate. The data regarding larval diapause (induction and termination), different stages of diapause as pre-diapause (Spinning of silken cocoon), post-diapause (cutting of silken cocoon), larval mortality in the larvae (1st to 4th instar), pupal mortality and adult emergence in the larvae and adults were recorded in the corresponding treatments of temperature and photoperiods. The statistical analysis of the data was performed using computer based statistical programme the "Statistica" to check the significance of the treatments, compare means by Tukey's HSD followed by Post-hoc at probability of 5.00%.

Table 1: Treatments tested on pink bollworm (*Pectinophora gossypiella*) larvae under laboratory conditions

Sr. No	Treatments	Description		
		Temperature ºC	**Photoperiod (L: D)**	**Relative Humidity %**
1	**T_1**	14.00	15:09 (Constant)	65±05 (Constant)
2	**T_2**	18.00	15:09 (Constant)	65±05 (Constant)
3	**T_3**	22.00	15:09(Constant)	65±05 (Constant)
4	**T_4**	26.00	15:09 (Constant)	65±05 (Constant)
5	**T_5 Control**	26±02 (constant)	15:09 (Constant)	65±05 (Constant)
6	**T_1**	26±02 (constant)	07: 17	65±05 (Constant)
7	**T_2**	26±02 (constant)	09: 15	65±05 (Constant)
8	**T_3**	26±02 (constant)	11: 13	65±05 (Constant)
9	**T_4**	26±02 (constant)	13:11	65±05 (Constant)
10	**T_5 Control**	26±02 (constant)	15: 09 (Constant)	65±05 (Constant)

RESULTS AND DISCUSSION

The larvae of pink bollworms (4th instar) were exposed to different temperature ranges keeping a constant photoperiod and relative humidity of 15L: 09D and 65±05% respectively. Generally, the diapause induction increases as the duration for exposure to particular set of conditions (various limits of temperature) at fixed photoperiod and relative humidity. Nonetheless, variation in temperature from 14 to 26 °C caused most to the larvae to be active rather to go under diapause irrespective of the time of exposure. Larval mortality (%) at the 4th instar stage in pink bollworms decreased over time (number of days). As the temperature changed from 14 to 18, 22 and 26 °C, larval mortality increased when the conditions maintained for a period of one day and four days but decreased when extended for seven days. In the study, maximum number of larvae (68.00±1.83%) observed the condition of diapause at 14 °C on day-7 at constant photoperiod and relative humidity of 15L: 09D and 65±05% respectively. While a minimum of (24.02±1.87%) larvae were found to be in diapause at the same conditions on day-1 (Table. 2).

Table 2. Diapause & mortality in Pink bollworm (*Pectinophora gossypiella*) larvae under different temperature limits

Sr. #	(Temperature)	Day-1		Day-4		Day-7	
		Diapause Induction (%)	**Larval Mortality (%)**	**Diapause Induction (%)**	**Larval Mortality (%)**	**Diapause Induction (%)**	**Larval Mortality (%)**
T_1	14.00 °C	24.02±1.87	11.25±0.45	48.51±3.47	9.54±0.02	68.21±1.83	8.85±1.87
T_2	18.00 °C	17.50±1.22	36.09±1.82	27.85±3.52	16.42±1.64	41.27±0.29	8.21±2.45
T_3	22.00 °C	6.12±1.87	70.88 ±0.16	21.49±1.50	37.90±2.39	20.28±0.16	4.22±1.87
T_4	26.00 °C	0.00±0.00	87.92±0.74	3.11±1.03	47.97±3.33	21.96±0.13	2.05±0.33
T_5	Control	3.12±0.22	60.98±0.56	9.36±2.09	38.14±1.38	5.23±0.58	5.25±1.58

Another batch of larvae derived from a homogenous laboratory population of pink bollworm was subjected to a condition of constant temperature and relative humidity (26.00±2.00 °C and 65±05%) followed by different episodes of photoperiods (07: 17, 09:15, 11:13 and 13:11/ L: D) accordingly. The diapause trend for day-1, day-4 and day-7, increases at all the test photoperiod phases but dropped when recorded at 07:17 to 13:11/ L:D on all exposure intervals. Maximum number (28.01±0.20%) of the larvae were in the state of diapause when the photo phase of (07:17/ L:D) was maintained for seven days. While minimum (9.21 ±0.24%) at photoperiod of 13:11/ L:D within the same duration. At all the test conditions of photoperiods spells (07:17, 09:15, 11:13 and 13:11/ L: D) and exposure duration (day1, day-4 and day-7), larval mortality reduced horizontally and vertically (Table. 3).

Table 3. Diapause & mortality in Pink bollworm (*Pectinophora gossypiella*) larvae at different episodes of photoperiod

Sr. #	(Photoperiod)	Day-1		Day-4		Day-7	
		Diapause Induction (%)	**Larval Mortality (%)**	**Diapause Induction (%)**	**Larval Mortality (%)**	**Diapause Induction (%)**	**Larval Mortality (%)**
T_1	07: 17 (L:D)	17.85±0.89	32.01±0.22	21.08±0.40	27.46±3.16	28.01±0.20	18.22±2.52
T_2	09: 15 (L:D)	12.25±0.83	22.02±0.01	14.02±0.12	19.86±1.90	18.20±1.87	14.16±1.82
T_3	11: 13 (L:D)	5.68±0.45	19.01±0.25	09.33±0.52	15.70±1.60	10.35±1.58	11.32±1.18
T_4	13:11 (L:D)	4.31±0.96	10.01±0.02	8.05±0.25	9.15±0.15	9.21 ±0.24	6.22±0.06
T_5	Control	3.32±0.97	3.01±0.74	4.05±0.80	2.50±1.48	2.01±0.22	1.73±1.32

The research investigations were extended to explore the impact of the same set of treatment conditions (temperature regimes and photo phases) on pink bollworm adult emergence and mortality (%). The change in temperature (14, 18, 22 and 26 °C) and exposure time (day-7, day-10 and day-14) resulted in increase in adult emergence (%). Highest adult emergence (92.13±3.74%) was recorded at a fixed temperature of 26 °C when the pupae kept for 14 days. None of the adults emerged at 14 °C on the first week of exposure but reached to (23.44±1.88%) when kept for 14 days at the same temperature. A gradual decline in mortality (%) was found when the pupae were kept at different temperature (14, 18, 22 and 26 °C) for different exposure duration (7, 10 and 14 days). Whereas pupal mortality increased as the time of exposure increased (7, 10 and 14 days). Highest mortality (31.66±4.59%) was recorded at 14 °C when pupae where incubated for 14 days (Table. 4).

Table 4: Adult emergence & mortality in Pink bollworm (*Pectinophora gossypiella*) under different temperature limits

Sr. #	**(Temperature)**	**Day-7**		**Day-10**		**Day-14**	
		Adult Emergence (%)	**Adult Mortality (%)**	**Adult Emergence (%)**	**Adult Mortality (%)**	**Adult Emergence (%)**	**Adult Mortality (%)**
T_1	**14.00 °C**	0.00±0.00	13.55±1.01	09.58±0.73	22.37±1.56	23.44±1.88	31.66±4.59
T_2	**18.00 °C**	12.85±0.22	09.25±0.55	19.36±1.22	14.25±1.73	36.45±2.35	26.72±2.82
T_3	**22.00 °C**	14.25±0.87	07.05±0.45	38.70±1.30	09.28±0.33	59.89±1.45	13.11±1.22
T_4	**26.00 °C**	31.96±1.32	02.20±0.22	75.18±1.30	02.84±0.53	92.13±3.74	08.25±0.74
T_5	**Control**	18.37±1.55	10.25±1.58	59.09±0.99	11.64±0.48	70.11±7.19	15.66±1.52

Adult emergence (%) from the pupae of the pink bollworms was recorded at different limits of photoperiod (07: 17, 9: 15, 11: 13 and 31: 11/ L: D) at fixed temperature of 26±2 °C and 65±5% relative humidity. Adult emergence (%) and mortality (%) was increased as the exposure duration increased (7 to 14 days) and different episodes of photo phases. Nonetheless, pupal mortality (%) decreased within test photoperiod conditions. Maximum of 75.65±1.87% and 28.68±1.27% adult emergence and mortality were recorded when a photoperiod of 13:11 and 07: 17/ L:D was maintained at 14 days respectively (Table. 5).

Table 5: Adult emergence & mortality in Pink bollworm (*Pectinophora gossypiella*) at different episodes of photoperiod

Sr. #	**(Photoperiod)**	**Day-7**		**Day-10**		**Day-14**	
		Adult Emergence (%)	**Adult Mortality (%)**	**Adult Emergence (%)**	**Adult Mortality (%)**	**Adult Emergence (%)**	**Adult Mortality (%)**
T_1	07: 17 (L:D)	01.05±0.50	9.96±0.10	09.37±0.96	14.38±2.72	29.88±0.49	28.68±1.27
T_2	09: 15 (L:D)	06.15±0.87	7.85±0.55	19.84±1.13	13.28±1.34	44.56±0.84	22.02±1.71
T_3	11: 13 (L:D)	13.84±1.01	5.05±0.45	49.62±1.57	09.86±2.84	59.61±1.36	11.72±0.57
T_4	13:11 (L:D)	24.45±1.05	3.98±1.12	68.57±2.72	07.76±1.69	75.65±1.87	09.15±0.25
T_5	Control	16.65±1.92	2.22±1.87	51.57±2.34	71.56±2.53	70.12±1.91	81.54±0.10

The impact of a wide range of temperature and different episodes of photoperiod previously explored by a number of scientists who reported induction and termination of diapause in the 4th instar larvae of pink bollworms. The findings of Gutierrez *et al.*, (1981) do not conform to the present results with difference of temperature influencing diapause to varied extent. Our results also do not resemble to those reported by Adkisson *et al.,* (1963) who found 11.7, 19.1,46.0, 59.1, 83.9, 89.9% and 5.9, 9.6, 23.0, 29.5, 42.7, 45.8% larvae in diapause state at 33, 27 and 20°C (constant and variable temperature) and 12:12, 14:10/ L:D (photoperiod) respectively. However, the effect of diet ingredients with special emphasis on oil contents in the diet, (cottonseed and wheat germ meals-based diet) in the diet was found to be influencing diapause in pink bollworm when compared with the results of present findings. At the same time, the results found to be coinciding with Hummel *et al.,* (1973), and Foster and Crowder (1976) who reported that pink bollworm growth and development were similarly at their best when the temperature was in the right range of 26ºC, and 20°C, 11:13/L: D and 30% relative humidity respectively. Pink bollworm larvae were reared at 33°C, and mortality was found to be around 56.31% at this temperature. Further analysis of the present investigations indicated that rise in temperature from 14.00 to 26.00ºC, hinder to reach in the state of diapause which resembles to those reported by Sgolastra *et al.,* (2010) who found that at temperature of above 20ºC maximum diapause termination occurred. Watson et al., (1973), Tauber et al., (1986), furnished similar reports. El-Saadany *et al.*, (2003) and Pradhan *et al.,* (2022), who found that in Pink bollworms high mortality and delayed development, were seen at extremely hot and low temperatures.

The effect of different episodes of photoperiods (light: dark) on the termination of pupal diapause adult mortality was recorded at constant temperature (26±02ºC) showed an overall effect of decline in pupal diapause (increased adult emergence) as the light spells were enhanced. Adkisson *et al.,* (1963), Kaltsa *et al.,* (2006) and Mohapatra (2007) and many other studies reported that increasing the light ratio to dark of 24 hours intricate the reduction in diapause in pink bollworm.

The adult emergence from pupae and their survival potentials investigated under the influence of temperature and photoperiod variations showed an increase in both parameters which are supported by Khalifa *et al.,* (1975), Gergis *et al.,* (1990), Renault *et al.,* (2002), Fand *et al.,* (2020), Shrinivas *et al.,* (2019) and Venette *et al.,* (2000).

The present research provide more comprehensive than that of past regarding the depth knowledge of diapause induction, termination, adult emergence at particular temperature and photoperiod. These verdicts found to be novel in nature in terms of introducing a controlled mass production system for laboratory rearing of pink bollworm.

CONCLUSION

It is concluded that variation in temperature and photoperiod greatly affect the diapause in pink bollworms (*Pectinophora gossypiella*)

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