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Research Paper

Effect of elevated CO_2 and temperature on the life cycle of pink bollworm, *Pectinophora gossypiella* (Saunders) mediated by *Bt* cotton

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ABSTRACT

Pink bollworm (PBW), *Pectinophora gossypiella* (Saunders) is a notorious monophagous pest of cotton. Any changes in the nutritional quality of cotton have direct influences on the life cycle of the pink bollworm. In this regard, the host-mediated effect of elevated carbon dioxide (CO_2) and temperature on the life cycle of pink bollworm, *P. gossypiella* on *Bt* cotton was studied in the open top chambers at the Center for Agro-climatic Studies, UAS, Raichur. The results revealed that variations in phyto-chemistry of *Bt* cotton particularly the *Cry*toxin expression and nitrogen have reduced in elevated CO_2 and temperature conditions and also reduced the larval, pupal period, adult longevity, fecundity and incubation period. However, there was no significant difference in the biology of PBW among the climate change treatments. Similar results were noticed when PBW was reared on *Bt* cotton collected from different open top chambers and reared separately in growth chambers and the same larvae were analyzed for their enzyme activity. The results showed that there was significant increase in the activity of midgut proteases (trypsin and chymotrypsin) and carbohydrases (α-amylase) and decrease in the activity of lipase enzyme was noticed.

Key words: *Pectinophora gossypiella*, cotton, open top chambers, climate change, *cry*toxins

Cotton is the most important industrial fiber crop worldwide cultivated in an area of 33.38 million hectares with a production of 121.37 million bales and productivity of 792 kg per ha. In India cotton is cultivated in 125.84 lakh hectares with a production of 360 lakh bales in 2019-20 (CAB, 2019). Worldwide adoption of *Bt* cotton with no proper regulation of its cultivation (without refugees) has invited more pest problems, particularly pink bollworm. Since three to four years or so, pink bollworm, *Pectinophora gossypiella* (Saunders) has aggravated on *Bt* cotton as it has developed resistance to insecticides and to cry toxin (*Cry1Ac* and *Cry1Ab)* in India of *Bt* cotton (Tabashnik *et al*., 2008). Higher temperatures and drought can alter the expression of *Bt* toxin by changing plant architecture and physiology, which may influence the pest behavior (Jurat-Fuentes *et al*, 2017). Environmental conditions can drastically affect the efficacy of *Bt* cotton by reducing the expression and can increase susceptibility to pest pressure and reduce yields (Calles-Torrez *et al*., 2019).

Climate change is one of the greatest concerns and research challenge faced by agronomists, entomologists, conservation biologists, and plant biologists (IPCC, 2014). The current path of carbon dioxide would increase global temperature by as much as 4.4 ºC by the end of the century (IPCC, 2021). Das *et al*., (2020) reported that the growth and yield of rice was found to be better under elevated CO_2-temperature levels when transplanted in first fortnight of July in NE India. The combined effect of elevated carbon dioxide and temperature may have a direct and indirect effect on the tri-trophic interaction between host plants, herbivores, and their natural enemies (Stiling *et al*., 2002). Climate change affects the insects either directly by altering their physiological mechanisms (Amarasekare and Coutinho 2014, Reuman *et al.* 2013), developmental rate and phenology (Rudolf and Singh, 2013). Wherein, increased growth and physiological parameter were noticed in most of the plant species, particularly in C_3 plants (Bazzaz, 1990) and indirectly by decreasing the nutritional quality of plants and changing the plant hormone composition (Zavala *et al.,* 2013) thereby affecting the insect-plant interaction. An increase in CO_2 reduces transpiration rate, stomatal conductance, and increases the photosynthetic rate. Elevated CO_2 has positive effect on plant growth and yield attributes in both cultivars of bell pepper. However,

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under interactive effect of elevated CO_2 and elevated temperature, rising temperature negated the positive effects of elevated CO_2 on crop production (Kumari *et al*., 2019). Climate change will directly affect insect reproduction, development, survival, dispersal which indirectly correlates between pests and their natural enemies. However, the response of different insect species to climate change varies and may result in pest incidence or outbreaks (Parmesan, 2006). Hence, the present investigation was carried out to know the host-mediated effect of eCO_2 and temperature on the growth and development of pink bollworm, *P. gossypiella* which will help to formulate effective management strategies in the future.

MATERIALS AND METHODS

Investigations on the effect of climate change variables on pink bollworm biology were carried out at the Centre for Agro-climatic Studies, Main Agricultural Research Station, University of Agricultural Sciences, Raichur, 16.2044° N, 77.3341° E. Four circular OTC's (Open Top Chambers) were constructed by genesis group of technology, Mumbai with dimensions of five meters diameter and four meters height. In these different climate change treatments were set and the *Bt* cotton crop was sown.

These Open Top Chamber's were connected to Supervisory Control and Data Acquisition (SCADA) software system which could able to supply desired concentration of CO_2 and set temperature inside OTC's for 12 hours (morning 6 am to evening 6 pm). However, in the night hours due to the closure of the stomata the gaseous exchange is almost nil. Different climate change treatments simulated in different open top chambers are as follows,

T_1: Ambient CO_2 and elevated temperature (410 ppm +35 ºC),

T_2: Elevated CO_2 and elevated temperature (550 ppm +35 ºC),

T_3: Elevated CO_2 and ambient temperature (550 ppm + 33 ºC),

T_4: Ambient CO_2 and ambient temperature (410 ppm +33 ºC) and

T_5: Reference plot.

The popular *Bt* cotton hybrid Everest-II (Bio-seed company, Hyderabad) was raised individually in different climate change treatments (OTCs) in 10 cement pots of size 42 x 32 sq. cm containing a mixture of FYM, vermin compost, and soil in the ratio of 1:1:2. All agronomic practices for raising crop were followed as per the package of the University of Agricultural Sciences, Raichur. Cry toxin estimation was carried out by the ELISA method (Grothaus *et al.,* 2006) to quantify *Bt* toxin expression in leaves and bolls at 90 and 120 days of crop age from the respective treatments.

Effect eCO_2 and temperature on bionomics of pink bollworm on Bt cotton grown under OTC's

Maximum population of pink bollworm, *P. gossypiella* larvae were maintained on a cotton seed-based artificial diet (Dharajothi *et al.,* 2016) in the National Food Security Mission laboratory, University of Agricultural Sciences, Raichur.

Bt cotton plants were raised in cement pots in different open top chambers. When plants were 60 days old, 2nd instar larvae of pink bollworm, *Pectinophora gossypiella* (ten neonates) were released on five plants and covered with nylon net (size0.15 x 0.15 sq. cm). Later the bolls (after 15 days) containing pupae were collected from each OTC as well as a reference plot. Further, pupae were sexed and moths (1:1) ratio were placed in plastic containers (20 x 10 cm) having 10 % honey solution for adults. Four replications were maintained for each treatment. Further, the observations were recorded on different life stages (egg, larval, pupal and adult period).

Direct effect of eCO_2 and temperature on bionomics of pink bollworm under growth chambers

The detached boll assay was used to study the effect of elevated CO_2 and temperature on *P. gossypiella* under laboratory conditions (Sharma *et al.,* 2005). Young bolls were collected from *Bt* cotton plants grown under different OTC's and fed to 20 neonates (2nd instar) which was replicated thrice separately under growth chambers having set parameters similar to different treatments of open top chambers. Observations on growth and reproductive parameters were recorded. Midgut extract for analyzing digestive enzyme activity (α-amylase, Trypsin activity and chymotrypsin activity, lipase activity) was measured by the method as described by Tukaram, 2014.

Data analysis

Combined effects of CO_2 and temperature on cry toxin expression in *Bt* cotton were analyzed using a one-way analysis of variance. Treatment means were compared and separated using the least significant difference (LSD) at $p < 0.01$. The data on larval weight, larval duration, pupal weight, pupal duration, moth emergence, fecundity was analyzed using ANOVA. Statistical analysis was done by using SPSS software (version 16.0).

RESULTS AND DISCUSSION

The concentration of *Bt* toxin, Cry1Ac in leaves in reference plot was 3.35 ± 0.03 µg/g with 2.48 ± 0.09 µg/g in boll rind at 90 days after sowing which was significantly more (Write value recorded in reference). Wherein, climate change treatments *viz.,* eCO_2+33ºC and eCO_2+35ºCrecorded the minimum concentration of Cry1Ac in leaves and boll rind. A similar trend was noticed at Cry1Ac concentration recorded at 120 days after sowing was presented in Fig. 1.

Likewise, toxin ($Cry2Ab_2$) quantified in leaves and boll rind at 90 days after sowing showed maximum concentration in reference plot (60.17 ± 0.96 µg in leaves and 54.81 ± 1.83 µg/g in boll rind) which was on par with the ambient CO_2+33 ºC followed by eCO_2+35ºC treatment. Contrastingly, the minimum concentration of $Cry2Ab_2$ in leaves (39.99 ± 1.17 µg) and boll rind (27.55 ± 2.65 µg) was recorded at eCO_2+33 ºC followed by eCO_2+35 ºC treatment (Fig 2).

The present findings on the effect of eCO_2 and temperature on *Bt* toxin have resulted in reduced toxin expression with the increased CO_2 condition. Similarly, present findings are in accordance with Gang *et al.,* 2007, who reported that *Bt* toxin

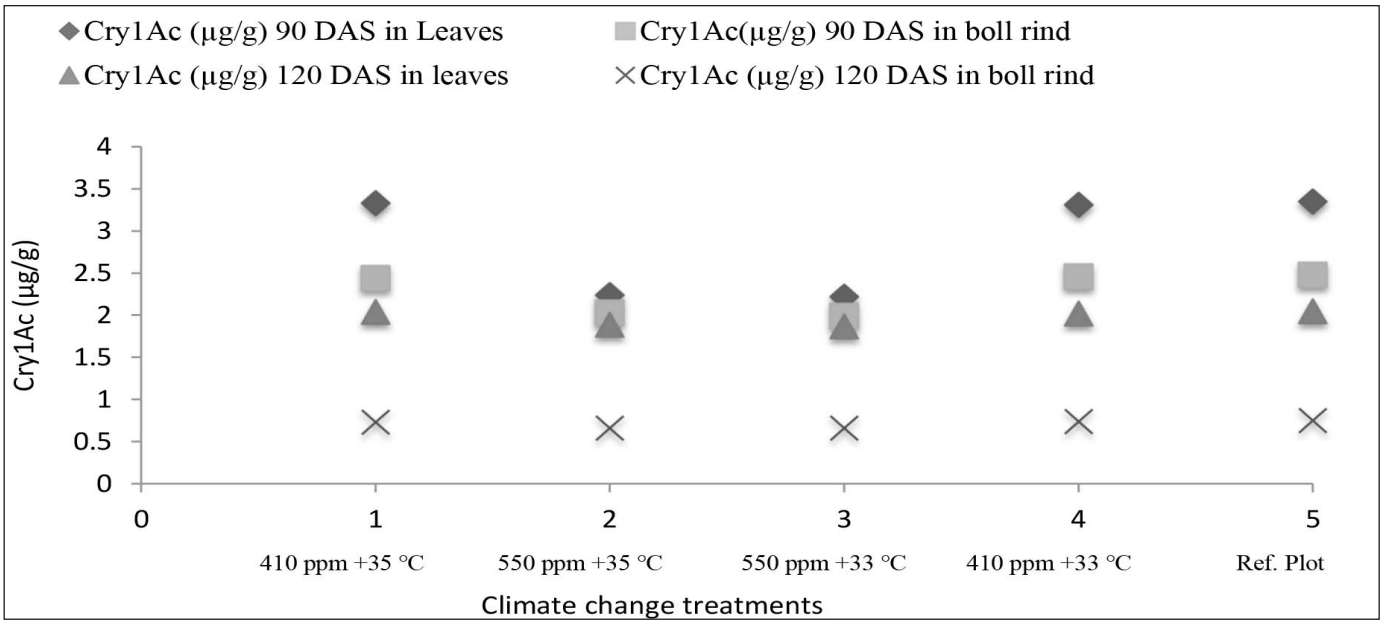

Fig. 1: Effect of elevated CO_2 and temperature on Cry1Ac *Bt* toxin expression

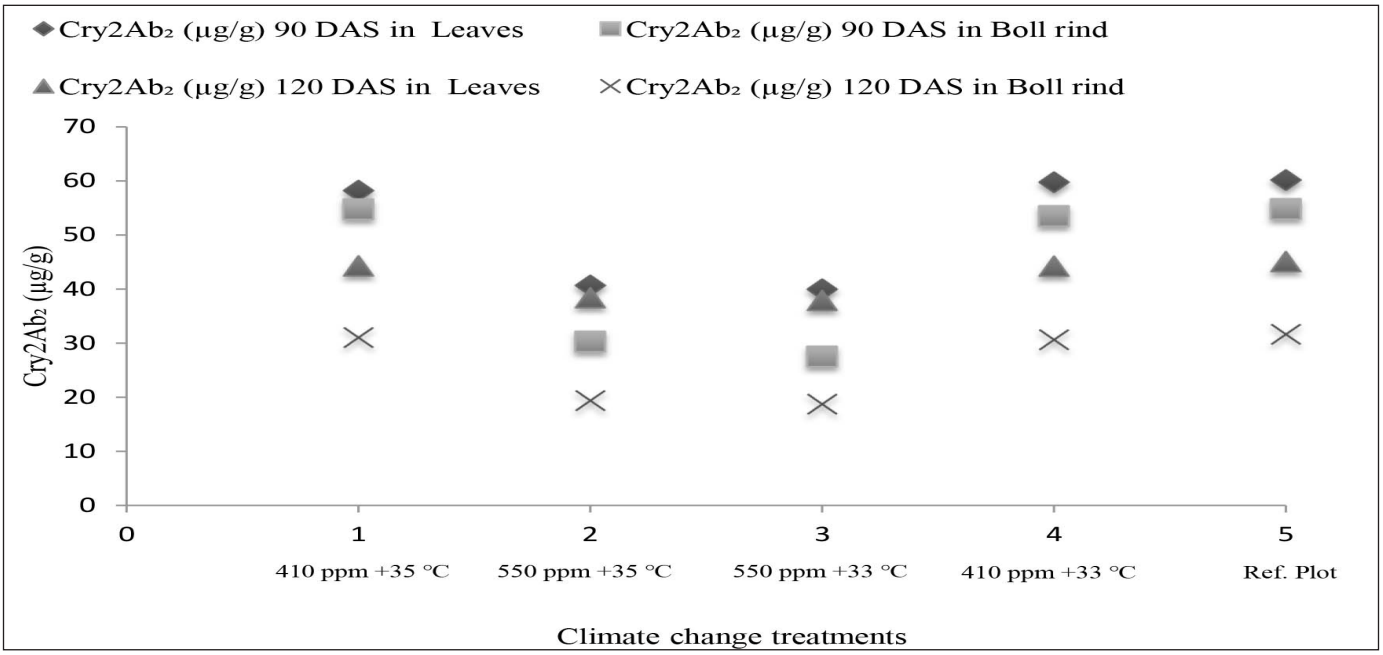

Fig. 2: Effect of elevated CO_2 and temperature on $Cry2Ab_2$ *Bt* toxin expression

decreased by 4 percent and 2.5 percent in transgenic *Bt* cotton in two consecutive years which is presumed that eCO_2 (double-ambient) can alter the plant growth and ultimately the phenotype allocation to foliar chemical components of transgenic *Bt* cotton, which may, in turn, affect the plant-herbivore interactions (Gang *et al.,* 2007). High temperature also affected the insecticidal properties of *Bt* cotton which resulted in the degradation of soluble proteins in the leaf, which resulted in a decline in Cry1AC toxin even eCO_2 decreased *Bt* toxin (Shreevani 2015). John and Cassey (2009) have reported that growing transgenic plants in elevated carbon dioxide conditions resulted in a nearly 25 percent reduction of expression of these proteins.

Effect of elevated CO_2 and temperature on growth and development of pink bollworm mediated by Bt cotton host

The variations in phyto-chemistry and cry toxin expression of the *Bt* cotton plant due to the indirect effect of elevated CO_2 and temperature have influenced the changes in growth and development of *P. gossypiella* when reared on *Bt* cotton under different treatments.

Adult longevity

Average adult longevity of males and females in different climate change treatments (OTCs with a different set of carbon dioxide and temperature) varied from 5.09 ± 0.36 to8.00 ± 0.16 in males and 5.58 ± 0.54 to 8.03 ± 0.33 days in females. The elevated treatment particularlyeCO_2+33ºC recorded minimum longevity of

Table 1: Indirect effect of elevated CO_2 and temperature on bionomics of *P. gossypiella*mediated by *Bt* cotton host

Treatment details	Adult longevity (days) Male	Adult longevity (days) Female	Oviposition period (days)	Fecundity/ Female	Incubation period (days)	Larval weight (mg)	Total larval period (days)	Pupal weight (mg)	Total pupal period (days)	Total life cycle (days)
aCO_2 + eTemp (410 ppm + 35ºC)	7.90 ± 0.54[a]	8.01 ± 0.25[a]	4.00 ± 0.41[a]	111.54 ± 3.89[a]	4.24 ± 0.40[a]	31.70 ± 1.41[a]	22.59 ± 1.85[b]	20.54 ± 1.32[a]	8.00 ± 0.71[a]	42.78 ± 2.53[a]
eCO_2 + eTemp (550 ppm + 35ºC)	5.25 ± 0.65[b]	5.73 ± 0.50[b]	3.17 ± 0.71[b]	92.17 ± 4.72[b]	3.82 ± 0.22[b]	27.98 ± 1.56[b]	25.34 ± 0.61[a]	16.15 ± ٠٫47[b]	6.68 ± 0.26[b]	41.32 ± 1.00[a]
eCO_2+ Temp (550 ppm+ 33ºC)	5.09 ± 0.36[b]	5.58 ± 0.54[b]	3.00 ± 0.71[b]	93.05 ± 5.09[b]	3.74 ± 0.18[b]	27.21 ± 1.46[b]	25.87 ± 0.73[a]	16.13 ± 0.74[b]	6.52 ± 0.74[b]	41.61 ± 0.58[a]
aCO_2+ Temp (410 ppm+ 33ºC Reference OTC)	7.86 ± 0.27[a]	7.94 ± 0.17[a]	3.88 ± 0.29[a]	110.03± 6.38[a]	4.23 ± 0.18[a]	31.49 ± 0.79[a]	23.10 ± 0.70[b]	20.50 ± 0.88[a]	7.95 ± 0.42[a]	43.10 ± 1.05[a]
Reference plot	8.00 ± 0.16[a]	8.03 ± 0.33[a]	4.05 ± 0.42[a]	112.15 ± 4.88[a]	4.25 ± 0.42[a]	32.04 ± 0.79[a]	22.75 ± 1.99[b]	21.25 ± 0.93[a]	8.03 ± 0.38[a]	43.04 ± 2.04[a]
$F_{4,15}$	48.81**	44.31**	3.45**	16.40**	2.83**	12.64**	5.49**	31.43**	8.23**	1.08*
S.Em (±)	0.22	0.19	0.27	2.53	0.15	0.65	0.66	0.45	0.27	0.81
CD (p=0.01)	0.90	0.80	1.12	10.54	0.63	2.69	2.76	1.90	1.12	3.36

Mean ± SD are separated by Least significant difference *Significant @ 1%
Means denoted by same letters in vertical column are not significantly different by DMRT

Table 2: Direct effect of elevated CO_2 and temperature on bionomics of *P. gossypiella* under plant growth chambers

Treatments	Adult longevity (days) Male	Adult longevity (days) Female	Oviposition period (days)	Fecundity/ Female	Incubation period (days)	Larval weight (mg)	Total larval period (days)	Pupal weight (mg)	Total pupal period (days)	Total life cycle (days)
aCO_2 + eTemp (410 ppm +35ºC)	7.97 ± 0.33[a]	7.99 ± 0.21[a]	3.95 ± 0.25[a]	109.26 ± 5.72[a]	4.16 ± 0.35[a]	33.87 ± 1.48[a]	20.23 ± 0.85[b]	20.80 ± 1.23[a]	7.82 ± 0.75[a]	39.08 ±1.94[c]
eCO_2 + eTemp (550 ppm + 35ºC)	5.75 ± 0.54[b]	6.00 ± 0.93[b]	2.97 ± 0.35[b]	97.83 ± 3.63[b]	3.29 ± 0.11[b]	26.09 ± 0.33[b]	26.22 ± 0.71[a]	16.57 ± 0.34[b]	6.38 ± 0.97[b]	41.45 ± 0.89[b]
eCO_2+ Temp (550 ppm+ 33ºC)	5.31± 0.55[b]	5.81 ± 0.43[b]	2.91 ± 0.34[b]	97.46 ± 3.88[b]	3.18 ± 0.22[b]	25.18 ± 0.46[b]	26.72 ± 0.37[a]	16.46 ± 0.50[b]	6.33 ± 0.58[b]	43.21 ± 0.33[a]
aCO_2+ Temp (410 ppm+ 33ºC Reference OTC)	7.93 ± 0.41[a]	7.81 ± 0.50[a]	3.92 ± 0.17[a]	108.05 ± 9.55[a]	4.08 ± 0.21[a]	33.32 ± 0.84[a]	21.29 ± 1.04[b]	20.35 ± 0.65[a]	7.39 ± 0.51[a]	40.66 ± 1.34[b]
Reference plot	8.00 ± 0.32[a]	8.06 ± 0.15[a]	4.06 ± 1.15[a]	111.46 ± 7.93[a]	4.20 ± 0.30[a]	34.13 ± 1.43[a]	21.19± 0.69[b]	21.11 ± 1.19[a]	7.88 ± 0.32[a]	41.29 ±0.61[b]
$F_{4,15}$	36.91**	39.82**	18.85**	4.12**	15.87**	76.35**	65.13**	29.39**	5.28**	11.90**
S.Em (±)	0.22	0.18	0.13	3.28	0.13	0.51	0.38	0.43	0.33	0.45
CD (p=0.01)	0.92	0.75	0.55	13.66	0.52	2.14	1.59	1.79	1.38	1.86

Mean ± SD are separated by Least significant difference, **Significant @ 1%
Mean denoted by same letters in vertical column are not significantly different by DMRT

5.09 ± 0.36 days whereas, the reference plot recorded maximum longevity of 8.00 ± 0.16 days. A similar kind of result was obtained from the pink bollworm reared on the same set of treatments in growth chambers (Table 1 and 2).

Results of the present study have contradicted the findings of Mironidis and Savopoulou-soultani (2014), who reported that adult longevity of *H. armigera* increased when the temperature got decreased.

Fecundity

Fecundity per female was more in ambient conditions particularly in the reference plot (112.15 ± 4.88) compared to elevated treatments (550 ppm+ 33ºC) 92.17 ± 4.72.

Larval and pupal weight

The average larval and pupal weight varied from 27.21 ± 1.46to 32.04 ± 0.79 mg and 16.13 ± 0.76 to 21.25 ± 0.93 mg, respectively across treatments. The highest larval weight (32.04 ± 0.79 mg) and pupal weight (21.25 ± 0.93) was recorded in ambient conditions (reference plot) than the elevated climate change treatments (Table 1).Similar result was obtained in different growth chamber treatments (Table 2).

The results are comparable with Abdul (2012) who observed that *H. armigera* (Hubner) fed with chickpea leaves obtained from elevated CO_2 conditions resulted in long larval duration and reduced pupal weight as compared to ambient

Table 3: Impact of elevated CO_2 and temperature on digestive enzyme activity of pink bollworm, *P. gossypiella*

Treatments	Digestive enzyme activity (mU/mL)			
	Trypsin	Chymotrypsin	α-Amylase	Lipase
aCO_2 + eTemp @ 410 ppm + 35ºC	18.99 ± 1.43[b]	27.59 ± 0.47[b]	5.22 ± 0.20[b]	9.58 ± 0.43[a]
eCO_2 + eTemp @ 550 ppm + 35ºC	23.59 ± 0.73[a]	39.48 ± 1.60[a]	8.45 ± 0.08a	5.16 ± 0.08[b]
eCO_2+ Temp 550 ppm+ 33ºC	24.13 ± 0.18[a]	41.08 ± 1.53[a]	8.52 ± 0.62[a]	4.78 ± 0.28[b]
aCO_2+ Temp 410 ppm+ 33ºC (Reference OTC)	18.46 ± 0.73[b]	26.89 ± 2.26[b]	5.26 ± 0.60[b]	9.66 ± 0.57[a]
Reference plot (Open plot)	19.33 ± 0.56[b]	27.77 ± 1.36[b]	5.24 ± 0.42[b]	10.01 ± 0.32[a]
$F_{4, 15}$	42.84**	82.69**	64.70**	203.62**
S. Em(±)	0.42	0.78	0.22	0.19
CD (p = 0.01)	1.73	3.24	0.92	0.77

Mean ± SD are separated by Least significant difference **Significant @ 1%

Means denoted by same letters in vertical column are not significantly different by DMRT

conditions which resulted in reduced fecundity per female (373.25 and 383.87 eggs) under elevated-I CO_2 (550 ± 25 ppm) and elevated-II CO_2 (700 ± 25 ppm) conditions, respectively compared to ambient conditions (463.87 eggs/female).

Larval and pupal duration

Long larval duration was recorded in eCO_2+35 ºC treatment (25.87 ± 0.73 days) which was found to be on par with eCO_2+33ºC treatment (25.34 ± 0.61days). The short larval duration was noticed in aCO_2+35 ºC treatment (22.59 ± 1.85days) followed by reference plot and reference open top chamber. In contrary to larval duration, long pupal duration was noticed in the reference plot (8.03 ± 0.38 days) which was the non-significant difference with reference open top chamber and aCO_2+35 ºC treatments. Short pupal duration was recorded in eCO_2+33ºC treatment (6.52 ± 0.74 days) and eCO_2+35ºC treatments (6.68 ± 0.26 days) (Table 1).Similar kinds of results were obtained when pink bollworm larvae were reared in the growth chamber (Table 2).

Total life cycle (days)

The total life span of pink bollworm from egg stage to death of adults varied from 41.32 ± 1.00 to 43.10 ± 1.05 days across treatments. The average life span was more in reference open top chamber (43.10 ± 1.05 days) and lowest in eCO_2+35 ºC treatment (41.32 ± 1.00 days) (Table 1). In contrast to this pupa reared in a growth, chamber recorded more life span in elevated carbon dioxide and temperature treatments (43.21 ± 0.33 days) as compared to ambient carbon dioxide and temperature treatments (39.08 ±1.94 days) (Table-2).

Host mediated effect of elevated CO_2 and temperature on digestive enzyme activity of P. gossypiella

Under eCO_2 conditions (eCO_2 alone and in combination with temperature) activities of midgut proteases (trypsin activity of 24.13 ± 0.18 mU/mL and chymotrypsin activity of 41.08 ± 1.53 mU/mL), carbohydrases (α-amylase activity of ٠,٦٢ ± ٨,٥٢ mU/mL) have significantly increased as compared to ambient conditions (Table 3). Whereas, lipase activity (4.78 ± 0.28 mU/mL) was drastically decreased in eCO_2 treatments over and reference plot treatment (10.01 ± 0.32 mU/mL) and aCO_2 (9.66 ± 0.57 mU/mL) treatments (Table 3).

The nutritional requirements of the insects are obtained from proper digestion of ingested food and by utilizing food from the environment. The digestive enzyme activity in the midgut is influenced by a change in concentration of carbon dioxide and temperature which intern affect the survival and development of *P. gossypiella.* Results of the present studies are in accordance with the findings of Akbar *et al* (2015) who reported that *H. armigera* caused more damage to crops due to increased food consumption because of increased temperature and carbon dioxide (eCO_2) which influenced the metabolism of larvae by enhancing the activity of carbohydrases (amylase and cellulase, midgut protease (trypsin and chymotrypsin) and mitochondrial enzymes.

CONCLUSION

The combined effect of elevated carbon dioxide and temperature can alter pest behavior and weaken the expression of *Bt* toxin by changing the plant chemistry. The variation in cry toxin expression of the *Bt* cotton plant has influenced the growth and development of *P. gossypiella* when reared in environmental growth chambers. Under eCO_2 conditions (eCO_2 alone and in combination with temperature) activities of midgut proteases and carbohydrases have significantly increased as compared to ambient conditions. Whereas, lipase activity was drastically decreased in eCO_2 treatments as compared to reference plot treatment. This indicated the increased metabolism and digestive efficiency of pink bollworm larvae. But weight gain by the larvae and pupae was negatively correlated with increased activities of midgut proteases (trypsin and chemo trypsin) due to decreased conversion of digested food into body matter.

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Conflict of Interest Statement: The author (s) declares (s) that there is no conflict of interest.

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